Prevalence of Heavy Prenatal Alcohol Exposure in Uganda via Analysis of Fatty Acid Ethyl Esters in Meconium

by

Ira Justin Nightingale

A thesis submitted in conformity with the requirements for the degree of Master of Science

Leslie Dan Faculty of Pharmacy, Pharmaceutical Sciences
University of Toronto

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2016

Abstract

Fetal alcohol spectrum disorder (FASD) manifests as a continuum of permanent birth defects and neurodevelopmental impairments that originate from maternal alcohol use during pregnancy. The number of recognized FASD cases in parts of Sub-Saharan Africa is growing, which is a cause for concern. Prenatal alcohol exposure was examined via regional hospital population-based collection of meconium, and analysis of the fatty acid ethyl esters (FAEEs) contained within. Meconium samples were collected from five hundred ten neonates over a two-month period at Mbarara Regional Referral Hospital. Each meconium sample was accompanied by a maternal questionnaire. The prevalence of prenatal alcohol exposure using the maternal questionnaire was 16.1% (81 of 510). The overall prevalence of prenatal alcohol exposure via FAEE-meconium analysis in this population was 1.6% (8 of 510). This is the first population-based study of an entire local neonatal population examining fetal alcohol exposure via meconium-FAEE analysis conducted in Uganda.
Acknowledgments

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Chapter 1
INTRODUCTION

1.1 Study Rationale

Fetal alcohol spectrum disorder (FASD) manifests as a wide range of physical, cognitive and neurobehavioural deficits that result from prenatal alcohol exposure (Chudley et al., 2005). Diagnostic signs of FASD become evident between the ages 4 and 14 years, thus attempting to identify FASD in newborns can be problematic (Little et al., 1990). Children affected by FASD may not show the physical symptoms attributed to FASD and diagnosis may be delayed or missed entirely. Only 10% of alcohol-exposed children exhibit the dysmorphic craniofacial features associated with FASD, leaving 90% of potentially affected individuals without obvious physical abnormalities (Abel, 1995; Sampson et al., 1997). Due to the limited awareness and resources allocated to addressing FASD, only a fraction of those affected currently receive a diagnosis, likely leading to underestimates of population FASD burden (Lupton et al., 2004; Chudley et al., 2005).

Overall, diagnosis and identification of FASD using maternal self-reporting is an unreliable method, due to the social stigma associated with verified alcohol and drug use during pregnancy (Littner & Bearer, 2007). Consequently, there is a strong demand to identify a reliable biological marker for in utero alcohol exposure, especially in cases of heavy prenatal exposure. The availability of a biological marker to detect prenatal alcohol exposure would allow for early intervention, prevention and minimization of the adverse effects that can arise from ‘secondary
disabilities’ (Zelner et al., 2010), which include trouble with the law leading to incarceration, drug dependency, unemployment, and disrupted school experiences (Streissguth, 1996; Streissguth et al., 2004).

In order to overcome this ambiguity in maternal self-reporting, the use of biological markers has emerged as a potentially feasible method for early identification of prenatal alcohol exposure (Goh et al., 2010). Fatty acid ethyl esters (FAEES) present in meconium have been established as viable biological markers for fetal alcohol exposure (Moore et al., 2003; Bearer et al., 1999).

This study seeks to determine the prevalence of heavy prenatal alcohol exposure through the analysis of FAEEs in meconium over a two-month study period in a local hospital in Uganda. The identification of specific populations in need of basic epidemiologic research on the prevalence of prenatal alcohol exposure is urgently required in order to create awareness of the problem and reduce the devastating personal and social impacts of FASD, especially in developing nations.

1.2 Study Objectives

I. To determine the prevalence of heavy prenatal alcohol exposure during pregnancy by meconium FAEE analysis in both urban and rural Ugandan women.

II. To determine maternal self-reported alcohol consumption during pregnancy by using a detailed questionnaire.
1.3 Research Hypotheses

*Hypothesis #1:* The use of an objective meconium-FAEE analysis will expose a higher measure of the prevalence of heavy prenatal alcohol exposure compared to maternal alcohol self-report.

*Rationale #1:* Maternal self-reporting of alcohol use remains an unreliable measure of consumption due to recall bias and social stigma. Mothers are less willing to report any use of alcohol during pregnancy due to the fear of being judged, and of child apprehension issues. Using an objective biomarker to detect alcohol consumption during pregnancy should yield a higher accuracy in detecting ethanol exposure to the fetus compared to reliance on a subjective technique.

*Hypothesis #2:* The prevalence of heavy prenatal alcohol exposure in the studied Ugandan population will be higher compared to other published study populations in which prevalence is determined by meconium-FAEE analysis.

*Rationale #2:* Given the evidence of elevated alcohol consumption rates among the adult population in Uganda compared to published global figures, the prevalence of prenatal alcohol exposure is expected to be much higher in this study population.

1.4 Literature Review

1.4.1 FASD Etiology

Even though the term *fetal alcohol spectrum disorder* (FASD) was only coined within the past three decades (Clarren, 2005), it had been observed many centuries ago, notably by the
Greek philosopher-scientist Aristotle, and referred to in the Biblical Book of Judges 13:2-14 (Abel EL, 1999; Zondervan, 1978). However, more recent historical mentions of the recognition of the effects of alcohol on the developing fetus also exist (Hoyme et al., 2005). In the 1700’s, a group of English physicians described children born from alcoholics as being “feeble and distempered, and born weak and silly, shrieveled and old” (Royal College of Physicians of London, 1726; Goodacre K. 1965). In more recent times, a French group (Lemoine et al., 2012) revisited the misunderstood but crucial concept of the adverse effects of heavy prenatal alcohol exposure on children. They concluded that among over 100 children of women who drank heavily during pregnancy, all showed similar behavioural and physical patterns of development. However, these findings did not provide any practical diagnostic tools that may have potentially led to the recognition of FASD at the time.

Ultimately, Jones, Smith and colleagues published the initial diagnostic criteria for the condition they defined, fetal alcohol syndrome (Jones et al., 1973; Jones & Smith, 1973; Ulleland, 1972). Complete FAS was characterized by specific physical abnormalities (prenatal growth deficiency, minor facial anomalies, neurocognitive deficits) in conjunction with a confirmed report of maternal alcohol intake during pregnancy (Stratton et al., 1996). Eventually, the need for a diagnosis to describe children with less severe phenotypes led to the term fetal alcohol effects (FAE; Hoyme et al., 2005). For many years, the term FAE remained problematic due the lack of consistency with regard to its interpretation. The term FAE legitimized a more general interpretation by clinicians, rendering it diagnostically inadequate (Hoyme et al., 2005). This led to considerable frustration among health care providers and families alike, which led to the unfortunate consequence of disregarding alcohol as an overall contributing factor to this illness (Aase JM, Jones KL & Clarren SK, 1995). In 1996, the U.S. Congress authorized a study on FAS that generated the first set of diagnostic criteria (Institute of Medicine, IOM of the
National Academy of Sciences) for diagnosing children prenatally exposed to alcohol. (Stratton et al., 1996).

Fetal alcohol spectrum disorder (FASD) originates from maternal alcohol use during pregnancy (Chudley et al., 2005). People affected by FASD exhibit an extensive array of physical and cognitive manifestations, varying from major physical abnormalities and severe developmental delays to more subtle deficiencies that can negatively affect an individual’s capability to function within society (Streissguth et al., 1996). The hallmark, clinically apparent physical symptoms of FASD include: microcephaly, intrauterine growth restriction (IUGR), short palpebral fissures, short nose, low nasal bridge, flat midface, smooth philtrum, thin vermilion border, and ear anomalies, (Wattendorf and Muenke, 2005). In addition to these physical anomalies, children exposed to large amounts of alcohol in utero can have serious neurocognitive impairment (Lemoine, 2003). Although numerous theoretical mechanisms have been proposed to explain the etiology of FASD, the molecular targets of alcohol toxicity during early development still remain unknown (Muralidharan et al., 2013).

The proposed mechanisms include cell-signaling defects, cell death (apoptotic and necrotic), epigenetic changes related to altered gene expression, vitamin A deficiency, folic acid insufficiencies, and many others (Li et al., 2007; Smith 1997; Muralidharan et al., 2013). Low levels in alcoholic women of an essential nutrient in the human diet, choline, may also lead to the development of abnormalities that are representative of FASD (Zeisel 2011).

Choline availability during pregnancy has direct epigenetic influences on DNA and histones, which can modify the expression of genes required for neural tube development (Zeisel 2011). Vitamin A (retinol) deficiency is also linked to a number of diseases, including FASD: it is oxidized into retinoic acid (RA) in the developing embryo, a process highly regulated by the
expression of genes encoding alcohol/aldehyde dehydrogenase enzymes (ADH/ALDH). Studies have shown that in the presence of ethanol, RA levels are reduced due to competitive inhibition of retinol oxidation (Mezey et al., 1971). This results in a downregulation of the RA synthetic pathway, potentially resulting in embryonic malformations that are synonymous with the dysmorphology found in FASD cases (Duester, 1991; Keir, 1991).

Epidemiological studies suggest that the prevalence of FASD in the U.S. is as high as 2-5%, while the prevalence is even higher in lower socioeconomic populations (Muralidharan et al., 2013). Differences in dosage, timing of ethanol exposure, and environmental and genetic factors may explain the wide spectrum of physical and mental deficits seen in FASD (Feldman et al., 2012; Muralidharan et al., 2013).

It is important to note that not all children exposed to heavy amounts of ethanol in utero will be affected to the same degree. Recent statistics show that among children exposed to ethanol during pregnancy, 40% will be detectably affected and of this subpopulation, 4% will exhibit the physical craniofacial dysmorphism attributed to full-blown FAS (Abel, 1995).

Although it has been assumed that paternal alcohol intake is not teratogenic to the fetus, studies indicate that paternal drinking of a certain magnitude may encourage similar alcohol consumption behaviours in their pregnant partners, thereby exerting an indirect effect, and increasing the risk of the fetus being exposed to clinically significant amounts of ethanol (Bakhireva et al., 2011). However more recently, there has been speculation that paternal preconception ethanol consumption may induce a spectrum of morphological and cognitive deficits in the offspring via epigenetic factors (Finegersh et al., 2015). Several studies have shown that children born to fathers with alcohol use disorders (AUD) had a higher risk of psychiatric disorders (Ozkaragoz et al., 1997; Pihl et al., 1996), decreased IQ test scores (Ervin et
al., 1984), decreased intracranial volumes (Gilman et al., 2007), and electroencephalographic changes (Ramsey & Finn, 1997).

1.4.2 FASD Clinical Diagnosis

Fetal alcohol spectrum disorder (FASD) results from a wide array of adverse effects that alcohol has on the developing fetus, which include a spectrum of physical abnormalities and neurocognitive deficits (Barr, 2001). The diagnosis of FASD among children has been an ongoing challenge for physicians (Hoyme et al., 2005). Some phenotypes of FASD include readily apparent growth impairment with regards to a child’s height and weight, abnormal facial features, cognitive deficits, and social impairment (Streissguth et al., 2004). However, on the mild end of the spectrum, a child may only show subtle neurocognitive deficits in one or more neuropsychological domains, which include overall intellectual functioning, executive function, memory and learning, language skills, visual-spatial ability, motor function, and attention. It is these children, who show less severe phenotypes, who are at greater risk of being misdiagnosed or overlooked altogether (Astley & Clarren, 2000).

In addition, children with FASD may exhibit behavioural problems, including adaptive dysfunction, academic difficulties, and increased rates of psychiatric disorders (Mattson et al., 2011). Children who display these subtle phenotypes are the most common among the entire spectrum, and may comprise up to 1% of all children born in the U.S. (Sampson et al., 1997). These large groups of individuals often remain undiagnosed and untreated, leading to further negative outcomes secondary to the neurocognitive deficits. These ‘secondary disabilities’ include substance addiction, trouble with the law leading to potential incarceration, high
unemployment rates, high rates of school dropout, child apprehension, and premature death (Streissguth, 1996).

In 1996, the U.S. Congress mandated a study of FAS, which resulted in the first set of diagnostic criteria, developed by the Institute of Medicine of the National Academy of Sciences (Hoyme et al., 2005). The results were published by the IOM study group, which encompassed diagnostic categories for FAS and alcohol-related effects (Table 1.1; Stratton et al., 1996).

Table 1.1. IOM (1996) FASD Diagnosis Guidelines (Institute of Medicine, 1996).

<table>
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<th>Component</th>
<th>Criteria</th>
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<tr>
<td>Growth</td>
<td>At least one of: 1) low birth weight; 2) low weight for height; 3) decelerating weight</td>
</tr>
<tr>
<td>Face</td>
<td>Distinguishing pattern including: 1) short PFL; 2) flat upper lip; 3) indistinct philtrum; 4) flat midface</td>
</tr>
<tr>
<td>CNS</td>
<td>At least one of: 1) microcephaly; 2) neurological deficit; 3) abnormal structure (partial/complete agenesis of corpus callosum, cerebellar hypoplasia)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Confirmed-excessive or unknown</td>
</tr>
</tbody>
</table>

PFL: Palpebral fissure length

Shortly after the IOM report on Diagnostic Criteria for FAS and alcohol-related effects was released, Astley and Clarren established the 4-Digit Diagnostic Code in 1999 (Astley and Clarren, 1999). This new diagnostic tool was tested and compared to the standard subjective method for FASD diagnosis. The 4-Digit Diagnostic Code presented more accurate diagnoses compared to the standard method due to the integration of quantitative, objective measurement scales and concrete definitions (Astley and Clarren, 1999). Generally, the 4 digits in the diagnostic code represent the 4 major symptomatic features of FASD in the following order: 1) growth deficiency, 2) FAS facial phenotype, 3) CNS damage/dysfunction, and 4) gestational alcohol exposure (Astley and Clarren, 1999). The degree of expression for each digit is given a code ranging from 1 (complete absence of the FASD feature) to 4 (strong presence of the FASD feature) (Astley and Clarren, 1999; Table 1.2).
### Table 1.2 4-Digit Diagnostic Code (Astley and Clarren, 1999).

<table>
<thead>
<tr>
<th>Rank</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td>Growth Deficiency (Height-weight)</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>≥10th centile</td>
<td>&lt;10th centile</td>
<td>&lt;10th centile</td>
<td>&lt;3rd centile</td>
</tr>
<tr>
<td>FAS Facial Phenotype (Palpebral fissures/Philtrum/Upper lip)</td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>Zero features present</td>
<td>Normally 1 of 3 features present</td>
<td>Normally 2 of 3 features present</td>
<td>All features present</td>
</tr>
<tr>
<td>CNS Damage</td>
<td>Absent</td>
<td>Possible</td>
<td>Probable</td>
<td>Definite</td>
</tr>
<tr>
<td></td>
<td>No problems likely to reflect brain damage are present</td>
<td>Suggestive brain damage (neurobehavioural disorder)</td>
<td>Three or more areas affected (encephalopathy)</td>
<td>Microcephaly (OFC ≤ -2 SD). Evidence of persistent neurological deficits (static encephalopathy)</td>
</tr>
<tr>
<td>Prenatal Alcohol Exposure (PAE)</td>
<td>No risk</td>
<td>Unknown</td>
<td>Some risk</td>
<td>High risk</td>
</tr>
<tr>
<td></td>
<td>Confirmed absence</td>
<td>In utero exposure to alcohol not reliable</td>
<td>Confirmed exposure. Exact amounts remain unknown</td>
<td>Confirmed exposure. High peak BAC levels</td>
</tr>
</tbody>
</table>

OFC = occipitofrontal circumference; BAC = blood alcohol levels; SD = standard deviation

In 2005, Chudley et al. released the Canadian guidelines for FASD diagnosis, which integrated the diagnostic categories and terminology proposed by the IOM committee in 1996, and the 4-digit diagnostic code presented by Astley and Clarren in 1999. The Canadian guideline recommends that individuals meeting certain criteria should be referred for a potential FASD-related diagnosis (Table 1.3).

### Table 1.3 Canadian Guideline Recommendations for FASD Diagnosis Referrals (Chudley et al., 2005)

1. Presence of 3 characteristic facial features (short palpebral fissures, indistinct philtrum, thin upper lip)
2. Confirmed heavy PAE (levels associated with physical/developmental effects)
3. Presence of ≥1 facial abnormality + growth deficit + confirmed PAE
4. Presence of ≥1 facial abnormality + ≥1 CNS deficits + confirmed PAE
5. Presence of ≥1 facial abnormality + pre/post-natal growth deficit (at ≤10th centile) + ≥1 CNS deficits + confirmed PAE

10th centile = 1.5 SD below the mean
1.4.3 FASD Treatment and Intervention

Considering the low percentage of individuals displaying the physical craniofacial abnormalities found in alcohol-exposed neonates, it is imperative to obtain accurate maternal self-reports of alcohol use during pregnancy when diagnosing individuals for FASD (Chudley et al., 2005). Once the neonate is exposed to ethanol in utero, its effects on the developing fetus are presumably irreversible. It is essential to identify the degree of in utero ethanol exposure early on in the diagnostic process. According to Streissguth et al., the earlier a child is identified to have been exposed to high amounts of ethanol, the earlier one can intervene and refer the child for close neurodevelopmental follow-up to assess for diagnostic signs of FASD. While this will not reverse the neurodevelopmental deficits or craniofacial abnormalities already present, it will help to provide appropriate medical and social interventions and lessen the chances of suffering from the ‘secondary disabilities’ attributed to FASD (Streissguth et al., 1996; Streissguth et al., 2004).

Previous animals studies have shown similar results that can be translated to human development. Long-Evans rats exposed prenatally to alcohol which were then reared in isolation presented with a dysmetric stride length suggestive of ataxic gait. However, when reared in enriched environments (reared in groups of 8 in large cages, provided with ‘toys’, and handled daily), the rats exposed prenatally to alcohol did not show any signs of ataxia. These results may suggest that the postnatal environment can influence the expression of alcohol related birth defects (ARBD) in rats (Hannigan et al., 1993). Furthermore, rodent studies demonstrate that when allowed access to a running wheel, voluntary exercise can mitigate deficits in spatial memory and long-term potentiation, induced by alcohol exposure in utero (Christie et al., 2005). It may be reasonable to speculate that early intervention and treatment in infancy could assist in refining neurocognitive development in humans.
Although there is no specific treatment protocol for individuals with FASD, there have been many pilot studies designed to measure the effectiveness of specific interventions that have been shown to ameliorate the neurocognitive impairments related to prenatal alcohol exposure (Paley & O’Connor, 2009). Implementation of specific teaching strategies has been speculated to aid in learning in individuals with FASD (Watson and Westby, 2003; Green, 2007; Laugeson et al., 2007). Examples of these teaching strategies include: 1) implementation of daily scheduled routines or tasks, 2) teaching of self-directed speech and problem solving, and 3) use of visual cues and aids to supplement verbal directions (Watson and Westby, 2003). In addition to appropriate teaching strategies aimed at children with FASD, modifying aspects of the classroom environment may also facilitate improved learning in individuals with FASD. These include: 1) minimizing visual and auditory distractions, 2) designating activity centers, and 3) using daily schedules that are visually presentable (Kalberg and Buckley, 2007). Several researchers have also explored potential interventions outside the realm of the classroom, particularly computer game interventions (Green CR. et al., CanFASD). Preliminary findings suggest that continuous, repetitive exercises stimulated by computer game interventions can improve attention and memory, which may translate into improvements in general intelligence, mathematical ability, reading comprehension and overall reaction times (Green CR. et al., CanFASD).

1.4.4 Prenatal Alcohol Exposure & Hormonal Effects on the Fetus

Jones and Smith (1973) were the first to introduce the term *fetal alcohol syndrome* (FAS), when they noticed a consistent trend of craniofacial abnormalities linked to prenatal alcohol exposure. In the past 40 years, there have been numerous studies that clearly associate prenatal alcohol exposure to a spectrum of anomalies present in children, denoted as FASD (Feldman et al., 2012). Exposure during the first month of pregnancy has been connected to
various birth outcomes, including low birth weight (<2,500g), low birth length, and small head circumference (Day et al., 1989). There is no known ‘safe’ level of drinking during pregnancy, and there is a lack of reliable information regarding patterns of dosing and timing of alcohol exposure (Feldman et al., 2012). Research data imply that larger amounts of alcohol consumption (i.e., binge drinking: ≥4 drinks/occasion) will put the fetus at higher risk for developing the known phenotypes within the FASD continuum (Maier SE. & West JR., 2001). Conversely, smaller amounts of alcohol consumption during pregnancy have shown to induce subtler phenotypes along the FASD continuum (Hamilton et al., 2014; Day et al., 2013).

Alcohol consumption during pregnancy can have direct effects on the fetus, as ethanol can readily cross the placental tissue into fetal circulation (Gabriel et al., 1998). Once ethanol reaches the fetal compartment, the fetus will enzymatically metabolize the ethanol; however, the fetal metabolic pathway used for ethanol is very different compared to maternal metabolic pathway. This subject will be discussed in more depth in the next chapter.

Prenatal alcohol exposure can impair the functioning of many different endocrine hormones in the developing fetus such as those of the hypothalamic-pituitary-adrenal axis (HPA axis), growth hormone, and insulin-like growth factors (Gabriel et al., 1998). Alcohol consumption has been shown to activate the HPA axis, which induces glucocorticoid release. This stimulus is similar to events of stress, where the HPA axis is triggered, producing cascades of hormones that result in glucocorticoid release. Studies show that prolonged stressful events can have pathological consequences due to direct metabolic effects and redistribution of metabolic resources that leads to growth retardation, underdevelopment in children, and immune suppression (Gabriel et al., 1998). The HPA axis undergoes regulation through negative feedback loops: stress induces release of hypothalamic corticotropin-releasing hormone (CRH)
which triggers release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, ultimately leading to cortisol synthesis and release from the adrenal cortex. Cortisol, in turn, feeds back to the hypothalamus, decreasing CRH release.

In the context of pregnancy, alcohol acts as a stress signal, activating the maternal HPA axis, resulting in cortisol release. However, in addition to its effects on maternal physiology, cortisol crosses placental tissue and into fetal circulation, suppressing the HPA axis. Simultaneously, ethanol crossing into fetal circulation stimulates the HPA axis. These conflicting signals may permanently alter fetal HPA activity, potentially leading to behavioural changes in infancy and childhood (Gabriel et al., 1998). Animal studies of this pathway suggest that prenatal alcohol exposure may delay maturation of the HPA axis and/or pathways that stimulate hypothalamic activity in response to stress (Weinberg, 1993).

Several studies also indicate a role for the growth hormone (GH)/insulin-like growth factor (IGF) axis in the phenotypic presentation of FASD. GH, also called somatotropin, is a peptide hormone that stimulates cell growth and division. GH is secreted by the anterior pituitary gland in response to hypothalamic growth hormone-releasing hormone (GHRH) stimulation. It has direct mitogenic effects on target tissues including chondrocytes and skeletal muscle, and indirect effects on others through secondary growth factors. In particular, GH stimulates expression of insulin-like growth factor-1 (IGF-1), primarily from the liver; IGF-1 is a peptide hormone that exerts growth stimulatory effects on almost all tissues in the body, and is thought to play a critical role in the regulation of fetal development (Gabriel et al., 1998; Figure 1).
Animal models demonstrate that alcohol consumption in rats reduces circulating GH levels (Thadani and Schanberg, 1979), and that alcohol intake in pregnant rats reduces gene expression and circulating levels of IGF-1 and -2 (Singh et al., 1996; Mauceri et al., 1996). In addition, alcohol has been shown to reduce levels of IGF-binding proteins, and may play a role in the regulation and function of maternal IGFs (Gabriel et al., 1998).

Extrapolating these findings to human development, it is speculated that ethanol-induced reduction of IGF expression may contribute to the neurobehavioural phenotype(s) associated with FASD.
1.4.5 Pharmacokinetics of Ethanol

Aliphatic alcohols form a homologous series, beginning with methanol (CH$_3$OH, $M_w$ = 32 g/mol). Ethanol, or ethyl alcohol, contains a two-carbon chain (C$_2$H$_5$OH, $M_w$ = 46 g/mol) that is completely miscible with water and has a very low lipid/water partition coefficient (Kalant and Khanna, 1998). As the carbon chain length of this series increases, the water solubility decreases, leaving the larger alcohols considerably more toxic (Kalant and Khanna, 1998). Most of the shorter-chain alcohols are used as solvents; however, ethanol is relatively nontoxic (indeed, it is produced by some microbial fermentative processes in the GI tract) and therefore can be safely consumed as a beverage in small quantities (Kalant and Khanna, 1998).

The pharmacokinetics of ethanol in humans is very complex and is dependent on various factors such as: quantity of alcohol consumed, age, gender, hepatic blood flow, total body water percentage, gastric emptying rates and expression and activity of metabolic isoenzymes (Swift, 2003).

In order to generally determine the quantity of alcohol consumed by an individual, the ‘standard drink’ unit of alcoholic beverages is commonly used. In the U.S., one standard drink is equivalent to 12 grams (g) or 0.5 ounces (oz) of absolute ethanol. This is equal to 12 oz of beer (4% ethanol content), 6 oz of wine (10% ethanol content), or 1.25 oz of hard liquor (40% ethanol content). In Europe, one standard drink is defined as 10 g of absolute ethanol, while in Canada, one standard drink is contains 13.6 g of absolute ethanol, equivalent to 12 oz of beer (5% ethanol content), 5 oz of wine (12-15% ethanol content) or 1.5oz of hard liquor (40% ethanol content; Swift, 2003).
Absorption/Distribution

Ethanol is absorbed by mucosal surfaces in the gastrointestinal tract via simple diffusion (Kalant and Khanna, 1998). This will occur in the stomach, but is much more efficient in the intestinal lumen (80%; Swift, 2003). Very dilute or heavily concentrated alcohol can slow absorption and cause gastric irritation. A delay in gastric emptying due to the presence of food, and strenuous exercise can also slow absorption of ethanol (Swift, 2003; Kalant and Khanna, 1998). Lastly, the distribution of ethanol is primarily determined by the blood flow rate and diffusion gradient between the blood vessels and adjacent tissues (Kalant and Khanna, 1998). Since ethanol has a small molecular weight (46 g/mol), it will rapidly distribute throughout the body and reach equilibrium relatively quickly (Kalant and Khanna, 1998).

Metabolism/Elimination

Following the absorption of ethanol by the stomach and intestinal lumen, ethanol enters the liver via the mesenteric and portal veins (Swift, 2003). First pass metabolism through the liver eliminates a portion of the absorbed ethanol. It has been estimated that 85% of ethanol that enters the body will be oxidatively metabolized (Swift, 2003). Ethanol metabolism follows Michaelis-Menton kinetics, where exceptionally large ethanol concentrations in the liver can result in saturation of oxidative metabolism. This, in turn, results in maximal and constant ethanol biotransformation amounts, mimicking zero order elimination (Swift, 2003). At this maximal rate of elimination, the typical 70 kg human can metabolize and eliminate 7-10 g of ethanol per hour (Swift, 2003; Kalant and Khanna, 1998). Following Michaelis-Menton kinetics, this fixed amount will be eliminated per time until the total blood alcohol concentration (BAC) drops below 20 mg/dl, after which the rate of elimination will revert back to being concentration dependent (first-order kinetics; Swift, 2003).
The vast majority of ethanol enters circulation and is metabolized by a two-step oxidative enzymatic process that requires nicotinamide adenine dinucleotide (NAD$^+$) to serve as a co-factor (Swift, 2003). The first step involves the enzyme alcohol dehydrogenase (ADH), which converts ethanol into acetaldehyde, while reducing NAD$^+$ to NADH in the process (Swift, 2003). The second step is mediated by aldehyde dehydrogenase (ALDH), which converts acetaldehyde, into acetate (acetic acid), while reducing another molecule of NAD$^+$ to NADH in the process (Swift, 2003). Acetate is then utilized in cellular respiration via the Kreb’s cycle, and converted into carbon dioxide and water (Swift, 2003; Figure 2). According to the literature, there are multiple isoforms of ALDH enzymes that may explain the disparities in ethanol tolerance and toxicities in ethnically distinct populations (Osier et al., 2002).

![Figure 2. Major Oxidative Pathway for Ethanol Metabolism – ADH/ALDH Reaction](image_url)

In addition to the previous metabolic pathway, there are two alternative minor oxidative pathways used for metabolizing ethanol into acetaldehyde: the cytochrome P450 2E1 (CYP2E1) and catalase (Swift, 2003). The CYP2E1 pathway is more readily observed in the heavy chronic drinking population due to the inducible nature of the enzyme (Swift, 2003). Catalase serves a relatively insignificant role in total ethanol metabolism; catalases stimulate the oxidative reaction of ethanol and hydrogen peroxides (oxygen radicals), into acetaldehyde (Swift, 2003; Figure 3).
Even though oxidative metabolism comprises the majority of ethanol metabolism and elimination, there are several non-oxidative pathways active in the human body. Esterase enzymes located in the adipose tissues of the brain, heart and pancreas can utilize ethanol and fatty acids as reactants to produce fatty acid ethyl esters (FAEEs). This is directed by the reactivity of the hydroxyl moiety on ethanol, reacting with organic acids to form chemically stable FAEEs (Swift, 2003). FAEEs are known to persist in the human body for hours to days after ethanol consumption and elimination (Swift, 2003). Ethanol can also react with glucuronic acid, sulfate or phospholipids to form ethyl glucuronide, ethyl sulfates and phosphotidylethanol, respectively (Swift, 2003).

1.4.6 Biomarkers of Prenatal Alcohol Exposure

There are various techniques used widely to measure acute exposures to alcohol. The most common procedures used today include measurements of blood alcohol concentration (BACs), breathe alcohol concentration (BrACs), and urine alcohol concentration (UAC; Helander et al., 1996; Helander, 2001). Unfortunately, these tests have a narrow time window of detection and are only effective when measuring acute exposures to alcohol. Due to the relatively short half-life of ethanol, these cannot be accurately when testing for chronic exposures over a broader timeline. To this date, fatty acid ethyl esters (FAEEs) measured in the hair and meconium have been used as reliable markers of ethanol consumption during pregnancy (Bearer
et al., 1999; Klein et al., 1999; Chan et al., 2003; Garcia-Algar et al., 2008). Aside from measuring gestational ethanol consumption via FAEEs in the meconium, ethyl glucuronide (EtG) and ethyl sulfate (EtS) have been used as an alternative (Morini et al., 2008). These two compounds have been previously found in other biological matrices in attempt to measure acute (blood and urine) and chronic (hair) ethanol consumption in adults (Wurst et al., 2008; Politi et al., 2007; Politi et al., 2008; Morini et al., 2009; Pichini et al., 2009).

Following consumption of ethanol, the majority of the dose is metabolized in the liver via a two-stage oxidation pathway (first to acetaldehyde by ADH; second to acetic acid by ALDH; Helander & Beck, 2005). However, a small amount of ethanol will undergo a phase II conjugation reaction with glucuronic acid catalyzed via the endoplasmic reticulum UDP-glucuronosyltransferase enzyme to produce ethyl glucuronide, which can be detected in the various matrices mentioned above (Dahl et al., 2002). The formation of ethyl sulfate (EtS) was determined by incubating liver tissue from a rat and found that ethanol may also undergo sulfate conjugation via sulfotransferase (SULT) enzyme to produce EtS (Vestermark & Bostrom, 1959; Bostrom & Vestermark, 1960; Helander & Beck, 2004).

A study comparing EtG, EtS, and FAEEs in meconium found that total FAEEs concentrations did no correlate quantitatively with EtG or EtS (Morini et al., 2010). Conversely, ethyl stearate showed a respectable correlation with both metabolites, where applying a cut-off of 1.5 nmol/g for EtG resulted in all positive samples showing EtS levels above 0.012 nmol/g (Morini et al., 2010). Overall, it appears that EtG and EtS in meconium remain promising biomarkers for intrauterine exposure to ethanol, however a baseline population study would be necessary to take the next step.
1.4.6.1 Fatty Acid Ethyl Esters (FAEEs)

Fatty acid ethyl esters (FAEEs) are created via the enzymatic reaction of ethanol with free fatty acids in the blood and tissues. FAEEs are a product of enzyme-mediated esterification of fatty acids or fatty acyl-CoA via fatty acid ethyl ester synthase (FAEES) or acyl-CoA:ethanol O-acyltransferase (AEAT), respectively (Figure 4) (Diczfalusy et al., 2001). A study investigating the activity levels of FAEES in tissues found the highest activity in the liver, duodenal mucosa, and pancreas (Diczfalusy et al., 2001). Autopsy studies verify that the liver, heart and brain are the organs with the highest FAEE concentrations following ethanol intake, and also express the highest concentrations of FAEE synthase enzymes (Laposata et al., 1986). Variability in the carbon chain-length and saturation points of the fatty acids ethyl esters result in many FAEE species (Table 1.4). The four most prevalent FAEE species in alcohol-exposed neonates are: ethyl palmitate, ethyl oleate, ethyl linoleate, and ethyl stearate.

**Figure 4. Production of FAEE & AEAT Pathways**
Adapted from Best & Laposata, 2003
Table 1.4. FAEE Species used in Meconium-FAEE Analysis (Moore et al., 2003; Hutson et al., 2011).

<table>
<thead>
<tr>
<th>FAEE</th>
<th>Configuration</th>
<th>Molecular weight (Mw)</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl palmitate</td>
<td>E16:0</td>
<td>284.5</td>
<td>C_{18H_{36}O_2}</td>
</tr>
<tr>
<td>Ethyl oleate</td>
<td>E18:1</td>
<td>310.5</td>
<td>C_{20H_{38}O_2}</td>
</tr>
<tr>
<td>Ethyl linoleate</td>
<td>E18:2</td>
<td>308.5</td>
<td>C_{20H_{36}O_2}</td>
</tr>
<tr>
<td>Ethyl stearate</td>
<td>E18:0</td>
<td>312.5</td>
<td>C_{20H_{40}O_2}</td>
</tr>
</tbody>
</table>

In an animal model, the use of FAEEs as a biological marker identified neonates at risk for major organ dysfunction due to in utero ethanol exposure that did not result in explicit physical signs of ethanol toxicity (Zelner et al., 2013). Studies on FAEEs have shown that they work as facilitators of organ damage in humans (Werner et al., 1997) and act as teratogens among neonates (Bearer et al., 1992). FAEEs have also been studied to distinguish between chronic alcoholics versus episodic binge drinkers. In one study, the concentration of fatty acid ethyl oleate was much higher in chronic alcoholics than in episodic binge drinkers, where the concentration of ethyl oleate in chronic alcoholics and binge drinkers 24 hours after ingestion was significantly different (Soderberg et al., 2001).

1.4.6.2 Meconium as a Biological Matrix

Meconium has been previously used as a matrix for detecting in utero neonatal drug exposure (Moore et al., 1998). Meconium is the first several stools passed by the neonate, and can be distinguished from normal feces by its lack of odour and tar-like consistency (Gareri,
Klein and Koren, 2006). Its greenish-black colouring originates from the large quantity of bile that is excreted during the beginning of the second trimester (Grand et al., 1976). Meconium production occurs in the fetal GI tract from early as the 12th week of gestation (Moore et al., 1998) to as late as the 20th week of gestation. Fetal swallowing is thought to be the primary mechanism by which compounds are integrated and concentrated into the meconium (Gareri, Klein and Koren, 2006). Xenobiotics and metabolites are deposited into the meconium via the biliary route or by swallowing amniotic fluid containing these substances (Ostrea et al., 1989).

Meconium is usually excreted shortly following birth (up to as long as 48 hours post-delivery) (Griffin and Beattie, 2001). The neonate’s excretions progressively transition from the dark, tar-like texture of meconium to postnatally produced stool (Zelner et al., 2012). This transition is triggered by intra-intestinal contamination with ingested dietary components arising from the metabolism of milk by the neonate (Zelner et al., 2012). The composition of the amniotic fluid during the early phases of pregnancy is relatively similar to maternal serum. Following the initiation of fetal urination, the composition of the amniotic fluid changes and will contain anything filtered from the fetal plasma into the urine (Hutson J.R., 2007). Therefore, any substance that is present in the maternal plasma and has the ability to cross the placental tissue can enter into fetal circulation, and deposit into the meconium (Hutson, 2007).

Meconium is usually passed within the first 24-48 hours following birth in 90-99% of healthy term neonates (Rudolph, 2003). For preterm and unhealthy neonates, the time of the first meconium excretion may be delayed up to 100 hours (Kumar and Dhanireddy, 1995). Collection of meconium samples excreted further into the postpartum period (>48 hours) may lead to an increased probability of endogenously produced FAEEs entering the meconium (Zelner et al., 2012), and not be reflective of maternal ethanol consumption during pregnancy. This is due to contamination of postnatally produced stool with dietary constituents and the presence of
ethanol-producing microorganisms that colonize the gut within the first 48 hours of life (Zelner et al., 2012). Therefore, it is essential to calculate the time elapsed from birth to first meconium excretion due to the chemical instability of FAEEs in the meconium. Meconium serving as a biological matrix for detection of prenatal alcohol exposure has performed well due to its stability with drugs, non-invasive collection protocol, and broader time window of detection compared to other biological matrices, such as neonatal blood and urine (Ostrea et al., 1999).

1.4.7 Advancement of Meconium-FAEE as a Biomarker

Over the past several decades, elevated levels of FAEEs in the meconium have been quantified and subsequently utilized as a biomarker for heavy prenatal alcohol exposure, spanning a window of detection from within the 2nd to the 3rd trimester of pregnancy. The study leading to the development of the meconium-FAEE assay was published by by Bearer et al. (1992), who reported on the accumulation of FAEEs in placental tissue following maternal consumption of ethanol. Subsequent studies showed that specific FAEEs were present in the meconium, demonstrating alcohol consumption levels consistent with maternal self-reports (Bearer et al., 1999; Klein et al., 1999). A population baseline study was conducted, using meconium-FAEE analysis, on infants born to non-drinking women to assess the basal levels of FAEEs in the absence of gestational alcohol exposure (Chan et al., 2003). Unsurprisingly, but importantly, this group found low levels of FAEEs among this cohort of women. Furthermore, baseline meconium-FAEE levels were determined among a small cohort of women (n = 6) who had confirmed maternal alcohol consumption during pregnancy across two drinking groups (chronic drinkers, ≥ 2 drinks/day; binge drinkers, ≥ 5 drinks/occasion). Lastly, neonates born to women defined as social drinkers (1 drink/occasion or 1 drink/month) could not be distinguished
from the baseline levels of endogenous FAEEs (Chan et al., 2003). For clinical purposes, a positive cut-off of 2 nmol/g of FAEEs in meconium yielded the highest sensitivity and specificity, 100% and 98.4%, respectively (Chan et al., 2003).

There are many different FAEE species that are produced endogenously by the body and exogenously by alcohol consumption. Various studies show conflicting results regarding the use of individual FAEEs (ethyl linolate, ethyl oleate, and ethyl palmitate) as fetal ethanol exposure biomarkers (Bearer et al., 1999; Bearer et al., 2003). Other studies suggest that the cumulative quantification of different FAEEs in meconium serves as an improved biomarker due to the lack of redundancy and variability between the different FAEE species (Klein et al., 1999; Chan et al., 2003). FAEEs have been shown to not cross the placental tissue from maternal circulation to fetal circulation and vice versa. Therefore, FAEEs found in the meconium are a direct measure of prenatal alcohol exposure via the fetal FAEE synthase enzyme activity (Chan et al., 2004).

Animal studies conducted to test the effectiveness of detecting FAEEs in meconium discovered a negative correlation between FAEE levels in the meconium and neonatal brain weights in term fetal guinea pigs that were subjected to chronic prenatal alcohol exposure at maternal doses comparable to human alcoholics (Brien et al., 2006).

1.4.8 Alcohol Consumption during Pregnancy in Africa

There is a substantial imbalance with regard to the amount of research on FASD between the developed and developing world. It is important to note that FASD is not a unique disorder that only affects populations in the developed world. However, due to limited diagnostic
infrastructure and healthcare systems, FASD is rarely reported in developing countries such as Uganda.

Research examining alcohol consumption among pregnant sub-Saharan African women is very limited. According to a systematic review of alcohol exposure among pregnant women residing in sub-Saharan Africa, there is a high prevalence of general alcohol use among this population (Culley et al., 2013). In addition to this finding, Uganda has steadily ranked in the top percentile by the World Health Organization (WHO) of the highest per-capita alcohol consumption in the world (World Health Organization, 2004). The prevalence of alcohol consumption during pregnancy in Uganda is still unknown; however, the overall prevalence of alcohol consumption is increasing over time (World Health Organization 2004). In the 2004 WHO Global Status report, it was estimated that the average adult per capita consumption of pure ethanol in Uganda was 19.7 L/year, compared to Canada’s consumption rate of 8.6 L/year, which positions Uganda as one of the highest in the world (World Health Organization 2004).

One study investigated the consumption of alcoholic beverages among pregnant urban Ugandan women and found that 30% of women drank alcohol at least monthly before recognition of pregnancy. Of this 30% of women who drank, 33% reported average consumption of at least one alcoholic beverage type at equal quantities of binging levels in women. The 30% of women who reported regular drinking (at least monthly drinking) prior to recognition of pregnancy were asked to comment on their drinking behaviour after realizing they were pregnant. Investigators found that, within this group, 18.3% of women abstained from drinking, 68.3% of women substantially decreased their alcohol intake, 4.4% of women reported no change in drinking behaviours, and an astonishing 11.1% of women increased their alcohol intake (Namagembe et al., 2010). When questioned further, the 11.1% of women who increased
alcohol intake after recognition of pregnancy, provided various rationales staggered across a spectrum of motives such as: drinking beer will increase the size of the baby; drinking a locally-produced spirit will decrease the size of the baby, which would provide them with an easier vaginal delivery; and drinking alcohol would relieve their heartburn symptoms (Namagembe et al., 2010).

A systematic review of alcohol exposure among pregnant women in Sub-Saharan Africa investigated twelve cross-sectional studies with varying study populations, including South Africa, Nigeria, Ghana, and Uganda (Culley et al., 2013). Among the studies from South Africa were five studies from the province of Western Cape, which reported prevalence rates of prenatal alcohol exposure (post-recognition of pregnancy) variously ranging from 20.2% to 87.4%, and four studies from provinces other than Western Cape (Mpumalanga, KwaZulu-Natal, Limpopo, and Gauteng) with prevalence rates ranging from 2.2% to 6.5% (Croxford & Vilijoen, 1999; Vythilingum et al., 2012; Tomlinson et al., 2013; O’Connor et al., 2011; Eaton et al., 2012; Matseke et al., 2012; Desmond et al., 2012; Mostert et al., 2005; Ramchandani et al., 2010). Lastly, the remaining studies (conducted outside South Africa, in Uganda, Nigeria, and Ghana) reported prevalence rates ranging from 2.5% to 24.8% (Namagembe et al., 2010; Abasiubong et al., 2012; Adusi-Poku et al., 2012). It is evident that the burden of alcohol use during pregnancy in African countries is an important issue; there still exists a large discrepancy between the amounts of research conducted in South Africa compared to other parts of Sub-Saharan Africa (Culley et al., 2013).

Of the limited studies published examining alcohol consumption among Ugandan adults, none were exclusively focused on pregnant women (Namagembe et al., 2010). Given the well-understood fetal effects of alcohol during pregnancy, there is a distinct lack of empirical research
examining the prevalence and/or prevalence of prenatal alcohol exposure among this high-risk Ugandan population.

1.4.9 Legislation on Alcohol Use in Uganda

Current law regarding alcohol use in Uganda, called the “Enguli Act” of 1964, is very different compared to alcohol use laws in North America. The Enguli Act covers various components, such as the liquor act, manufacture and licensing act, potable spirits act, shop hours act, and premises for sale of alcohol (UYDEL, 2008). In Uganda, these laws are hardly enforced and are obsolete, and require an immediate review with respect to manufacturing and the sale of alcohol, licensing regulations, regulation of hours of sale, and provisions relating to under age citizens (UYDEL, 2008).

Uganda’s economic growth relies heavily on their agricultural production, where there lies an abundant resource and tradition of local brewing and distilling using millet, maize, sugar, bananas and various crops grown in Uganda. Quality control has been an interesting topic of discussion with regards to the Ugandan traditional alcohol industry. Aside from enguli, the main form of alcohol consumed in Uganda, typical Ugandans create fermented home-made drinks, distilled in their villages and homes, which together make up about 80% of the total alcohol consumed in Uganda (World Health Organization, 2004). The most universally consumed traditional brews in Uganda are tonto (6-11% v/v alcohol) made from bananas, ajon (6-8% v/v alcohol) made from finger millet, and omuramba made from sorghum (Mwesigye and Okurut, 1995). Various other traditional brews include kweete made from maize and millet, and waragi (up to 40% v/v alcohol) made from molasses or as a distillate from local brews (Mwesigye and
Okurut, 1995). Due to the countless brewing techniques, which originate from older generations of families passed down to the new generations, the home brews are a result of mixed-culture fermentation, where each family will have different fermentation processes and additional ingredients. These variables add another dimension to the issue on quality control of alcohol production in Uganda (Mwesigye and Okurut, 1995). Among the five most common traditional homebrews produced in Uganda, the beverage most widely known to be dangerous due its distillation processes and common adulteration, is waragi. A number of hazardous substances such as battery acid, formaldehyde and methanol are sometimes used as ingredients in waragi, which in the past has led to tragedy (World Health Organization, 2004).

The Uganda National Bureau of Standards (UNBS) was introduced in 1983 to serve as a government statutory body to regulate the quality and standard of all goods produced in Uganda, including alcohol. The UNBS is responsible for ensuring the products coming into the market for consumption pass certain safety tests and that they meet minimum safety requirements. In terms of the quality of alcohol on market, the UNBS faces a number of challenges in controlling these standards. First off, the UNBS has only four divisions in Uganda, thus limiting the surveillance capacity in more remote areas (UYDEL, 2008). This results in a number of manufacturers that don’t meet UNBS standards and compliance measures, which in turn leads to illicit products findings their way into the market (UYDEL, 2008). A large issue that the UNBS faces is that the alcohol suppliers and producers do not put warning labels of the harmful effects of alcohol (particularly during pregnancy) on the containers of their products, and in fact actually promote consumption (UYDEL, 2008). The lack of warning labels on alcoholic beverages can have a detrimental effect on the pregnant population, as there are no precautionary signs present to deter the mother from drinking during pregnancy. The current laws in Uganda do not support the UNBS to regulate quality control and standards of alcoholic goods produced. In order to
overcome this barrier, the UNBS require appropriate assistance in training, equipment, and minimum testing requirements (UYDEL, 2008). The market is constantly changing, and the UNBS needs to be regularly reviewed to bring them up to speed with these changes.

Legislation and regulatory measures on alcohol use exist in the majority of African countries. However there is some leniency in terms of enforcement of these regulatory measures in approximately 70% of African countries (UYDEL, 2008). Regulation of the availability of alcohol via restricted hours of sale, and reducing the demand for alcohol through taxation and pricing strategies have been the two most cost-effective strategies to reduce and prevent harm of excessive alcohol consumption in Uganda (UYDEL, 2008).

Chapter 2
METHODS AND MATERIALS

2.1 Samples Collection

2.1.1 Study Design

This is a population-based, cross-sectional study that includes anonymous study participants and subsequent meconium specimen collection and maternal questionnaires. The meconium underwent FAEE analysis from all neonates born between September 23 and November 23, 2013, at the Mbarara Regional Referral Hospital (MRRH) maternity ward in Mbarara, Uganda.
2.1.2 Study Site and Population

Mbarara Regional Referral Hospital was the primary site used for recruitment of study participants. The MRRH maternity ward sees an estimated 10,000 deliveries per year, 20% of which are preformed via caesarian sections. The demographic of our study population consisted of 50/50 rural and urban mothers. Dr. Godfrey Muyenyi and Dr. Matthew Wiens were in charge of supervising data collection protocols conducted by the research midwives.

2.1.3 Subjects & Inclusion/Exclusion Criteria

All women who delivered at MRRH during the time period between September 23, 2013 and November 23, 2013, were eligible for inclusion in our study. An equal number of rural and urban mothers were enrolled in the study, therefore after reaching the quota for a certain demographic (rural or urban), only women of the remaining demographic were permitted to be included in the study. The inclusion criteria consisted of all consented neonates born during the two months. The exclusion criteria included complicated deliveries, neonatal and/or maternal fatalities, and meconium passed outside study hours.

2.1.4 Consenting Process

Two separate time points were used in order to collect informed consent from the study participants. Consent was collected at the first time point when the mothers were present in early phases of labor. If the mother was already in a later stage of labor, we did not approach her to
obtain consent. The second time point followed delivery after an initial period of rest, which usually lasted around a few hours post-delivery or when the mother was deemed to be in the right mindset to discuss the details of the study. Study participants were informed that they would remain anonymous.

2.1.5 Maternal Questionnaire

After obtaining informed consent from the study participants, the research midwife was responsible for initiating and administering an eight-page maternal questionnaire. The questionnaire gathered information on demographics, pregnancy histories, substance use histories (including alcohol, tobacco and illicit drug use), TWEAK screening test (an alcohol-use screening instrument for pregnant women), previous medical history, HIV status, and prenatal care (Appendix A). The questionnaire also included a detailed report on the meconium collection process (date/time of birth, date/time of collection, number of meconium excretions collected). All data was entered onto a web-based form via REDCap (Research Electronic Data Capture).

2.1.6 Meconium Collection

Meconium specimens were anonymously collected from participating neonates born at MRRH, Mbarara, Uganda. Each meconium sample was given a study ID number that was linked to the corresponding maternal questionnaire. The research midwife harvested the meconium sample from the soiled diaper and transferred the specimen to a plastic container to be stored in a -20°C freezer. It was critical for the midwife to document the date(s) and time(s) of collection and to specify if it was the first, second, or third meconium excretion. All meconium samples
were to be collected within the first 24-48 hours of life. Following completion of the study period, all meconium specimens collected were couriered together on dry ice to the Motherisk Laboratory at The Hospital for Sick Children in Toronto, Canada. Upon arrival, the meconium samples were immediately transferred into the –80°C freezer for storage, until FAEE analyses were executed.

2.1.7 Sample Size Calculations

Due to the lack of extensive research conducted on the prevalence of heavy prenatal alcohol use in pregnancy in southwestern Uganda, we were not able to estimate a clear-cut sample size for this population. The study population represents a convenience sample set, where I was blinded to the recruitment phase of the study. The main objective of this study was to assess the prevalence of heavy PAE in this population therefore, sample size calculations was not required.

2.1.8 Ethical Considerations/Approvals

Ethics approval was obtained from the four main affiliations: University of British Columbia (May 7, 2014; File #H13-00453), Mbarara University of Science and Technology (March 13, 2013), The Republic of Uganda Office of the President (June 28, 2013; ADM 154/212/01), and The Hospital for Sick Children/University of Toronto (June 8, 2015; REB application #1000049917/REB protocol #1000035947) (Appendix B). The current study did not subject the study participants to any risk, due to the fact that it was strictly a cross-sectional observational study and did not consist of any active intervention or follow-up procedures. This
study was solely a screening study and all collected data and analysis remained anonymous. A general consent form was created (Appendix C) to inform the study subject that general information regarding maternal exposure to alcohol, tobacco and other drugs was to be collected for the study. All laboratory and statistical analyses were kept de-identified and the results were not reported back to the participant. There was no monetary compensation issued during the recruitment phase of the study.

2.2 Analytical Procedures/Analysis

2.2.1 Materials and Supplies

Heptane, ethyl palmitate, ethyl oleate, ethyl linoleate, and ethyl stearate were obtained from Sigma-Aldrich (St. Louis, MO). Acetone was obtained from British Drug Houses Chemicals (BDH Chemicals; VWR International; Mississauga, ON). The deuterated FAEEs ($d_5$-FAEE) used for internal standards were obtained from Toronto Research Chemicals (TRC; Toronto, ON).

The gas-chromatograph-mass spectrometer (GCMS-QP2010 Plus) was acquired from Shimadzu with an auto-sampler (KOC-5000). The SPME software was obtained from Varian Inc. (Mississauga, ON). The SPME 65µM Polydimethylsiloxane-Divinylbenzene fiber, and GC injection port septum were obtained from Supelco (St. Louis, MO). The GC column (VF-Xms capillary column) was obtained from Agilent Technologies (Santa Clara, CA). The Pasteur pipettes used to transfer organic layer into SPME vials were purchased from VWE International (Mississauga, ON) and disposable centrifuge tubes (15 mL) were obtained from Fisher Scientific (Ottawa, ON).
2.2.2 Method Validation

This method was validated according to the criteria set by the CLSI and MDTL 4010/01 Validation Report written by the Motherisk Laboratory (CLSI document C43-A2: Gas Chromatography/Mass Spectrometry Confirmation of Drugs- Approved Guideline; Westgard et al., 2006; Hutson et al., 2011). The following parameters were test: specificity, recovery, carryover, accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ) using patient samples.

The specificity of the method was assessed by spiking blank meconium with 400ng of each FAEE species in triplicates. The peak area from the resulting chromatogram was compared with samples that underwent identical extraction but without meconium. The coefficient of variation (CV%) of the peak area ratios of FAEE/IS-FAEE ranged from 3.2% - 11.1%, which was within the acceptance criteria of 20% (Hutson et al., 2011).

Recovery was calculated by dividing peak area of FAEE or IS-FAEE from spiked samples (400ng) of each FAEE or IS-FAEE when extracted with or without meconium. Recovery of extracted FAEE and IS-FAEE was 3.2% - 19.5%. This set was done in triplicates (Hutson et al., 2011).

Carryover was conducted by spiking meconium with high FAEE concentrations and then running a blank meconium sample immediately following. Chromatograms were assessed to determine if FAEEs found after the spike were above LOD in the blank samples. Meconium with FAEE concentrations up to 10,000 ng/g did not result in any detectable FAEE in the subsequent sample (Hutson et al., 2011).
Calculating the concentration and measuring the overall percent recovery of FAEEs at two differing concentrations, 100 and 400ng demonstrated accuracy. Six replicates at each concentration were spiked with the internal standard from a different lot than the calibrators. The calculated amount of FAEEs was divided by the expected amounts to determine the overall percent recovery (Hutson et al., 2011). The %CV for the 100 and 400ng samples were 3.547 and 4.401 nmol/g, respectively. The recovery percentage for the 100 and 400ng samples were 100.4 and 101.1, respectively. Both parameters fit within the acceptance criteria.

Precision was conducted by comparing the ratio between FAEE and IS-FAEE area counts of 3 spiked concentrations (75, 200, and 400ng) of FAEEs. This is prepared in quadruplicates. This would demonstrate that the method could provide similar results for the same measure under the same conditions in a shorter time frame (Hutson et al., 2011).

The linearity of the method was demonstrated by measuring the ratio between FAEE and IS-FAEE area counts as a function of FAEE concentrations at six levels. This was conducted in triplicates of 0, 50, 75, 200, 400, and 1000ng of spiked FAEEs. Calibration curves were generated for each individual FAEE species and regression analysis was preformed. The linearity was determined by regression line coefficients, slopes, and intercepts (Hutson et al., 2011). The linearity of all four individual FAEE species had an R² value within the acceptance criteria (EP 0.987; EL 0.998; EO 0.999; ES 0.998).

The LOD and LOQ were determined by spiking nine levels of low FAEE concentrations (5, 10, 15, 20, 25, 30, 35, 40, and 50ng) in triplicates. The FAEE/IS-FAEE area ratios were plotted as a function of FAEE concentrations. Regression analysis was conducted to find LOD and LOQ. The average of the 3 sets were calculated to result in the LOD and LOQs of each individual FAEE (Hutson et al., 2011). For analytical purposes, values below the LOQ were
defined as ‘trace’ levels and values above the ULOQ were defined as >ULOQ for the specific individual FAEE.

For statistical purposes, a value of 0 was given to the concentrations that fell below the LOD for each individual FAEE (Table 2.1). The reason for this is because the LOD is the lowest amount of analyte that can be detected but not necessarily quantified. For concentrations that fell between the LOQ and LOD of each individual FAEE, the value attributed for this concentration was the LOD of their corresponding FAEE. For concentrations above the ULOQ, they were given a value of their corresponding FAEE, because of the level of uncertainty and saturation of the detectors on the machinery; therefore, we cannot be 100% accurate on the exact concentrations of these samples at the extreme ends of detection and quantification.

Table 2.1. LOD/LOQ/ULOQ of each individual FAEE

<table>
<thead>
<tr>
<th>FAEE Species</th>
<th>LOD (nmol/g)</th>
<th>LOQ (nmol/g)</th>
<th>ULOQ (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate</td>
<td>0.041</td>
<td>0.124</td>
<td>10.546</td>
</tr>
<tr>
<td>Linoleate</td>
<td>0.039</td>
<td>0.116</td>
<td>9.724</td>
</tr>
<tr>
<td>Oleate</td>
<td>0.025</td>
<td>0.074</td>
<td>9.662</td>
</tr>
<tr>
<td>Stearate</td>
<td>0.020</td>
<td>0.060</td>
<td>9.599</td>
</tr>
</tbody>
</table>

Any concentration testing below each individual LOD was given a value of 0. Any concentration testing below each individual LOQ was given a value equal to the LOD. Any concentration testing above the upper LOQ was given a value equal to the ULOQ.

2.2.3 Sample Preparation & Headspace Solid Phase Micro Extraction (HS-SPME)

Meconium sample preparation begins with a thawing out period of 20-30 minutes or until the meconium reaches room temperature, followed by homogenization of the sample and weighing out of ~0.50 g of meconium into a 15 mL centrifuge tube (Fisher Scientific Disposable Centrifuge Tube). The mass of each sample was recorded on the meconium specimen manifest. Next, two calibration standards were prepared by adding 40 µL of $d_5$-FAEE internal standard (10
µg/ml) to a vial containing blank meconium. These vials serve as a calibrator to ensure reliability of the instrument and that the readings are consistent with the clinical meconium specimens being quantified. A duplicate of the 400 ng point sample on the calibration curve was used as the calibration standard, where 40 µL of FAEE stock solution and 40 µL of d5-FAEE are added to each vial containing blank meconium. Following these calibration standard steps, 40 µL of d5-FAEE IS solution was added to each vial containing the meconium specimen. Subsequently, the internal standards and 5 mL of the extraction solvent (heptane:acetone 5:2 v/v) were added to each meconium vial. Each sample was vortexed for 1 minute (2 sets) and then centrifuged at 3,500 rpm for 15 minutes at 4°C. After vortexing and centrifuging, the upper organic layer (heptane) was removed by glass pipetting. The heptane organic layer was transferred into a 10 mL HS-SPME vial, and dried under a constant flow of nitrogen gas (THERMOLYNE Dri-Bath Model) at 35°C. This was continued until the liquid phase had completely evaporated, leaving a residue on the walls of the 10 mL HS-SPME vial. Each sample was reconstituted in 1ml of 0.1M phosphate buffer (PBS; pH 7.6), capped using a steel screw cap with a polytetrafluoroethylene (PTFE)/silicon septum, and placed in the GC auto-sampler (KOC-5000) rack.

The HS-SPME process proceeded with sample preheating, sample agitation, headspace adsorption, and desorption in the GC injection port, programmed via a KOC-5000 auto-sampler (Shimadzu, Columbia, MD). A 65-µm polydimethylsiloxanedivinylbenzene fiber (Supelco, Bellefonte, PA) was used for the HS-SPME of FAEEs, which was previously determined to provide optimal levels of function (Pragst et al., 2001).
2.2.4 Chromatographic Analysis of FAEE

The analysis was carried out using a gas chromatograph-mass spectrometer, GC-MS QP-2010 PLUS equipped with GCMSSolutions software (Shimadzu). The FAEEs were extracted using a FactorFour Capillary Column (30 m × 0.25 mm × 0.25 µm; Varian, Palo Alto, CA), and helium carrier gas (He; 1.0 mL/min). The injection mode was set at split-less and the temperature at 260°C. The oven temperature in the GC was programmed at 70°C for 2 minutes, following by a ramping rate of 20°C/minute until it reached the maximum temperature of 300°C, which was held for 1 minute.

2.2.5 FAEE Quantification

The quantification of the individual samples was accomplished by comparing the peak ratios of each individual FAEE species to the internal standards from the calibration curve. The ratio of peak area of the molecular ions (m/z) was compared to their respective deuterated standards. In a previously published study from our lab, the m/z values of EP, EL, EO, and ES were determined to be 284, 308, 310, and 312, respectively (Hutson et al., 2011). The m/z for the deuterated internal standards, d5-ethyl palmitate, d5-ethyl linoleate, d5-ethyl oleate, and d5-ethyl stearate were determined to be 289, 313, 315, and 317, respectively (Hutson et al., 2011). Each individual FAEE species has a specific retention time that is used to identify it on the chromatogram. The retention times for EP, EL, EO, and ES were found to be 11.223, 12.050, 12.053, and 12.142, respectively (Hutson et al., 2011). The corresponding retention times for d5-ethyl palmitate, d5-ethyl linoleate, d5-ethyl oleate, and d5-ethyl stearate were 11.208, 12.027, 12.036, and 12.120, respectively (Hutson et al., 2011). The area counts of each corresponding FAEE were established using the GCMSSolutions software (Shimadzu). The ratio of the peak
area of the molecular ions (m/z) for each of the four FAEEs to their respective deuterated standard was used to quantify each of the four FAEEs. Each individual FAEE measurement was added up to achieve a cumulative [FAEE] for each specimen. A sample with a cumulative [FAEE] of $>2.00 \text{ nmol/g}$ was deemed a positive test result (Chan et al., 2003).

2.2.6 Statistical Analysis

All statistical analyses were performed using IBM© SPSS© Statistics Version 20. The entire data set was subjected to a normality test (Kolmogorov-Smirnov test). Once verified, Mann-Whitney Rank Sum tests were conducted to compare the data. If data were normally distributed, an independent t-test was conducted. To compare the differences between the educational statuses of each mother to the cumulative FAEE concentration mean of the groups, a one-way ANOVA with a post-hoc Tukey’s test was conducted. A Pearson chi-square analysis was conducted to compare the fetal ethanol exposure rates determined by the maternal self-report and the laboratory-based meconium-FAEE analysis.

Chapter 3

Results

2.3 Population Participation

Over the span of a two-month period (September 23, 2013 – November 23, 2013), a total of 612 mothers delivered at Mbarara Regional Referral Hospital In Mbarara, Uganda. A total of 510 meconium samples were successfully collected giving an 83.3% study participation rate. The remaining 102 (16.7%) mothers were excluded from the study due to various reasons such as: discharge prior to passing of meconium, neonatal death, neonate too sick for meconium
collection, mother too sick to consent (Table 3.1). Among the 510 samples, 7 (1.4%) samples were deemed not analyzable due to insufficient quantities of meconium. A total of 503 samples were successfully analyzed, representing 82.6% of the original study population.

Table 3.1. Distribution of Study Exclusion Criteria Among Study Participants (n=100*)

<table>
<thead>
<tr>
<th>Reasons for Exclusion</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharged/ left before meconium passed</td>
<td>77</td>
<td>77%</td>
</tr>
<tr>
<td>Neonatal fatality</td>
<td>10</td>
<td>10%</td>
</tr>
<tr>
<td>Neonatal illness</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td>Maternal illness</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>7%</td>
</tr>
</tbody>
</table>

*Total n = 100; missing 2 subjects (not reported)
Other = Already seen, discharged during night, mother left before seen, passed meconium outside study hours

2.4 Maternal Characteristics

2.4.1 Demographics

The demographic information collected from the mothers in this study included age, marital status, maternal place of residence (while pregnant) and the highest level of education completed by the mother (Table 3.2). With regard to the distribution of maternal age, 31.9% of the mothers were 21 years of age or younger, 64.7% of mothers were between 22 and 36 years of age, whereas 3.4% were between the ages of 37 and 45 (Figure 5) The modal age of mothers was 20 years, which comprised 11.1% (n=56) of the cohort.
Table 3.2. Maternal Demographic Information (n=505)

<table>
<thead>
<tr>
<th>Demographic Questionnaire Result (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (mean)</strong> 24.94 years</td>
<td></td>
</tr>
<tr>
<td><strong>Total (n)</strong> 504</td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>467</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>6</td>
</tr>
<tr>
<td>Single</td>
<td>28</td>
</tr>
<tr>
<td>Divorced</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total (n)</strong> 503</td>
<td></td>
</tr>
<tr>
<td><strong>Urban</strong></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>236</td>
</tr>
<tr>
<td><strong>Rural</strong></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>269</td>
</tr>
<tr>
<td><strong>Total (n)</strong> 505</td>
<td></td>
</tr>
<tr>
<td>Educational Status</td>
<td></td>
</tr>
<tr>
<td>Completed Primary School</td>
<td>277</td>
</tr>
<tr>
<td>Completed Secondary School</td>
<td>154</td>
</tr>
<tr>
<td>Completed University/Technical School</td>
<td>74</td>
</tr>
</tbody>
</table>

*Urban = Mbarara Town, Other major town (such as Banda, Bushenyi, Lyantonde, etc.)*
*Rural = in village more than 5km outside a major town, in trade center more than 5km outside a major town*
*Educational Status: Primary school = less than P4, P4, P7*
*Secondary school = S4, S6*

Figure 5. Age of Mothers (n=504)
Pregnancy history, including the number of previous pregnancies, number of miscarriages/abortions (<20 weeks) and number of pre-term (20-36 weeks) and term (36-40 weeks) births were gathered in the maternal questionnaire (Table 3.3-3.4; Figure 6-7). The majority of women enrolled in the study had 1 previous pregnancy, 0 miscarriages/abortions, 0 pre-term births, and 1 term birth. There were 35 women who reported having a range of 7-11 previous pregnancies. There was one mother who reported having 8 miscarriages (Table 3.3).

![Figure 6. Frequency of Previous Pregnancies Among Study Participants (n=504)](image_url)

**Table 3.3. Frequency of Miscarriages/Abortions Among Study Participants (n=504)**

<table>
<thead>
<tr>
<th>Number of Miscarriages/Abortions</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>460</td>
<td>91.2</td>
</tr>
<tr>
<td>1</td>
<td>32</td>
<td>6.35</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>1.19</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.79</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Miscarriages/Abortions = <20 weeks (<5 months) at birth
Table 3.4. Frequency of Pre-Term Births Among Study Participants (n=504)

<table>
<thead>
<tr>
<th>Number of Pre-Term Births</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>478</td>
<td>94.8</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>4.56</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.40</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Pre-Term Birth = 20-36 (5-8 months) at birth

Figure 7. Frequency of Term Births Among Study Participants (n=503)

Full-Term Birth = 36-40 weeks (9 months) at birth

2.4.2 Maternal Self-Report of Alcohol Use Before Pregnancy

According to the maternal self-report on alcohol use before and after learning of pregnancy, 81.5% (n = 409) of mothers abstained from alcohol a year before becoming pregnant, whereas 18.5% (n = 93) of mothers consumed alcohol during the year before becoming pregnant. The maternal questionnaire asked each mother to identify the type(s) of alcoholic beverages consumed, the frequency of use on a daily, weekly, and monthly basis, and how many estimated standard drinks consumed per drinking episode. The typical alcoholic beverages consumed by the study cohort consisted of beer, wine, spirits, local spirits and local brew. When asked to
recall the alcoholic beverage of their choice prior to becoming pregnant, 66.67% consumed beer, 5.38% consumed wine, 2.15% consumed spirits, 17.20% consumed local spirit (waragi), 8.60% consumed local brew (tonto, etc). When asked to recall the frequency of alcohol use on a daily, weekly, and monthly basis, 2.17% of women reported consuming alcohol daily or almost daily in the year before becoming pregnant (Table 3.5). Fifty-four percent of mothers drank 1-2 drinks per episode, while 28.0% of mothers drank 3-4 drinks per episode. Two women (2.15%) reported drinking an amount per occasion at binge levels (≥5 drinks).

Table 3.5. Total Maternal Self-Report of Alcohol Use Before Pregnancy (n=92)

<table>
<thead>
<tr>
<th>Drinking Pattern</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once per month</td>
<td>31</td>
<td>33.7</td>
</tr>
<tr>
<td>Once or twice per month</td>
<td>36</td>
<td>39.1</td>
</tr>
<tr>
<td>Once or twice per week</td>
<td>20</td>
<td>21.7</td>
</tr>
<tr>
<td>Several times per week</td>
<td>3</td>
<td>3.26</td>
</tr>
<tr>
<td>Daily or almost daily</td>
<td>2</td>
<td>2.17</td>
</tr>
</tbody>
</table>

Light drinking = less than once per month, once or twice per month
Moderate drinking = once or twice per week
Heavy drinking = daily or almost daily, several times per week

2.4.3 Maternal Self-Report of Alcohol Use During Pregnancy

The mothers were asked a series of questions synonymous to the maternal self-report on alcohol use the year before pregnancy. When questioned if they ever consumed alcohol while they knew (or suspected) they were pregnant, 83.9% (n = 421) reported “no”, where the remaining 16.1% (n = 81) reported “yes”. Fifty percent of those mothers who responded positively reported local spirits (waragi) as their drink of choice during pregnancy, 2.47% consumed wine, 11.1% consumed local brews and 35.8% consumed beer. Refer to Tables 3.6.1-3.6.4 to review the frequency of alcohol use during pregnancy per trimester (1st trimester pre/post-recognition, 2nd trimester, 3rd trimester) on a daily, weekly, and monthly basis. Women who responded positively to drinking during pregnancy, but did not report use in a specific pregnancy phase were listed as “not reported”. Most notably, 4.94 – 9.88 of women reported
using alcohol daily or almost daily (i.e., heavy drinking) during pregnancy (Tables 3.6.1-3.6.4). Lastly, when asked to recall the number of standard drinks consumed per drinking episode, 40.7% of mothers reported drinking 1-2 drinks per episode, where 17.3% of mothers consumed 3-4 drinks per episode. One woman (1.2%) reported drinking binge levels (≥5) per occasion, even when she suspected or knew that she was pregnant.

Table 3.6.1. Maternal Self-Report of Alcohol Use During Pregnancy – 1st Trimester Pre-recognition (n=81)

<table>
<thead>
<tr>
<th>Drinking Pattern</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once per month</td>
<td>2</td>
<td>2.47</td>
</tr>
<tr>
<td>Once or twice per month</td>
<td>5</td>
<td>6.17</td>
</tr>
<tr>
<td>Once or twice per week</td>
<td>5</td>
<td>6.17</td>
</tr>
<tr>
<td>Several times per week</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Daily or almost daily</td>
<td>4</td>
<td>4.94</td>
</tr>
<tr>
<td>Not reported</td>
<td>65</td>
<td>80.3</td>
</tr>
</tbody>
</table>

Light drinking = less than once per month, once or twice per month
Moderate drinking = once or twice per week
Heavy drinking = daily or almost daily, several times per week

Table 3.6.2. Maternal Self-Report of Alcohol Use During Pregnancy – 1st Trimester Post-recognition (n=81)

<table>
<thead>
<tr>
<th>Drinking Pattern</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once per month</td>
<td>6</td>
<td>7.41</td>
</tr>
<tr>
<td>Once or twice per month</td>
<td>11</td>
<td>13.6</td>
</tr>
<tr>
<td>Once or twice per week</td>
<td>11</td>
<td>13.6</td>
</tr>
<tr>
<td>Several times per week</td>
<td>2</td>
<td>2.47</td>
</tr>
<tr>
<td>Daily or almost daily</td>
<td>4</td>
<td>4.94</td>
</tr>
<tr>
<td>Not reported</td>
<td>47</td>
<td>58.1</td>
</tr>
</tbody>
</table>

Light drinking = less than once per month, once or twice per month
Moderate drinking = once or twice per week
Heavy drinking = daily or almost daily, several times per week
### Table 3.6.3. Maternal Self-Report of Alcohol Use During Pregnancy – 2\textsuperscript{nd} Trimester (n=81)

<table>
<thead>
<tr>
<th>Drinking Pattern</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once per month</td>
<td>4</td>
<td>4.94</td>
</tr>
<tr>
<td>Once or twice per month</td>
<td>21</td>
<td>25.9</td>
</tr>
<tr>
<td>Once or twice per week</td>
<td>11</td>
<td>13.6</td>
</tr>
<tr>
<td>Several times per week</td>
<td>2</td>
<td>2.47</td>
</tr>
<tr>
<td>Daily or almost daily</td>
<td>5</td>
<td>6.17</td>
</tr>
<tr>
<td>Not reported</td>
<td>38</td>
<td>46.9</td>
</tr>
</tbody>
</table>

Light drinking = less than once per month, once or twice per month  
Moderate drinking = once or twice per week  
Heavy drinking = daily or almost daily, several times per week

### Table 3.6.4. Maternal Self-Report of Alcohol Use During Pregnancy – 3\textsuperscript{rd} Trimester (n=81)

<table>
<thead>
<tr>
<th>Drinking Pattern</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once per month</td>
<td>15</td>
<td>18.5</td>
</tr>
<tr>
<td>Once or twice per month</td>
<td>18</td>
<td>22.2</td>
</tr>
<tr>
<td>Once or twice per week</td>
<td>13</td>
<td>16.1</td>
</tr>
<tr>
<td>Several times per week</td>
<td>2</td>
<td>2.47</td>
</tr>
<tr>
<td>Daily or almost daily</td>
<td>6</td>
<td>7.41</td>
</tr>
<tr>
<td>Not reported</td>
<td>27</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Light drinking = less than once per month, once or twice per month  
Moderate drinking = once or twice per week  
Heavy drinking = daily or almost daily, several times per week

When comparing total maternal self-report of alcohol consumption when mothers knew or suspected themselves to be pregnant, versus their consumption history the year before they became pregnant, the data yielded some unexpected results. Seventy-five percent of women (n=378) reported no alcohol consumption the year before they became pregnant and throughout pregnancy. Eight percent of women (n=41) reported alcohol consumption the year before they became pregnant, but abstained from alcohol consumption during pregnancy. Six percent of women (n=29) reported no alcohol consumption the year before they became pregnant, but increased alcohol consumption throughout the pregnancy. Lastly, 11% of women (n=52) reported consuming alcohol the year before they became pregnant, and continued to consume alcohol throughout their pregnancy.
Subdividing the maternal self-report on alcohol use into trimesters among the population of mothers recruited, 3.14% (n=16) of women reported drinking during the 1st trimester pre-recognition phase, 6.67% (n=34) of women reported drinking during the 1st trimester post-recognition phase, 8.43% (n=43) of women reported drinking during the 2nd trimester, and 10.6% (n=54) of women reported drinking during the 3rd trimester.

2.4.4 Maternal & Paternal Self Report of Alcohol Use

Sixty-one percent of women participating in the study had partners who reportedly abstained from alcohol consumption, while the remaining 38.7% of husbands/partners had confirmed alcohol consumption histories. Looking at the association between paternal versus maternal drinking habits during pregnancy, 55% of mothers and partners did not consume alcohol. Six percent of mothers drank during pregnancy when their partners did not drink alcohol. Twenty-nine percent of mothers abstained from alcohol while their corresponding partners drank alcohol throughout the pregnancy. Lastly, 10% of mothers and husbands/partners consumed alcohol together.

2.5 FAEE Analysis

2.5.1 Summary of cumulative FAEE concentrations

The HS-SPME and GC-MS analysis of the four FAEE species (ethyl palmitate, MW = 284.48g/mol; ethyl oleate, MW = 308.50g/mol; ethyl linolate, MW = 310.51g/mol; and ethyl stearate, MW = 312.53g/mol) from the entire study population of 503 meconium specimens produced a cumulative mean FAEE concentration of 0.246 ± 0.065 nmol/g ranging from 0.000 – 26.760 nmol/g (Table 3.7). A total of 493 meconium samples were confirmed negative for measurable amounts of FAEEs (ΣFAEEs < 2.000 nmol/g) with a mean concentration of 0.105 ±
0.012 nmol/g ranging from 0.000 – 1.962 nmol/g. A total of 10 meconium samples tested positive for FAEEs (ΣFAEEs > 2.00 nmol/g) indicating heavy prenatal alcohol exposure to the neonates, with a mean concentration of 7.212 ± 2.434 nmol/g, ranging from 2.089 – 26.760 nmol/g.

Table 3.7. Overview of cumulative FAEE concentrations in the sample population

<table>
<thead>
<tr>
<th>[FAEE] Group</th>
<th>n</th>
<th>nmol/g</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Range</td>
<td>Interquartile Range (25-75%)</td>
</tr>
<tr>
<td>Negative (&lt;2.000)</td>
<td>493</td>
<td>0.105 ± 0.012</td>
<td>0.000</td>
<td>0.000 – 1.962</td>
<td>0.000 – 0.042</td>
</tr>
<tr>
<td>All Samples</td>
<td>503</td>
<td>0.246 ± 0.065</td>
<td>0.000</td>
<td>0.000 – 26.760</td>
<td>0.000 – 0.043</td>
</tr>
</tbody>
</table>

2.5.2 Summary of Individual FAEE Concentrations

The mean and median concentrations, ranges and interquartile ranges for the concentrations of the four individual FAEEs in meconium were found to be quite variable (Table 3.8). Looking across all samples, ethyl palmitate exhibited the highest mean concentration (0.105 ± 0.024), where ethyl stearate exhibited the lowest mean concentration (0.00 ± 0.002) among all the groups (Table 3.8). Within the FAEE-negative group, ethyl palmitate exhibited the highest mean concentration (0.057 ± 0.007) (Table 3.9), while in the positive group, ethyl oleate displayed the highest mean concentration (3.328 ± 1.064) (Table 3.10). Mann-Whitney Rank Sum analyses were conducted to determine if there was a significant difference in the levels of individual FAEEs between the negative group and the positive group. The results from the Mann-Whitney Rank Sum analyses demonstrated a significant difference across all FAEE
species concentrations, where there was an increased concentration of each individual FAEE in the positive group (Figure 8).

Table 3.8. Expression of Individual FAEEs for All Samples (n=503)

<table>
<thead>
<tr>
<th>FAEE Species</th>
<th>nmol/g</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile Range (25%-75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Palmitate</td>
<td>0.105 ± 0.024</td>
<td>0.000</td>
<td>0.000 – 10.339</td>
<td>0.000 - 0.041</td>
<td></td>
</tr>
<tr>
<td>Ethyl Linoleate</td>
<td>0.035 ± 0.011</td>
<td>0.000</td>
<td>0.000 - 6.632</td>
<td>0.000 - 0.000</td>
<td></td>
</tr>
<tr>
<td>Ethyl Oleate</td>
<td>0.101 ± 0.029</td>
<td>0.000</td>
<td>0.000 – 9.700</td>
<td>0.000 - 0.000</td>
<td></td>
</tr>
<tr>
<td>Ethyl Stearate</td>
<td>0.005 ± 0.002</td>
<td>0.000</td>
<td>0.000 - 0.471</td>
<td>0.000 - 0.000</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.9. Expression of Individual FAEEs in the Negative Group (n=493)

<table>
<thead>
<tr>
<th>FAEE Species</th>
<th>nmol/g</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile Range (25%-75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Palmitate</td>
<td>0.057 ± 0.007</td>
<td>0.000</td>
<td>0.000 – 1.549</td>
<td>0.000 – 0.041</td>
<td></td>
</tr>
<tr>
<td>Ethyl Linoleate</td>
<td>0.010 ± 0.002</td>
<td>0.000</td>
<td>0.000 – 0.350</td>
<td>0.000 – 0.000</td>
<td></td>
</tr>
<tr>
<td>Ethyl Oleate</td>
<td>0.036 ± 0.006</td>
<td>0.000</td>
<td>0.000 – 1.155</td>
<td>0.000 – 0.000</td>
<td></td>
</tr>
<tr>
<td>Ethyl Stearate</td>
<td>0.002 ± 0.001</td>
<td>0.000</td>
<td>0.000 – 0.324</td>
<td>0.000 – 0.000</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.10. Expression of Individual FAEEs in the Positive (>2.00 nmol/g) Group (n=10)

<table>
<thead>
<tr>
<th>FAEE Species</th>
<th>nmol/g</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile Range (25%-75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Palmitate</td>
<td>2.482 ± 0.927</td>
<td>1.684</td>
<td>0.258 – 10.339</td>
<td>1.071 - 2.014</td>
<td></td>
</tr>
<tr>
<td>Ethyl Linoleate</td>
<td>1.240 ± 0.636</td>
<td>0.424</td>
<td>0.039 - 6.632</td>
<td>0.282 - 0.795</td>
<td></td>
</tr>
<tr>
<td>Ethyl Oleate</td>
<td>3.328 ± 1.064</td>
<td>1.969</td>
<td>0.624 – 9.700</td>
<td>1.395 – 2.847</td>
<td></td>
</tr>
<tr>
<td>Ethyl Stearate</td>
<td>0.163 ± 0.051</td>
<td>0.129</td>
<td>0.000 - 0.471</td>
<td>0.020 - 0.274</td>
<td></td>
</tr>
</tbody>
</table>
Within the FAEE-negative group, the most prevalent individual FAEE species found above the LOQ was ethyl palmitate (14.8%), followed by ethyl oleate (10.3%). Among the 10 positive samples, the two most prevalent FAEE species above the LOQ were ethyl palmitate and ethyl oleate, both found at a frequency of 100% in all positive cases (Figure 9).
Figure 9. Prevalence of Meconium-FAEE samples that had individual levels above their corresponding LOQs.

2.5.3 Maternal Demographics Compared to FAEE in Meconium

Mothers who came from urban and rural locations did not show a significant difference in cumulative FAEE concentrations (p=0.210; Table 3.1). In contrast, focusing on the mother’s educational status, there was an overall significant difference when correlating the mean FAEE concentrations to educational status, where an increased FAEE concentration was present among the infants of mothers who attended university or technical school (p=0.042; Table 3.1). However, the gravidity and the age of the mother did not correlate with FAEE-positive or FAEE-negative meconium status (p=0.831, p=0.070, respectively; Table 3.1).

Table 3.1. Educational Status vs. Cumulative [FAEE] Means in Meconium

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cumulative [FAEE] (nmol/g) (Mean ± SEM)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Educational Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary (n=275)</td>
<td>aPrimary vs. bSecondary</td>
<td></td>
</tr>
<tr>
<td>Secondary (n=152)</td>
<td>a0.26 ± 0.07</td>
<td>b0.08 ± 0.02</td>
</tr>
<tr>
<td>College (n=72)</td>
<td>aPrimary vs. cCollege</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a0.26 ± 0.07</td>
<td>c0.58 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>bSecondary vs. cCollege</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b0.08 ± 0.02</td>
<td>c0.58 ± 0.37</td>
</tr>
<tr>
<td>2Geographic location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban (n=235)</td>
<td>uUrban vs. rRural</td>
<td></td>
</tr>
<tr>
<td>Rural (n=264)</td>
<td>u0.22 ± 0.12</td>
<td>r0.27 ± 0.07</td>
</tr>
</tbody>
</table>

* = Significant difference, α ≤ 0.05
1One-way ANOVA w/ Tukey’s Post hoc test
2Mann Whitney Rank Sum Analysis conducted
Table 3.12. Maternal Demographics of Corresponding Infants Testing Positive & Negative for FAEEs in Meconium

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Negative FAEE (n=493)</th>
<th>Positive FAEE (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Age (years) (n=501) (x̄ ± SEM)</td>
<td>25.00 ± 5.38</td>
<td>21.90 ± 3.70</td>
<td>0.070</td>
</tr>
<tr>
<td>2Gravidity (n=500) (x̄ ± SEM)</td>
<td>2.70 ± 2.87</td>
<td>2.57 ± 1.81</td>
<td>0.831</td>
</tr>
</tbody>
</table>

1, 2Independent t-test was conducted

2.5.4 Maternal Alcohol Self Report Compared to Meconium Analysis

Out of the 503 meconium samples and questionnaires obtained, 81 (16.1%) of mothers reported drinking alcohol during pregnancy. After successfully matching each meconium-FAEE sample result to the corresponding maternal self-report, a total of 502 meconium samples and questionnaires were reconciled. Of the 502 samples, 67 (13.4%) contained a positive maternal self-report of alcohol use during the 2nd/3rd trimesters of pregnancy. One sample was not included in the statistical test due to a missing questionnaire. A Pearson chi-square analysis was conducted, comparing the prevalence of prenatal alcohol exposure using the maternal self-report (67/502, 13.4%) against the determined prevalence of prenatal alcohol exposure using the laboratory GC-MS method (10/502, 2.00%). There was no agreement between the maternal self-reports of alcohol use during pregnancy associated with the meconium-FAEE laboratory analysis (Table 3.13; p=0.118).

Table 3.13. Maternal Self-Report of Alcohol Use During 2nd/3rd Trimesters of Pregnancy vs. GC-MS Meconium-FAEE Analysis (n=502)

<table>
<thead>
<tr>
<th>Maternal-Self Report</th>
<th>GC-MS Meconium-FAEE Analysis</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>No</td>
<td>428</td>
<td>7</td>
</tr>
<tr>
<td>Yes (2nd/3rd Trimester)</td>
<td>64</td>
<td>3</td>
</tr>
</tbody>
</table>
Chapter 4
DISCUSSION

3.1 Study Population

The region of Mbarara is in the southwestern part of Uganda, located approximately 290 kilometers southwest from the capital city, Kampala. The regional population is estimated to be 418,300, consisting of six main divisions with a total area of 1,778 km² (Mbarara Town 2011; UBOS 2014). The rural/urban split of the population is about 81%/19%. (Mbarara Town 2011).

The current study works in collaboration with Mbarara Regional Referral Hospital (MRRH) in Uganda, which served as the home base for recruitment of study participants. This is the largest
hospital within the city limits that is directly affiliated with Mbarara University of Science & Technology (MUST). There is a vast underrepresentation in the amount of dependable research conducted on maternal drinking rates during pregnancy in countries such as Uganda, compared to the immense number of reports coming out of South Africa. In the 2010 Annual Report on Alcohol, The World Health Organization (WHO) ranked Uganda as the country with the highest annual alcohol consumption rate per capita in the world (23.7 L, in people >15 years of age). Determining the prevalence of PAE in this region is critical in evaluating the overall burden of the problem in the majority of sub-Saharan African (SSA) countries. This is the first population-wide study establishing the total prevalence of PAE in Uganda using an objective biomarker of alcohol exposure. Study subjects recruited at MRRH came from a low socioeconomic status (SES) and a majority of the subjects had a poor educational background (15% completed post-secondary education, Table 3.2). The mean maternal age for the entire study population was 24.9 years: 25 years for the negative FAEE group and 22.1 years for the mothers who had corresponding positive meconium FAEE tests (Table 3.1). There was no significant difference between the age groups or number of previous pregnancies between positive and negative groups. However, there was a significant difference between the mean FAEE concentrations between mothers living in geographically distinct areas (rural, urban), though there was no significantly higher cumulative FAEE concentration in mothers living in urbanized cities, compared to rural districts (Table 3.11). This finding could have been due to lack of statistical power for this specific test. It does not confirm that there was no significant difference in drinking levels between mothers living in rural and urban locations.

This study did find a significant difference in cumulative [FAEE] levels between groups of mothers who completed a higher level of education (university/technical school) compared to mothers who had only completed secondary school (Table 3.11). According to previous
literature, there have been many published peer-reviewed scientific articles finding the same association as we did in the current study, with higher alcohol use in mothers who achieved a higher level of education (Tsai et al., 2007; Floyd et al., 1999; Chambers et al., 2005; Yamamoto et al., 2008).

3.2 Heavy Prenatal Alcohol Exposure

3.2.1 Evaluation of the Prevalence of Heavy Prenatal Alcohol Exposure

Based on the 503 samples that were successfully analyzed by GC-MS, 11 meconium samples tested above the cumulative FAEE concentration LOQ cutoff of 2.00 nmol/g, resulting in the prevalence rate of fetal ethanol exposure in this neonatal population to be estimated at a range of 1.2 – 2.0%. By using the most conservative approach, the study prevalence rate is reported in a range from a minimum value (1.2%) to a maximum value (2.0%) to compensate for the probability of improper pre-analytical procedures (i.e., meconium sample collected outside 24 hour time window), which could increase the degree of false-positivity in the FAEE-meconium analysis.

One of the main objectives of this study was to determine the prevalence of heavy prenatal alcohol exposure in the study population using an objective biomarker (meconium-FAEE analysis) and to compare these rates to the maternal self-report on alcohol consumption during pregnancy. We hypothesized that use of an objective approach in determining the prevalence of heavy PAE would provide a more accurate prevalence rate than maternal self-report (Gareri et al., 2008; Stoler et al., 1998). Considering our results, we determined a much higher maternal self-report rate of 16.1%, (13.3% in the 2nd/3rd trimester), compared to a meconium-FAEE prevalence rate of 1.2 – 2.0%. There was no agreement between mothers who
reported alcohol consumption throughout pregnancy and the corresponding FAEE-positive meconium test results (Table 3.14). One explanation for this discordance may be that some women self-reported alcohol consumption during pregnancy after consuming very few drinks per month (answering ‘yes’ to drinking less than once per month in the maternal questionnaire). Consumption of one drink or more than one drink per month cannot be distinguished by a positive meconium sample (Chan et al., 2003). Consequently, this will lead to a positive maternal self-report and a negative meconium-FAEE test. Another explanation may be that some women who responded positively on self-report only consumed alcohol in the first trimester of pregnancy, which would not produce a FAEE-positive meconium result. This is because meconium does not form until the 12-20\textsuperscript{th} week of gestation, well into the 2\textsuperscript{nd} trimester of pregnancy (Moore et al., 1998). Lastly, the sensitivity with the analytical instrument may have been too low which would explain why there was such a small prevalence rate of heavy PAE.

Quantitatively, there is no amount of alcohol that is deemed safe for consumption during pregnancy. The exact amount of ethanol that must be consumed to yield a FAEE-positive meconium test has not been determined, as many contributing factors such as inter-individual differences (in physiology, genetics, epigenetics, etc.) may play a role. A study investigating elevated FAEEs in meconium found that the mothers in the study group were consuming \( \geq 1 \) standard drink per week (1.5 ounces of pure ethanol) in the 3\textsuperscript{rd} trimester of pregnancy (Bearer et al., 1999).

### 3.2.2 Post Analytical Adjustment of the Study Prevalence Rate

Part of the rationale in determining the study prevalence rate of heavy PAE was to take into consideration the effect that time may have on the integrity of the meconium samples. Collection of meconium samples excreted further into the postpartum period may lead to an
increased probability of false-FAEE-positive meconium test results, due to contamination with dietary constituents of postnatally-produced stool and the presence of ethanol-producing microorganisms that develop in the gut within the first 48 hours of life (Zelner et al., 2012). Therefore, it is critical to collect the earliest meconium excretion (within the first 24 hours after birth is ideal) in order to maximize the integrity of the meconium samples and ensure that the FAEE content is representative of in utero ethanol exposure, and not due to endogenous FAEE production via ethanol-producing microorganisms. In the current study, 6 of 10 FAEE-positive meconium samples were collected within the 24-hour time period, while the remaining 4 FAEE-positive meconium samples were collected outside the 24 hour time period (Table 4.1). The minimum range, 1.2%, corresponds to the 6 of 503 meconium samples that tested positive; the maximum range, 2.0%, corresponds to the 10 of 503 meconium samples that include the 6 samples, which were collected inside the 24-hour time window.

Table 4.1. Temporal Distribution of Meconium Collection Positive Group (n=503).

<table>
<thead>
<tr>
<th>Time</th>
<th># of [FAEE] samples ≥2.00 nmol/g</th>
<th>Adjusted # of [FAEE] samples ≥2.00 nmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤24hrs after birth</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>25-48hrs after birth</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;48hrs after birth</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

In order to report the study prevalence rate as a single value, the adjusted prevalence rate was calculated based on the findings of Zelner et al. (2012). It was found that the degree of false positivity increases 2-fold when the meconium excretion occurred between 24-48 hours in the postpartum period (Zelner et al., 2012). In the current study, 1 of the 10 FAEE-positive meconium samples were collected within this time period. Since the probability of false-positivity doubles, the total number of positive samples collected within this time period (n=1) was halved, resulting in an adjusted total of 1 positive sample that was collected between 24-48
hours (rounding 0.5 to 1, as the value of this type of data must be a positive integer). Furthermore, Zelner’s study determined that the degree of false positivity increases 3-fold when the meconium excretion occurs >48 hours in the postpartum period (Zelner et al., 2012). In the current study, 3 of the 11 FAEE-positive meconium samples were collected within this time period, Subsequently, the probability of false-positivity increased by 3, therefore the total number of positive samples collected within this time period (n=3) was divided by 3, resulting in an adjusted total of 1 positive sample that was collected >48 hours in the postpartum period. Lastly, there was 1 FAEE-positive meconium sample that did not contain a documented excretion time; therefore we decided to exclude this FAEE-positive meconium sample from our overall adjusted prevalence rate. The final adjusted prevalence rate of heavy PAE is 1.6% (8/503 meconium samples).

3.2.3 Comparison of Heavy Prenatal Alcohol Exposure Around the World

Previous studies show an opposite trend when comparing the prevalence or prevalence rates of specific populations using the two different screening tools (maternal self-report, FAEE-meconium). Gareri and colleagues (2008) conducted a study examining the prevalence of PAE in the region of Grey Bruce, Ontario, Canada, by meconium-FAEE analysis and concluded the prevalence rate to be 2.5% compared to a maternal self-report prevalence rate of 0.5%, a 5-fold difference (Gareri et al., 2008). Similarly, Bryanton et al. conducted a similar study and found the prevalence rate using meconium-FAEE analysis to be 3-fold higher than by maternal self-report (Bryanton et al., 2014). Lastly, Goh et al., conducted a follow-up study in a high-risk obstetrics unit where they suspected a higher-than-reported prevalence of drug exposed
pregnancies, and found a 15-fold higher prevalence rate of PAE by meconium-FAEE analysis compared to maternal self-report (Goh et al., 2010).

The anonymous sample collection technique used in the current study allowed the investigators to identify affected children with a higher screening power, since the study participants remained anonymous. This made it easier for the mothers to give consent to participate in the study, which seemed to avoid the potential implications of confronting this social stigma.

This is the first ever study assessing the overall prevalence of heavy PAE via meconium-FAEE analysis in this clinical population. Five other population-based meconium-FAEE studies have been conducted to determine the prevalence or prevalence rates of heavy PAE in different geographic regions (Figure 11). The first was a study conducted by Moore et al., 2003, in two completely different geographic and cultural locations in the USA, namely Hawaii and Utah. In Hawaii, 436 meconium samples were collected anonymously from neonates born in a regional perinatal center in Hawaii, where 16.7% (n=73) meconium samples tested above the 10,000 ng/g cut-off for cumulative FAEEs. The 1.6% positive rate established in the Mbarara population is 10.4 times lower than the Hawaiian population and was found to be significantly different (0.14; 95% CI, 0.11 to 0.18; P<0.001). In Utah, 289 meconium samples were collected anonymously from neonates born in six NICUs within the state, where 12.1% (n=35) of meconium samples tested above the FAEE cutoff. The 1.6% positive rate from the Mbarara population is 7.5 times lower than the Utah population and was found to be significantly different (0.10; 95% CI, 0.07 to 0.15; P<0.001).

The second, a study conducted by Goh et al., 2010, examined meconium samples from a high-risk tertiary care facility in London Ontario, Canada. Fifty meconium samples were
obtained from the infants of consenting mothers; the analysis found a 30% (n=15) prevalence rate of neonates exposed to heavy PAE. The 1.6% positive rate in the Mbarara population is 18.7 times lower than the London population and was found to be significantly different (0.28; 95% CI, 0.17 to 0.42; P<0.001). However, it is worth pointing out that the high-risk tertiary care facility population is biased compared to the current Mbarara study population.

The third study, conducted by Hudson et al., 2010, analyzed 824 meconium samples from two hospitals in Montevideo, Uruguay where they found a total prevalence rate of 44% (n=362). The 1.6% positive rate in the Mbarara population is 27.5 times lower compared to the Montevideo population where it was found to be significantly different (0.42; 95% CI, 0.39 to 0.46; P<0.001).

The fourth study, involving a low socioeconomic status cohort, conducted by Garcia-Algar et al., 2008, in Barcelona, Spain, analyzed 353 meconium samples for FAEEs and found a 45% (n=159) prevalence rate of heavy PAE in this neonatal population. The 1.6% positive rate in the Mbarara populations is 28.1 times lower compared to the Barcelona population where the difference in proportions was found to be significantly different (0.43; 95% CI, 0.38 to 0.49; P<0.001).

Lastly, the fifth study conducted by Pichini et al., 2012, collected 607 meconium samples in 7 Italian cities to assess the prevalence of heavy PAE by determining cumulative FAEEs in meconium. They found an overall prevalence of 7.9% (n=48) among all 7 cities. The 1.6% positivity rate in the Mbarara population was found to be 4.9 times lower than the Italy population where the difference in proportions was significantly different (0.06; 95% CI, 0.04 to 0.09; P<0.001).
Comparing the prevalence and prevalence rates of previous studies to the current study involving a regional population in Uganda, there is a significantly lower rate of positivity in Mbarara, Uganda. Alcohol consumption rates were significantly lower in this Ugandan population, which contradicts the original hypothesis, where we would expect the overall prevalence of heavy prenatal alcohol exposure to be higher given the reportedly elevated alcohol consumption rates among the adult population in Uganda (World Health Organization, 2004; World Health Organization, 2014). The lower rate of alcohol consumption during pregnancy, determined by the meconium-FAEE analysis, in Uganda compared to these other parts of the world, may be the result of cultural, genetic, and public health differences, and warrants further investigation. Furthermore, FAEEs have been shown to accumulate unevenly in the meconium, reflecting the timing of exposure (Bearer et al., 2003). Therefore, in the current study, FAEEs in meconium may not have formed at the time that the reported drinking behavior occurred.
Although this has not been thoroughly investigated among this population, genetic polymorphisms in the alcohol dehydrogenase and acetaldehyde dehydrogenase enzymes of the mothers may play a role in the amount of alcohol and its metabolites to which fetuses were exposed. One can speculate that the capacity to metabolize ethanol more readily may decrease the amount of ethanol to which a fetus is exposed; conversely, maternal slow metabolizers may lead to increased PAE.

When comparing the current study’s prevalence rate of 1.6% to previous studies conducted around the world using identical analytical techniques, the rate found in the current study is surprisingly much lower compared to other countries where the epidemic of excessive alcohol use is less apparent. This contradicts the original hypothesis that was originally supported from the data collected in the WHO Annual Report in 2004. It was reported that Uganda ranked one of the countries with the highest adult per capita consumption of alcohol in the world (World Health Organization, 2004). One explanation for this discrepancy could be to the overall abstinence rate established in Uganda. In 2010, the proportion of alcohol abstainers in the previous 12 months was 58.7%, where females comprised the majority of this population (73.7%; World Health Organization, 2014). Furthermore, 54.9% of females were lifetime abstainers, compared to only 27.9% of males. In comparison, the proportion of alcohol abstainers in the previous 12 months in Canada and the United Kingdom were 22.9% and 16.1%, respectively. Among these populations, 26.1% of women from Canada and 19.0% of women from the UK abstained from alcohol in the prior 12 months (World Health Organization, 2014); within the male populations, 19.7% were abstainers in Canada, and 13.0% in the UK. In summary, there is an overall higher rate of alcohol abstinence in Uganda, compared to other countries. Furthermore, sensitivity studies conducted on the meconium-FAEE assay show that it detects more moderate/high drinking (7 drinks/week and/or 3 drinks/episode) compared to
lighter drinking (~1 drink/month) (Bearer et al., 2003; Chan et al., 2003). According to the WHO Annual Report on Alcohol 2014, the prevalence of heavy episodic drinking (consuming ≥60 g of pure ethanol per occasion in the past 30 days) for females was 0.1% compared to 6.8% in the male population (World Health Organization, 2014). Therefore, males show to have higher heavy episodic drinking rates compared to females in this population. Although, the Uganda population overall shows a high rate of overall alcohol consumption, this is heavily inflated by the male population, revealing a likely lower prevalence of heavy maternal drinking during pregnancy. Moreover, a large number of abstainers in Uganda in combination with the high per capita consumption of alcohol indicate that alcohol consumers in Uganda are drinking very large amounts. Ultimately, this is the first of many population-based studies that must be conducted in this potentially high-risk population in order to assess the underlying reasons behind the reportedly high rate of alcohol consumption in Uganda.

3.3 Meconium as an Effective Screening Technique

Overall, diagnosis and identification of FASD with the use of maternal self-reporting is an unreliable practice due to the social stigma that comes with maternal verification of alcohol and drug use during pregnancy (Littner and Bearer, 2007). In these cases, FASD diagnosis may either be delayed or overlooked by clinicians. Social stigmatization of alcohol use during pregnancy can affect both mothers and health care practitioners, where it can lead to high rates of underreporting of maternal alcohol consumption, often due to fears of child apprehension by Child Protection Services; on the other end, it can increase the unwillingness of health care providers to question expectant mothers about their drinking habits during pregnancy (Sarkar et al., 2009). Utilizing an objective biomarker to quantify the amount of pure ethanol that fetuses
are exposed to *in utero* increases the chances of identifying children who may have gone undetected when relying on less-reliable, subjective techniques, such as maternal questionnaires.

Comparing the use of meconium as an analytical matrix to other matrices such as urine and blood, one must consider the feasibility of sample collection, stability, and analysis. One of the most crucial components of an assay is the pre-analytical component (*i.e.*, collection and storage of the sample). Meconium is normally discarded as waste; therefore, its collection entails an easier, non-invasive process, compared to accumulating serial blood or urine samples. Additionally, the time window of detection of ethanol ingestion in urine and blood are narrow (EtG; <40-130 hours, and ≤14 hours, respectively) (Helander *et al.*, 2009; Helander *et al.*, 1999). Meconium has a much broader time window of detection (2nd to 3rd trimesters), which is a distinct advantage over the other matrices (Ostrea *et al.*, 1999). Given the current study participation rate of 83.3%, and the minimal number of refusals to participate, it is evident that meconium can be used quite readily as a prenatal screening matrix, with regard to high rates of subject participation. However, there are significant limitations: storage and stability of the sample must be taken into account when using meconium as an analytical matrix.

### 3.4 Individual FAEEs in Meconium

In the Ugandan population, ethyl palmitate was found to be the most prevalent FAEE species in all of the positive samples (Figure 9). The second most prevalent FAEE species found in both the positive and negative samples was ethyl oleate at 100% and 10.3%, respectively. According to Chan *et al.*, (2003), among the six heavy drinkers included in their study, each corresponding meconium sample contained ethyl palmitate as the most prevalent individual FAEE species. However, in a study by Hutson *et al.*, (2010), the most predominant FAEE
species found was ethyl oleate. The differences observed in the predominance of individual 
FAEEs among different populations may reflect ethnic and cultural disparities with respect to the 
FAEE synthetic processes. A variety of abundant FAEE species may be present in different 
populations due to their dietary preferences that may contain different fatty acids. Until further 
research is conducted on the prevalence of individual FAEE species among different 
populations, the cumulative sum of FAEEs against a specific cut-off are used in order to 
differentiate between a positive and negative meconium sample.

3.5 Risk for Developing Fetal Alcohol Spectrum Disorder

According to Abel (1995), the estimated mean percentage of infants frequently exposed 
to alcohol prenatally, who go on to develop FASD is 40%. Within our Ugandan study 
population, this would translate to 3 to 4 children at very high-risk in developing FASD. As 
indicated by its name, FASD is a spectrum of disorders that includes a wide array of physical and 
neurodevelopmental and/or cognitive delays in affected children. Included in this spectrum are 
“full-blown” FAS, which have been shown to affect 4.3% of infants exposed to heavy amounts 
of alcohol prenatally (Abel, 1995). Therefore, focusing on the positive population in the current 
study, an estimated 0.07% (1.6% × 4.3%) of the study population (≤1 infant) may ultimately 
develop physical features associated with FAS. Extreme caution is required in reading into this 
estimate as large deviations in the estimates of infants with FAS are seen across differing 
populations (Abel, 1995).

There are numerous demographic characteristics that play a major role in increasing the 
risk of infants developing FASD. Low SES, a characteristic that applies to the entire study 
population, has been shown to increase the risk of infants developing FASD (Abel and
Hannigan, 1995). In addition to infants born to low SES families, poor nutrition also plays a pivotal role in the susceptibility to developing FASD (Abel and Hannigan, 1995).

According to the Food and Agriculture Organization of the United Nations, vitamin A deficiencies are considered a severe public health issue among children in Uganda (FAO of the United Nations, 2010). Vitamin A supplementation remains a major issue among both children and women in Uganda, where only 36% and 33% of children and mothers, respectively, receive proper supplementation (FAO of the United Nations, 2010). Multiple studies demonstrate that ethanol alone does not explain the entire spectrum seen in individuals suffering with FASD (Ballard et al., 2012). Ethanol is known to inhibit vitamin A (retinoic acid) metabolism, where vitamin A deficiency can lead to embryonic malformations analogous to FAS (Duester, 1991; Keir, 1991). It has been speculated that vitamin A deficiency may play a role in putting a child at high risk of developing FASD, whereas supplementation with vitamin A may mitigate the effects of alcohol exposure and reduce the severity of FAS (Ballard et al., 2012).

In Uganda, the prevalence of chronic malnutrition among children (<5 years of age) was as high as 38% in 2006 (FAO of the United Nations, 2010). The recommended dietary energy supply (DES) in the population lacks a share in lipids and proteins (FAO of the United Nations, 2010). The average DES in Uganda is 2,384 kcal/capita/day. Within this, 10% is comprised of protein and 16% is comprised of lipids (FAO of the United Nations, 2010). In comparison, the average DES for an industrialized (developed) country was 3,380 kcal/capita/day (FAOSTAT, 2004). The lack of essential proteins and lipids in the Ugandan diet may have indirect effects on the fatty acid composition in this population. A large component of these fatty acids arise from poultry, meats, fish, and vegetable oils (Anderson et al., 1975; Anderson, 1976; Exler et al., 1975; McCance & Widdowson, 2002). A report from 2003-2005 determined that the major food
groups consumed among the Ugandan population were fruit/vegetables and starchy roots (FAO, FAOSTAT). In an animal model, Lopez-Pedrosa et al., (1998) found that the fatty acid composition in the intestine can be severely affected by protein-energy malnutrition (PEM), which can result in low amounts of certain intestinal long-chain polyunsaturated fatty acids (LC-PUFA). To summarize, the typical Ugandan diet does not contain large quantities of fatty acids; one can speculate that this low amount of specific dietary fatty acids, in conjunction with overall malnutrition, may have an effect on the FAEE concentration found through meconium analysis.

3.6 Study Limitations

All studies are subject to limitations, which can provide insight into the direction of future follow-up studies; this current study is no exception. First, this pilot study concentrates on prenatal ethanol exposure in a limited population of women in southwestern Uganda. Thus, this may be an overgeneralization of maternal alcohol consumption during pregnancy throughout the entire country. Second, this study focuses on measuring the proportion of women who consume moderate to high levels of alcohol during pregnancy, not the prevalence of FASD. While FASD is strongly associated with the amount of maternal ethanol intake during pregnancy, there is still no literature describing a safe amount of ethanol exposure; therefore this study may be over- or underestimating the population of neonates at risk of developing FASD.

Stability and integrity of the meconium matrix adds further limitations to the ability to properly assess the overall prevalence rates of PAE. The pre-analytical process for properly collecting and storing the meconium specimens must be optimal in order to accurately interpret the results and determine an overall study prevalence rate. Due to dietary contamination and elevation of endogenously produced FAEEs in postnatal days after 24 hours, one must be very
careful in documenting the number and timing of meconium samples collected, and ensure the samples are not kept at room temperature and/or in a light-rich environment for extended periods of time. Lastly, meconium FAEE analysis test results are only indicative of heavy ethanol exposure within the 2\textsuperscript{nd} and 3\textsuperscript{rd} trimesters of pregnancy, whereas it has been established that ethanol exposure in the 1\textsuperscript{st} trimester has the greatest developmental impact on the fetus.

3.7 Future Directions

This is the first population-based study assessing the prevalence of heavy PAE in Uganda. However, this study was conducted in a smaller town in Uganda that makes up a very small percentage of the total population; therefore, additional studies should be conducted in order to generalize a prevalence rate for this country. Moreover, collection and analysis of the traditional alcoholic beverages that are home-brewed in these regions should be conducted in order to quantify the absolute amount of pure ethanol contained in each beverage. This would aid in approximating standard drink estimates in the maternal self-reports.

Pharmacogenomics plays a crucial role in the metabolism of ethanol. Cytochrome P450 2E1 (CYP2E1) is an oxidative enzyme that is involved in the metabolism of ethanol into acetaldehyde (Swift, 2003). Fatty acid ethyl ester synthase is part of the non-oxidative branch of ethanol metabolism. One can speculate that the limiting reagent, ethanol, and its specific affinities to the oxidative and non-oxidative pathways of ethanol metabolism, may play an essential part in the amount of FAEEs ultimately produced. The CYP2E1 pathway is inducible in chronic ethanol consumers (Kalant and Khanna, 1998). In an animal model, an inverse relationship between FAEE synthase activity and CYP2E1 activity (p=0.002) was found, suggesting that the higher the induction of CYP2E1, the lower the amount of ethanol available
for non-oxidative metabolism, and vice versa (J. Gareri, Doctoral Thesis, 2015). Therefore, a study investigating the pharmacogenomics of specific CYP2E1 polymorphisms which could give rise to variable CYP2E1 inducers, may answer some questions about why the current study observed a lower amount of cumulative FAEEs in the alcohol exposed neonates.

Considerable variation in the genetic diversity of ethanol metabolizing enzymes (ADH/ALDH) has been reported among differing ethnic groups. It has been shown that polymorphisms in the ADH2 and ADH3 loci are likely the most important in relation to variation of ethanol metabolism (McCarver et al., 1997). The AHD2 locus has three possible alleles, (ADH2*1/ADH2*2/ADH2*3) that result in varying activities (Bostron et al., 1983a; Burnell et al., 1989; Ehrig et al., 1990). The ADH2*3 allele was found to protect against alcohol-related birth defects among the African American population (McCarver et al., 1997). The offspring of drinking women with an ADH2*3 allele had MDI (Mental Developmental Index) scores similar to nondrinking women. The protection of this allele may be secondary to its encoding of ADH β3 isoenzyme which does not become saturated at high levels of ethanol consumption (similar to binge quantities), suggesting more efficient alcohol metabolism at high blood alcohol concentrations (Burnell et al., 1989; McCarver et al., 1997).

A potential policy that should be mandated in Uganda to help eradicate the issue of excessive maternal drinking during pregnancy might be to introduce a law that legally requires health-warning labels on alcohol advertisements and beverage containers, however this would not completely eliminate this issue because of the increasing consumption of home-brewed ethanol products that could not be regulated. One can speculate that part of the issue could stem from the lack of educational services available to women of childbearing age, to warn them of the potential permanent damage alcohol can have on the developing fetus. Namagembe et al.
assessed the consumption of alcoholic beverages among pregnant urban Ugandan women via a series of questionnaires. Among the 404 women who had not previously consumed alcohol, four (1.0%) initiated drinking after learning of their pregnancies. When questioned as to why they initiated drinking during pregnancy, one woman reported, “drinking waragi relieves heartburn” and “drinking beer will make the baby grow larger” (Namagembe et al., 2010). These testimonies further verify the lack of proper education regarding alcohol use during pregnancy. The study by Namagembe et al. recommended that antenatal and general obstetrics and gynecology clinics integrate alcohol use education group sessions for all women of reproductive age (Namagembe et al., 2010).

A potential reason for why we observed a much lower prevalence rate of heavy prenatal alcohol exposure in this population could be due to the sensitivity of the meconium-FAEE analysis. The original sensitivity and specificity validation studies of the analysis were conducted among a North-American cohort of children. Due to the possibility of nutrition and genetics playing a role in the composition of fatty acids and amount of FAEEs detectable, the positive cut-off level of 2.00 nmol/g may be too high in this population, thus potentially underestimating the actual number of children exposed to heavy prenatal alcohol exposure, who may go on to develop FASD.

3.8 Summary and Conclusions

Uganda ranks as one of the top countries in the world with the highest adult per capita consumption of alcohol, which poses a high risk for developing fetal alcohol spectrum disorder. However, our data from this study demonstrated a lower-than-expected prevalence rate of heavy prenatal alcohol exposure using an objective biomarker, FAEE, in meconium. Currently, there is
little to no information on the prevalence of alcohol consumption in Uganda. Follow-up research and more data are needed in order to raise awareness of the current situation in Uganda.

This study successfully implemented a feasible approach for assessing population-based risks of prenatal alcohol exposure. Meconium FAEE analysis conducted by HS-SPME followed by GC-MS using a FAEE cutoff of $\Sigma \geq 2.00$ nmol/g resulted in an adjusted prevalence rate of heavy prenatal alcohol exposure of 1.6% (n=8). The prevalence of prenatal alcohol exposure by maternal self-report throughout the entire pregnancy, and 2nd/3rd trimesters of pregnancy, was 16.1% (n=81) and 13.3% (n=67), respectively, which was much higher than the observed prevalence by meconium analysis. Among the individual FAEEs detected in the study, ethyl palmitate and ethyl oleate were the two most prevalent FAEE species found in the meconium. Mothers with a higher educational status were more likely to have an alcohol-exposed infant testing positive for FAEEs in meconium (Table 3.12).

The limitations mentioned above should not discount the results found in this study, as they provide valuable epidemiologic insight into alcohol consumption patterns in this population of women. These data can be further used in the public health sector and educational campaigns designed to diminish maternal alcohol use during pregnancy in Uganda.
References


Gareri J. (2015). Translational studies of fatty acid ethyl esters (FAEE) in meconium and hair as biomarkers of prenatal ethanol exposure risk. Doctoral Thesis, Leslie Dan Faculty of Pharmacy, University of Toronto.


Streissguth A.P., Barr H., Kogan J., & Bookstein F. (1996). Understanding the Occurrence of Secondary Disabilities in Clients with Fetal Alcohol Syndrome (FAS) and Fetal Alcohol Effects (FAE). University of Washington School of Medicine: Fetal Alcohol and Drug Unit, Department of Psychiatry and Behavioral Sciences.


## Demographics

<table>
<thead>
<tr>
<th>Record ID</th>
<th>____________________________</th>
</tr>
</thead>
</table>
| Excluded? | □ Yes  
□ No |
| Reason for Exclusion | □ D/C’d or left before passing meconium  
□ Baby died  
□ Baby too sick to for meconium collection  
□ Refused consent  
□ Mother too sick to consent  
□ Other |
| Specify other | ____________________________ |
| Nurse | □ Clare  
□ Annette  
□ Justine  
□ Collins  
□ Other |
| Specify Other | ____________________________ |
| Date and Time Questionnaire | ____________________________ |
| Date and Time of Delivery | ____________________________ |
| Date and Time of Meconium Collection | ____________________________ |
| Date and time of meconium freezing | ____________________________ |
| Was meconium passed during labor/delivery? | □ Yes  
□ No |
| First Meconium after delivery? | □ Yes  
□ No  
□ Meconium was collected during delivery  
□ Unknown |
| Was meconium collected during day (Y/N)? | □ Yes  
□ No |
| Estimate time (in minutes) meconium spent at room temperature in diaper | □ less than 5 minutes  
□ less than 10 minutes  
□ less than 30 minutes  
□ less than 60 minutes  
□ less than 120 minutes  
□ less than 180 minutes  
□ less than 240 minutes  
□ more than 240 minutes  
□ unknown duration (specify maximum below) |
| If more than 4 hours (240 minutes), specify the maximum time (in hours) meconium may have been in diaper at room temperature | ____________________________ |
| Estimate time (in hours) meconium spent in ice-box | □ < 1h  
□ 1-2h  
□ 2-4h  
□ 4-8h  
□ 8-12h  
□ 12-18h  
□ >18h  
□ Unknown |
Please add in any additional meconium storage details you feel need to be stated

Maternal place of residence (where she generally lived while pregnant)?

- [ ] Mbarara Town
- [ ] Other major town (such as Ibunda, Bushenyi, Lyantonde etc.)
- [ ] In trading center more than 5km outside of a major town
- [ ] In village more than 5km outside of a major town

Highest level of education completed

- [ ] Less than P4
- [ ] P4
- [ ] P7
- [ ] S4
- [ ] S6
- [ ] Technical School
- [ ] University
Pregnancy history

Age of Mother (years)  

Age of Father (or suspected father) in years  

Marital Status  

- Married  
- Living together  
- Single  
- Divorced  

Number of Previous Pregnancies

- 1  
- 2  
- 3  
- 4  
- 5  
- 6  
- 7  
- 8  
- 9  
- 10  
- 11  
- 12  
- 13  
- 14  
- 15  

Year of first pregnancy (to calculate spacing)?

Number of births before 20 weeks (i.e. miscarriages/abortions)?

- 0  
- 1  
- 2  
- 3  
- 4  
- 5  
- 6  
- 7  
- 8  
- 9  
- 10  
- 11  
- 12  
- 13  
- 14  
- 15  

Number of births 20-36 weeks (5-8 months) - (i.e. pre-term births)?

- 0  
- 1  
- 2  
- 3  
- 4  
- 5  
- 6  
- 7  
- 8  
- 9  
- 10  
- 11  
- 12  
- 13  
- 14  
- 15
Number of term births (i.e. 36-40 weeks)?

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15

Number of currently living children?

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15

Date you found out (or suspected) you were pregnant (estimate if exact date not known)?

______________________________

Estimated Due Date

______________________________

How was estimated due date estimated

- LMP
- Ultrasound
- Other

Specify other

______________________________

Is date of LMP known?

- Yes
- No

Date of LMP (Date is the FIRST day of the LMP)

______________________________

Date of estimated LMP

______________________________

HIV status

- Known and negative
- Known and positive
- Unknown

Co-morbidities and year of diagnosis (including HIV)

- Tuberculosis
- Diabetes
- Cardiac disease (HTN, MI, Angina, Cholesterol etc.)
- Seizure disorder
- Psychiatric disorder (depression, bipolar, schizophrenia etc.)
- Ulcer/dyspepsia/heartburn
- Other

Specify other

______________________________
## Substance use

Did mother use any medications during pregnancy?

<table>
<thead>
<tr>
<th>Medication Type</th>
<th>First trimester</th>
<th>Second trimester</th>
<th>Third trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesics (paracetamol/ibuprofen)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>HIV medication</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Anti-malarial</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>TB medication</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Antibiotic (septrin, amoxicillin, ciprofloxacin etc.)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Pre-natal vitamin</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Other (specify)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Specify other

---

Do you use any herbal or "Natural" medications during pregnancy?

<table>
<thead>
<tr>
<th>Medication Type</th>
<th>First Trimester</th>
<th>Second Trimester</th>
<th>Third Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbal medication use</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Specify name and indication

In the year BEFORE you became pregnant, did you ever take alcohol?

- ☐ Yes
- ☐ No

In the year BEFORE you became pregnant, what kind of alcohol did you generally consume?

- Beer
- Wine
- Spirits
- Local spirit (waragi)
- Local brew (tonto etc.)

In the year BEFORE you were pregnant, how many days per month did you typically take alcohol?

- less than once per month
- once or twice per month
- once or twice per week
- several times per week
- daily or almost daily

In the year BEFORE you became pregnant, on average how many drinks did you consume each time you took alcohol?

- less than 1
- 1 or 2
- 3 or 4
- 5 or more

Did you ever take alcohol while you knew (or suspected) you were pregnant?

- ☐ Yes
- ☐ No
On average, how frequently did you consume alcohol during your pregnancy, according to trimester?

<table>
<thead>
<tr>
<th></th>
<th>less than once per month</th>
<th>once or twice per month</th>
<th>once or twice per week</th>
<th>several times per week</th>
<th>daily or almost daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester (pre-recognition)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>First trimester (post-recognition)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Second trimester</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Third trimester</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

While pregnant, what type of alcohol do you generally consume when you take alcohol?

☐ Beer  ☐ Wine  ☐ Spirits  ☐ Local spirit (waragi)  ☐ Local brew (tonto etc.)

On average, how many drinks did you consume each time you took alcohol while you knew (or suspected) you were pregnant?

☐ less than 1  ☐ 1 or 2  ☐ 3 or 4  ☐ 5 or more

During your pregnancy, did you ever consume 5 or more drinks during any single occasion

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester (pre-recognition)</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>First trimester (post-recognition)</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Second trimester</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Third trimester</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

On average, how often would you consume 5 or more drinks during any single occasion

☐ Only once or twice in total during pregnancy  ☐ less than once per month  ☐ once or twice per month  ☐ once or twice per week  ☐ several times per week  ☐ daily or almost daily

Do you smoke cigarettes?

☐ Yes  ☐ No

How much do you smoke

☐ a few cigarettes per week  ☐ a few cigarettes per day  ☐ half a pack of cigarettes per day  ☐ 1 pack of cigarettes per day  ☐ more than 1 pack of cigarettes per day

for about how many years have you been smoking?

__________________________________________

Does your husband/partner drink alcohol

☐ Yes  ☐ No

What is the mothers perception of the safety of alcohol use while pregnant

☐ Always safe in any quantity  ☐ Always safe in low quantities  ☐ Sometimes harmful  ☐ Always harmful in low quantity  ☐ Always harmful in high quantity

What is the mothers perception of what the harms of alcohol are

__________________________________________
**TWEAK alcohol abuse screening test**

How many drinks does it take to make you feel high? __________________________________________

Have close friends or relatives worried or complained about your drinking in the past year?  
☐ Yes  ☐ No

Do you sometimes take a drink in the morning when you first get up?  
☐ Yes  ☐ No

Has a friend or family member ever told you about things you said or did while you were drinking that you could not remember?  
☐ Yes  ☐ No

Do you sometimes feel the need to cut down on your drinking?  
☐ Yes  ☐ No
FASD indicators

1. Short palpebral fissure (Yes/No)
   - Yes
   - No

2. Erased upper lip and philtrum (Yes/No)
   - Yes
   - No

3. Length (cm)
   ____________________________

4. Weight (kg)
   ____________________________
Appendix B: Documentation of Ethical Approval

UBC C&W Research Ethics Board  
A2-136, 950 West 28th Avenue  
Vancouver, BC V5Z 4H4  
Tel: (604) 875-3103 Fax: (604) 875-2496  
Email: cwrseb@cw.bc.ca  
Website: http://www.cfrl.ca/research_support > Research Ethics

ETHICS CERTIFICATE OF DELEGATED APPROVAL: RENEWAL

<table>
<thead>
<tr>
<th>PRINCIPAL INVESTIGATOR:</th>
<th>DEPARTMENT:</th>
<th>UBC C&amp;W NUMBER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stuart MacLeod</td>
<td>UBC/Medicine, Faculty of Paediatrics</td>
<td>H13-00463</td>
</tr>
</tbody>
</table>

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

<table>
<thead>
<tr>
<th>Institution</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other locations where the research will be conducted:</td>
<td></td>
</tr>
<tr>
<td>Mbarara Regional Referral Center and the Kyabujimbi Health Center, both located in Southwestern Uganda</td>
<td></td>
</tr>
</tbody>
</table>

CO-INVESTIGATOR(S):

Brian E. Grunau

SPONSORING AGENCIES:

N/A

PROJECT TITLE:

Fetal alcohol exposure during pregnancy in Uganda

REMINDER: The current UBC Children’s and Women’s approval for this study expires: May 7, 2015

APPROVAL DATE: May 7, 2014

CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Children's and Women's Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Children's and Women's Research Ethics Board.

Approved by one of:

file:///Users/innightingale/Library/Containers/com.apple.mail/Data/Library/Mail%20%20Downloads/6E35978D-1196-4081-94D2-71E237274D5D/UBC%20approval%20.pdf
June 28, 2013

The Resident District Commissioner, Mbarara District
The Resident District Commissioner, Bushenyi District

This is to introduce to you Mugenyi Godfrey Rwambuka Nyarushanje a Researcher who will be carrying out a research entitled “Fetal alcohol exposure during pregnancy in South Western Uganda” for a period of 06 (six) months in your district.

He has undergone the necessary clearance to carry out the said project.

Please render him the necessary assistance.

By copy of this letter Mugenyi Godfrey Rwambuka Nyarushanje is requested to report to the Resident District Commissioners of the above districts before proceeding with the Research.

Alenga Rose
FOR: SECRETARY, OFFICE OF THE PRESIDENT

Copy to: Mugenyi Godfrey Rwambuka Nyarushanje
June 28, 2013

The Resident District Commissioner, Mbarara District
The Resident District Commissioner, Bushenyi District

This is to introduce to you Kiwanuka N Gertrude a Researcher who will be carrying out a research entitled “Fetal alcohol exposure during pregnancy in South Western Uganda” for a period of 06 (six) months in your district.

She has undergone the necessary clearance to carry out the said project.

Please render her the necessary assistance.

By copy of this letter Kiwanuka N Gertrude is requested to report to the Resident District Commissioners of the above districts before proceeding with the Research.

Alenga Rose
FOR: SECRETARY, OFFICE OF THE PRESIDENT

Copy to: Kiwanuka N Gertrude
ADM 154/212/01

June 28, 2013

The Resident District Commissioner, Mbarara District
The Resident District Commissioner, Bushenyi District

This is to introduce to you Ngonzi Joseph a Researcher who will be carrying out a research entitled “Fetal alcohol exposure during pregnancy in South Western Uganda” for a period of 06 (six) months in your district.

He has undergone the necessary clearance to carry out the said project.

Please render him the necessary assistance.

By copy of this letter Ngonzi Joseph is requested to report to the Resident District Commissioners of the above districts before proceeding with the Research.

Alenga Rose
FOR: SECRETARY, OFFICE OF THE PRESIDENT

Copy to: Ngonzi Joseph
The Resident District Commissioner, Mbarara District
The Resident District Commissioner, Bushenyi District

This is to introduce to you Wiens Matthew Owen Researcher who will be carrying out a research entitled **“Fetal Alcohol Exposure during pregnancy in South Western Uganda”** for a period of **06 (six) months** in your district.

He has undergone the necessary clearance to carry out the said project.

Please render him the necessary assistance.

By copy of this letter **Wiens Matthew Owen** is requested to report to the Resident District Commissioners of the above districts before proceeding with the Research.

Alenga Rose

FOR: SECRETARY, OFFICE OF THE PRESIDENT

Copy to: Wiens Matthew Owen
RESEARCH ETHICS BOARD
APPLICATION FORM
Form Version Date: October 1, 2012

REB APPLICATION # (for REB use only)
1000049917

REB protocol number (REB use only)
1000035947

PART 1: STUDY INFORMATION

1. PROJECT

Complete project title
Incidence of heavy prenatal alcohol exposure in Uganda via analysis of fatty acid ethyl esters in meconium

Short title (max 50 characters)
Uganda Meconium Study - Version #2

Lay summary (max 750 characters)
In Uganda, very little is known as to the true incidence and extent of alcohol use in pregnancy. Recently, objective markers of fetal alcohol exposure have been investigated for the diagnosis of pre-natal alcohol exposure. The purpose of this study is to conduct statistical analyses on the existing laboratory results and questionnaire database that was collected by our collaborators at the University of British Columbia (UBC) and at Mbarara University of Science & Technology (MUST) in association to the meconium-FAEE concentrations tested by the meconium-FAEE analyses. We will also be investigating the total incidence of heavy prenatal alcohol exposure in the study population.

**Please use protocol version #2 for the current study**

List in point form the objectives of your study
1. To determine the incidence of heavy prenatal alcohol exposure in this population using retrospective statistical analysis of the meconium-FAEE results from the current database.
2. To determine self-reported alcohol consumption during pregnancy by using the detailed maternal questionnaire located in the retrospective database.

Duration of study
Anticipated study start date: May 20th, 2015
Anticipated study completion date: Dec 31st, 2015

2. RESEARCH TEAM

All SickKids Staff, trainees, or volunteers must complete the mandatory online ethics training course (TCPS 2).
2a. Principal Investigator

Name: Dr. Shinya Itô
RI position: Senior Scientist
Institution: SickKids

Clinical division: Clinical Pharmacology & Toxicology
RI program: Physiology & Experimental Medicine

2b. Co-Investigators

Number of co-investigators (max 15 entries)

Co-investigator # 1

Name: Dr. Stuart MacLeod
Position: Other - Professor
SickKids badge number (if applicable)
TCPS 2 certificate? ☐ Attached ☑ Previously provided ☐ N/A

Co-investigator # 2

Name: Dr. Matt Wiens
Position: Post-Doctoral Fellow
SickKids badge number (if applicable)
TCPS 2 certificate? ☐ Attached ☑ Previously provided ☐ N/A

2c. Study Team Members

Number of team members (max 25 entries)

Pl: Research Ethics Board Application Form
Short Title:
Page 2 of 16
## 2b. Co-Investigators

### Number of co-investigators (max: 15 entries)

<table>
<thead>
<tr>
<th>Co-Investigator # 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
</tr>
<tr>
<td><strong>Position</strong></td>
</tr>
<tr>
<td><strong>Clinical division</strong></td>
</tr>
<tr>
<td><strong>Institution</strong></td>
</tr>
<tr>
<td><strong>SickKids badge number (if applicable)</strong></td>
</tr>
<tr>
<td><strong>Signature</strong></td>
</tr>
<tr>
<td><strong>TCPS 2 certificate?</strong></td>
</tr>
<tr>
<td><strong>Previously provided</strong></td>
</tr>
</tbody>
</table>

*Only if the individual is not a SickKids employee, trainee or volunteer.

### Co-Investigator # 2

<table>
<thead>
<tr>
<th>Co-Investigator # 2</th>
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<tbody>
<tr>
<td><strong>Name</strong></td>
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<td><strong>Position</strong></td>
</tr>
<tr>
<td><strong>Clinical division</strong></td>
</tr>
<tr>
<td><strong>Institution</strong></td>
</tr>
<tr>
<td><strong>SickKids badge number (if applicable)</strong></td>
</tr>
<tr>
<td><strong>Signature</strong></td>
</tr>
<tr>
<td><strong>TCPS 2 certificate?</strong></td>
</tr>
<tr>
<td><strong>Previously provided</strong></td>
</tr>
</tbody>
</table>

*Only if the individual is not a SickKids employee, trainee or volunteer.

## 2c. Study Team Members

Any other individuals that will be accessing study information should be listed here.

### Number of team members (max: 25 entries)

<table>
<thead>
<tr>
<th>Team member # 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
</tr>
<tr>
<td><strong>Position</strong></td>
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<tr>
<td><strong>Clinical division</strong></td>
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<td><strong>Institution</strong></td>
</tr>
<tr>
<td><strong>Credentials</strong></td>
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<td><strong>SickKids badge number (if applicable)</strong></td>
</tr>
<tr>
<td><strong>TCPS 2 certificate?</strong></td>
</tr>
<tr>
<td><strong>Previously provided</strong></td>
</tr>
</tbody>
</table>

*Only if the individual is not a SickKids employee, trainee or volunteer.

What responsibilities will this individual have in this research study

- Recruiting participants
- Obtaining consent
- Collecting data
- Interacting with participants
- Analyzing data
- Accessing personal health information

## 2d. Alternate Contacts
2b. Co-Investigators

<table>
<thead>
<tr>
<th>Number of co-investigators (max 15 entries)</th>
<th>2</th>
</tr>
</thead>
</table>

**Co-investigator # 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Dr. Stuart MacLeod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Other - Professor</td>
</tr>
<tr>
<td>SickKids badge number (if applicable)</td>
<td></td>
</tr>
<tr>
<td>Clinical division</td>
<td>No Clinical Appointment at SickKids</td>
</tr>
<tr>
<td>Institution</td>
<td>University of British Columbia</td>
</tr>
<tr>
<td>Signature</td>
<td>[Signature]</td>
</tr>
<tr>
<td>TCPS 2 certificate?</td>
<td>☑ Attached</td>
</tr>
</tbody>
</table>

* Only if the individual is not a SickKids employee, trainee or volunteer

**Co-investigator # 2**

<table>
<thead>
<tr>
<th>Name</th>
<th>Dr. Matt Wiens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Post-Doctoral Fellow</td>
</tr>
<tr>
<td>SickKids badge number (if applicable)</td>
<td></td>
</tr>
<tr>
<td>Clinical division</td>
<td>No Clinical Appointment at SickKids</td>
</tr>
<tr>
<td>Institution</td>
<td>University of British Columbia</td>
</tr>
<tr>
<td>Signature</td>
<td>[Signature]</td>
</tr>
<tr>
<td>TCPS 2 certificate?</td>
<td>☑ Attached</td>
</tr>
</tbody>
</table>

* Only if the individual is not a SickKids employee, trainee or volunteer

2c. Study Team Members

Any other individuals that will be accessing study information should be listed here.

<table>
<thead>
<tr>
<th>Number of team members (max 25 entries)</th>
<th>1</th>
</tr>
</thead>
</table>

**Team member # 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Ira Nightingale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Graduate Student</td>
</tr>
<tr>
<td>SickKids badge number (if applicable)</td>
<td>0 3 1 5 5</td>
</tr>
<tr>
<td>Clinical division</td>
<td>Clinical Pharmacology &amp; Toxicology</td>
</tr>
<tr>
<td>Institution</td>
<td>SickKids</td>
</tr>
<tr>
<td>Credentials</td>
<td>H.B.Sc. Specialization in Biology</td>
</tr>
<tr>
<td>TCPS 2 certificate?</td>
<td>☑ Attached</td>
</tr>
</tbody>
</table>

* Only if the individual is not a SickKids employee, trainee or volunteer

**What responsibilities will this individual have in this research study**

- [ ] Recruiting participants
- [ ] Obtaining consent
- [ ] Collecting data
- [ ] Interacting with participants
- [ ] Analyzing data
- [ ] Accessing personal health information

2d. Alternate Contacts
**Team member # 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Ira Nightingale</th>
<th>Clinical division</th>
<th>Clinical Pharmacology &amp; Toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Graduate Student</td>
<td>Institution</td>
<td>SickKids</td>
</tr>
<tr>
<td>SickKids badge number (if applicable)</td>
<td>0 3 1 5 7 5</td>
<td>Credentials</td>
<td>M.Sc Specialization in Biology</td>
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<tr>
<td>TCPS 2 certificate?</td>
<td>[ ] Attached [ ] Previously provided [ ] N/A</td>
<td>* Only if the individual is not a SickKids employee, trainee or volunteer</td>
<td></td>
</tr>
</tbody>
</table>

**What responsibilities will this individual have in this research study**

- [ ] Recruiting participants
- [ ] Obtaining consent
- [ ] Collecting data
- [ ] Interacting with participants
- [x] Analyzing data
- [ ] Accessing personal health information

**Alternate research contact**

You may provide an alternate contact, to whom questions about the research study can be directed, if the PI is not available.

*Please note that this contact must have a SickKids e-mail address*

<table>
<thead>
<tr>
<th>Name</th>
<th>Ira Nightingale</th>
<th>Clinical division</th>
<th>Child Health Evaluative Services</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institution</td>
<td>SickKids</td>
<td>Badge number</td>
<td>0 3 1 5 7 5</td>
</tr>
<tr>
<td>TCPS 2 certificate?</td>
<td>[ ] Attached [ ] Previously provided</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Alternate administrative contact**

You may provide an alternate contact, to whom all REB correspondence will also be sent, below.

*Please note that this contact must have a SickKids e-mail address*

<table>
<thead>
<tr>
<th>Name</th>
<th>Hila Halshott</th>
<th>Clinical division</th>
<th>Child Health Evaluative Services</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institution</td>
<td>SickKids</td>
<td>Badge number</td>
<td>0 1 8 9 4</td>
</tr>
<tr>
<td>TCPS 2 certificate?</td>
<td>[ ] Attached [ ] Previously provided</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**3. STUDY SPONSOR**

Who initiated/wrote the protocol for the study?

- [ ] SickKids principal investigator
- [ ] Principal investigator at another hospital / university
- [ ] Industry
- [ ] Cooperative research group
- [ ] Other

Name of the PI and/or institution, company or group

Dr. Stuart MacLeod, University of British Columbia

**4. PROJECT FUNDING**

Amount of funding needed for this study

<table>
<thead>
<tr>
<th>Funds needed for the overall study</th>
<th>N/A</th>
<th>Funds needed for the study at SickKids</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n/a if this information is unavailable to you)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PI**

Research Ethics Board Application Form

**Page 3 of 16**

Form: Version Date: October 1, 2012
Attach the budget for this study at SickKids

☐ Budget attached

Amount of funding available for this study (number only)

Funds available for the overall study
(N/A if this information is unavailable to you)  N/A

Funds available for the study at SickKids

Number of different funding sources (include internal, external and in kind donations)

☐ 0

5. OTHER SICKKIDS SERVICES

SickKids services that will be utilized for this research study

☐ No SickKids services are being utilized

6. SCIENTIFIC PEER REVIEW

See REB science review guidance document

Select one of the following options

☐ This study has undergone an internal science review

☐ This study was reviewed by one of the external scientific peer review agencies accepted by SickKids REB

☐ This study is a SickKids sponsored retrospective study that has not undergone a science review

Attach the following documents from your review

☐ Peer review form  ☐ Reviewer's comments  ☐ Itemized response to reviewer  ☐ Reviewer's sign-off

Does this study involve participants from the department of haematology/oncology?

☐ Yes  ☐ No

7. REGULATORY REQUIREMENTS

Does this study involve the use of a drug, biologic, natural health product or device?

☐ Yes  ☐ No

Has this study been registered on a public website? (e.g. clinicaltrials.gov, isrctn.org, etc.)

☐ Yes  ☐ No  ☐ Not applicable

8. OTHER SITES

PI:

Research Ethics Board Application Form

Short Title:

Page 4 of 16

Form Version Date: October 1, 2012
Will you be transferring funds, data, materials or personnel to other sites?

- [ ] Yes  - [ ] No

List the sites and indicate what will be transferred (Note: an agreement or contract may be required, please contact Legal Services)

<table>
<thead>
<tr>
<th>Add a site</th>
<th>Site</th>
<th>Transfer of funds</th>
<th>Transfer of data OUT of SickKids</th>
<th>Transfer of specimens OUT of SickKids</th>
<th>Transfer of equipment</th>
<th>Transfer of personnel or service</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delete this site</td>
<td>University of British Columbia</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

9. AGREEMENTS AND CONTRACTS

Are there any agreements or contracts related to this study?

- [ ] Yes  - [ ] No

Do you anticipate the study will result in a new invention or a new use for an existing product?

- [ ] Yes  - [ ] No

Meeting with Legal (Harpreet Hansra) determined contract not required for this stage.

- Only deidentified samples & questionnaires were analyzed for FAME (see OCF).

[Signature]

REB Vice Chair
June 12/15
**PART 2: ETHICAL INFORMATION**

### 10. RESEARCH CATEGORY, RISK LEVEL AND TYPE

#### 10a. Research Category

Indicate the research category and the level of continuing review by selecting only ONE of the boxes in the matrix below. If there are multiple components to your study, select the research category that applies to the most invasive type of contact that you will have with the participants.

*See REB continuing review guidelines*

<table>
<thead>
<tr>
<th>1. Research Category</th>
<th>LEVEL I Adverse events and annual REB Reports</th>
<th>LEVEL II</th>
<th>LEVEL III</th>
<th>LEVEL IV Observation of consent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Retrospective Data Collection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Studies Involving existing personal health information</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NO participant contact</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td><strong>B. Prospective Observational</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO physical exams but involves participant contact</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td></td>
</tr>
<tr>
<td><strong>C. Prospective Observational</strong></td>
<td></td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Physical exams, physiological assessments and/or imaging</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>NO biological specimens (blood, urine, tissue, saliva) taken</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td><strong>D. Observational Study of Biological Specimens</strong></td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Retrospective or Prospective</td>
<td>![ X ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>(blood, urine, tissue, saliva) taken</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>NO administration or use of a drug, biologic, natural health product, or device</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td><strong>E. Clinical Intervention Trial</strong></td>
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<td>![ ]</td>
<td>![ ]</td>
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</tr>
<tr>
<td>Drug</td>
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<tr>
<td>Natural health product</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
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</tbody>
</table>

#### 10b. Research Study Components

*Does this study have a retrospective chart review component?  *

☐ Yes ☐ No

*Does this study involve a retrospective analysis of previously collected tissue samples?  *

☐ Yes ☐ No

*Does this study have a prospective research component (including clinical interventions)?  *

☐ Yes ☐ No

*Does this study involve a clinical intervention or an invasive procedure?  *

☐ Yes ☐ No
### 11. RETROSPECTIVE CHART REVIEW INFORMATION (for studies that involve a chart review - as indicated in question 10b)

**11a. Protocol Information** (for studies with no prospective component - as indicated in question 10b)

Attach a separate protocol OR if there is not a protocol available, provide responses to the following question

- **Separate protocol attached**

**11b. Health Chart Screening**

Anticipated number of charts that will be screened for participant eligibility (approximate number)

| 503 |

How will you identify the charts that you will screen for participant eligibility? What search criteria will you be using?

- Mecconium samples arriving with corresponding questionnaires that were consented prior to collection

**11c. Enrollment in Health Chart Review**

Anticipated number of research participants to be enrolled in the health chart review (approximate number)

| 503 |

Eligibility criteria for enrollment in the health chart review

- Mecconium samples arriving with corresponding questionnaires that were consented prior to collection

**11d. Health Chart Dates**

What are the earliest and latest health chart entry dates that you will be accessing?

- **Earliest date**: Sep 23, 2013
- **Latest date**: Jun 2, 2015

Latest date can not exceed date of this REB application

**11e. Waiver of Consent for Health Chart Review**

The [Personal Health Information Protection Act (PHIPA)](https://www.ohri.ca/programs/ohri/ree/phipaguidelines/guidelines.html) does allow for research without participant consent in certain situations.

- The objectives of the research cannot be reasonably accomplished without using personal health information.
- There are adequate safeguards to protect the privacy of individuals.
- There is a public interest in this research while protecting the privacy of individuals.

*Please note, any waiver of consent excludes any record that has been “locked” by the participant. See hospital policy “Lockbox.”*

Are you requesting a waiver of consent for this health chart review?

- **Yes**
- **No**

Describe:

- **Who** will make initial contact and their relationship to the participant
- **How** initial contact will be made with prospective participants/parents: e.g., in person, by phone, by letter
Name and qualifications of all individuals who will be explaining the study and who will be obtaining consent. All of the individuals listed below must also be listed on your consent form.

Describe in detail how consent will be obtained.

12. RETROSPECTIVE BIOLOGIC SAMPLE ANALYSIS INFORMATION (for studies that involve analysis of previously collected biologic samples - as indicated in question 10b)

12a. Protocol Information (for studies with no prospective component - as indicated in question 10b)
Attach a separate protocol OR if there is not a protocol available, provide responses to the following question.
- Separate protocol attached

12b. Sample Identification

Anticipated number of samples that will be analyzed (approximate number)

503

How will you identify the samples that you will analyze in this study?
- Physician/care-giver of participant
- Existing database or tissue bank
- Health record search
- Other

What criteria are you using to select the samples that will be analyzed?

All meconium samples shipped to the PCCRL in Toronto that were consented and contained a questionnaire sheet

12c. Sample Source and Sample Type

What are the sources of the samples that will be analyzed in this study?

- Discarded or left over sample
- Research study bank of sample
- Archived pathology sample
- Other

Specify:
Meconium excretion from consented neonates
Type of samples (check all that apply)

- Excised organ
- Excised tissue
- Leftover biopsy
- Cell lines
- Blood
- Urine
- Saliva
- Bone marrow
- Other

Specify

Meconium

12d. Privacy and Ethical Concerns

Was consent for future use of these samples obtained from the tissue donors?

- Yes
- No
- Consent was waived (if sample was obtained as part of a research study)

Will you be doing genetic testing on any of the samples in this study?

- Yes
- No

Describe:
- Whether there is any anticipated linkage of the sample to information about the patient
- What measures that will be taken to protect privacy of the participants

All information in the questionnaires are anonymized and cannot be linked to the patient

Describe where the samples will be kept, the length of time they will be stored, and the process for disposal

The samples will be kept in a locked cryogenic freezer on the 10th floor of PGCR. They will be stored until the study is completed and closed by REB. The process for disposal will follow appropriate biological hazardous protocols instated by the institution

Describe the plan for handling any incidental findings that may arise

Handling of any incidental findings will be destroyed immediately via shredding any documentation containing this information

12e. Consent for Analysis of Previously Collected Biologic Samples

The **Personal Health Information Protection Act (PHIPA)** does allow for research without participant consent in certain situations

The following conditions must be met before a waiver of consent is considered:
- The objectives of the research cannot be reasonably accomplished without using personal health information
- There are adequate safeguards to protect the privacy of individuals
- There is a public interest in this research while protecting the privacy of individuals.

*Please note, any waiver of consent excludes any record that has been "locked" by the participant. See hospital policy 'Lockbox'.*

Are you requesting a waiver of consent for this analysis of previously collected biologic samples?

- Yes
- No

Describe:
- **Who** will make initial contact and their relationship to the participant
- **How** initial contact will be made with prospective participants/parents; e.g., in person, by phone, by letter

Name and qualifications of all individuals who will be explaining the study and who will be obtaining consent

All of the individuals listed below must also be listed on your consent form.
Describe in detail **how** consent will be obtained

---

### 13. PROSPECTIVE RESEARCH INFORMATION
(for studies that involve prospective research - as indicated in question 10b)

### 14. CLINICAL TRIALS INFORMATION
(for studies with a clinical intervention or invasive procedure - as indicated in question 10b)

### 15. SENSITIVE PERSONAL HEALTH INFORMATION AND RESEARCH DATA

The Research Ethics Board considers the following information to be sensitive personal health information, collection of which could result in identification of and/or harm to the participant (e.g. cause embarrassment, refusal of employment or insurance coverage, stigmatization). The collection of this information must be explicitly stated in the consent form.

**Indicate the sensitive personal health information you will be collecting for your study**

- No sensitive personal health information will be collected

<table>
<thead>
<tr>
<th>Biometric identifiers</th>
<th>Confidential legal information</th>
<th>Data with research participant identifiers</th>
<th>Date of birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of death</td>
<td>Dates of treatment, where treatment is rare or unique</td>
<td>Email address</td>
<td>Ethnicity</td>
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<tr>
<td>Family income</td>
<td>Family history</td>
<td>Genetic Information</td>
<td>Gender</td>
</tr>
<tr>
<td>Health card number</td>
<td>HIV status</td>
<td>Identifiable images</td>
<td>Initials</td>
</tr>
<tr>
<td>Local references (e.g. address, postal code, etc.)</td>
<td>Mental health status</td>
<td>Name</td>
<td>Race</td>
</tr>
<tr>
<td>Religious affiliation</td>
<td>SickKids medical record number</td>
<td>Social insurance number</td>
<td>Telephone or fax number</td>
</tr>
</tbody>
</table>

- **Other**

**If you have checked any of the sensitive information fields above, describe**

- Why the collection of each field is necessary to achieve the scientific objective of your study
- What measures you will be taking to protect the information

**HIV Status** - We wanted to collect HIV status for two reasons, a) risk factor to maternal drinking during pregnancy, b) safety precaution for sample handling and analysis

**Mental health status** - We wanted to use this information to see if mental health status may be a risk factor for maternal drinking

**Date of Birth** - We collected the DOB of the neonate in order to determine the time elapsed from birth to meconium collection. This is important to verify the integrity of the meconium sample.
16. DATA COLLECTION FORMS

Identifiable personal health information must not be included on data collection forms - i.e. the research participant names, initials, SickKids patient numbers, and other identifying information is strictly prohibited. Use of complete date of birth or full postal code must be justified. See the SickKids policy: "Privacy and Confidentiality of Information."

Each study participant must be assigned a unique study identifier code at the time of enrollment.

The code-breaking information must be kept securely and separately from the data collection files. The PI is responsible for ensuring that the code-breaking information is totally inaccessible to individuals who are not part of the research team, under double lock, using the principle of data minimization for data collection forms.

Include your data collection forms with this application

All data collection forms including identified and de-identified forms must be attached

☐ All data collection forms (version dated) attached

Will you be keeping data on participants that have been withdrawn or removed from the study?

☐ Yes ☐ No

17. DATA SOURCES

All individuals that will be accessing personal health information (e.g. health records, Electronic Patient Charts (EPC), clinic or research databases) must be listed in Section 2 (Research Team) above.

You will need to follow the appropriate processes for each data source and obtain permission from the health information custodian or database administrator in order to gain access to the data.

Health Records - Indicate all sources of data that you will be requesting permission to access for screening and/or for enrollment

☐ Paper charts - available only for data prior to May 1, 2000

☐ SickKids clinical information systems
  ☐ Electronic Patient Chart (EPC) ☐ KidCare ☐ PACS

☐ CIMS

Databases/Registries - Indicate all sources of data that you will be requesting permission to access for screening and/or for enrollment

☐ Clinical division/department database

☐ Research database/registry

☐ Data warehouse

Do you plan on linking locally collected data with any other data set (e.g. other hospitals OHIP, ICES data, etc.)?

☐ Yes ☐ No

Will you be using any other sources not listed above?

☐ Yes ☐ No
List all other sources of data that you will be using

Questionnaire database was entered into a generic Microsoft Excel spreadsheet that contains every study # and their corresponding questionnaire answers. The source of these questionnaires is from the UBC collaborators, Dr. Stuart MacLeod and Dr. Matthew Wiens. Attached to this REB application is the Uganda Meconium Study Questionnaire. The excel sheet is secured.

18. DATA STORAGE AND DESTRUCTION

Refer to the SickKids Policies "Information Security" and "Records Retention and Destruction"

Indicate the physical safeguards that you will be using to securely maintain your data
The SickKids Policy requires that PHI be stored behind two locks

☐ Locked office ☐ Locked storage unit ☐ Biometric authentication

☐ Cipher/coded locks ☐ Access cards

☐ Other

Specify

Questionnaire data stored on passworded computer file

Indicate the administrative safeguards that you will be using to securely maintain your data
The SickKids Policy requires that PHI be stored with two separate Passwords/Authentication methods

☐ Subjects coded ☐ Computer passwords ONLY with research team

☐ Locked folder on SickKids shared drive ☐ Designated individual responsible for controlling who has access to data

☐ Other

Indicate the technical safeguards that you will be using to securely maintain your data.
The SickKids Policy requires that PHI be stored with two separate Passwords/Authentication methods

☐ Files/folders password protected ☐ Computer password protected ☐ Firewalls

☐ Network Drive ☐ Encrypted laptop (CANNOT be a personal laptop) ☐ Encrypted USB key (CANNOT be a personal device)

☐ Other

How long will the data be stored?

☐ 7 years from last publication

☐ 25 years from end of study (if drug, biologic or natural health product trial approved by Health Canada)

☐ Other

Indicate the methods that will be used to destroy the data

☐ Paper records will be disposed in SickKids confidential disposal bins

☐ Electronic records will be destroyed by contacting SickKids IS help desk

☐ Old CDs, DVDs, videos, USB keys, external hard drives and other technology will be sent to the repair centre for destruction

☐ Other
Indicate the individuals that will have access to the data in the future once the study is complete.

- [x] Investigators
- [x] Staff/trainee
- [ ] External individuals - data MUST be de-identified
- [ ] Students/volunteers
- [ ] Other

19. CONFLICT OF INTEREST DECLARATION BY PRINCIPAL INVESTIGATOR

Explanation

Researchers hold trust relationships with research participants, research sponsors, The Hospital for Sick Children (SickKids), their professional bodies and society. Researchers, SickKids, and the REB are required to identify and address actual, potential and perceived conflicts of interest ("Conflicts of Interest") to maintain public confidence and trust, ensure the integrity of research, discharge professional obligations and ensure accountability.

A Conflict of Interest does not necessarily imply wrongdoing, as a Conflict of Interest depends upon the circumstances, not on the character of the staff member.

A Conflict of Interest does not mean that the research cannot proceed. Many (but not all) Conflicts of Interest can be managed, but always require identification of the Conflict of Interest, disclosure to research participants, and if required, other steps to manage the Conflict of Interest. It will be up to the REB to determine if the Conflict of Interest can be managed and if the proposed mitigation measures are adequate.

All Conflicts of Interest must be clearly identified by the Principal Investigator and the Principal Investigator is making this Declaration on behalf of himself/herself and the members of the research team (collectively referred to in the Declaration as "Researcher").

See REB "Conflict of Interest" Guidelines

Declaration

A. I have spoken with members of my research team and hereby declare that neither I nor (to the best of my knowledge) any members of my research team have an actual, potential or perceived conflict of interest ("Conflict of Interest" or "COI") with respect to the attached Application for Research.

- [x] Check this Box if applicable

OR

I have spoken with members of my research team and have identified a Conflict(s) of Interest with respect to this application for research in the following categories and as specified below:

(i) Financial
- [ ] Yes
- [x] No
- Member with Conflict of Interest

(ii) Status
- [ ] Yes
- [x] No
- Member with Conflict of Interest

(iii) Undue influence
- [ ] Yes
- [x] No
- Member with Conflict of Interest

(iv) Completing interest
- [ ] Yes
- [x] No
- Member with Conflict of Interest

Details of Conflict of Interest

If you have checked yes to any of the items in question A above, complete questions B and C, otherwise go to question D.

B. I intend to manage the Conflict(s) of Interest as set out below (e.g., disclosure in consent form, declining role/position with sponsor and additional monitoring strategies such as monitoring consent)
C. I have declared all Conflicts of Interest to the Research Institute

☐ Yes  ☐ No  ☑ N/A

Attach a copy of the Research Institute approval of the Conflict(s) of Interest
If you have not disclosed the Conflicts of Interest to the Research Institute or are not obliged to declare, explain why

D. Should a Conflict of Interest arise for me or any member of my research team during the course of the research, I shall declare this in writing to the Research Ethics Board

☐ Yes  ☐ No

E. I hereby declare that I have read this Declaration, have discussed this Declaration with the members of my research team, and that to the best of my knowledge and belief, my responses are true and complete

Dr. Shinya Ito
Name of Principal Investigator

Signature of Principal Investigator  June 5, 2015  Date

20. PRIVACY AND SECURITY ACKNOWLEDGEMENT

On behalf of my research team, I recognize the importance of maintaining the confidentiality of personal health information and the privacy of individuals with respect to that information. I will ensure that the confidentiality of personal health information used only as necessary, to fulfill the specific research objectives and related research questions described in this application and approved by the REB governing the use, security, disclosure, return or disposal of the research participants personal health information. I agree to take any further steps required by the REB or SickKids to ensure that the confidentiality and security of the personal health information is maintained in accordance with the Personal Health Information Protection Act (PHIPA - 2004), its accompanying regulation, the Tri-Council Policy Statement 2 and in accordance with SickKids Policies.

Dr. Shinya Ito
Name of Principal Investigator

Signature of Principal Investigator  June 5, 2015  Date

21. DEPARTMENT HEAD APPROVAL FOR THIS SUBMISSION

Department/Division Head

The signatures of division or department heads who are named as investigators in this application are not accepted here; sign-off in such cases is done by an existing (e.g., not created specifically for this research project) deputy, or by the person to whom the Head reports for patient care matters.

Dr. Meredith Irwin
Division Head Name

Division Head Signature  June 5, 2015  Date
22. REB APPROVAL FOR HEALTH CHART RESEARCH

<table>
<thead>
<tr>
<th>Research Ethics Board Approval for Retrospective Chart Research (REB use only)</th>
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<tbody>
<tr>
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<tr>
<td>Please note, any waiver of consent excludes any record that has been &quot;locked&quot; by the participant. See hospital policy &quot;Lockbox.&quot;</td>
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<table>
<thead>
<tr>
<th>Other comments</th>
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<tbody>
<tr>
<td>Deidentified meconium samples and questionnaires sent her solely to determine GAEE levels. Subjects consented and enrolled in the study in Uganda.</td>
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<table>
<thead>
<tr>
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<tr>
<th>Signature of REB Chair or delegate</th>
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<tr>
<td>[Signature] REB Virechai</td>
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<table>
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<tr>
<th>Date of approval</th>
<th>June 12/2015</th>
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<tbody>
<tr>
<td>Approval expires</td>
<td>June 12, 2016</td>
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</tbody>
</table>
23. REB APPROVAL FOR RETROSPECTIVE BIOLOGIC SAMPLE ANALYSIS

<table>
<thead>
<tr>
<th>Research Ethics Board Approval for Retrospective Biologic Sample Analysis</th>
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<tbody>
<tr>
<td>(REB use only)</td>
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</table>

Waiver of consent granted because the following criteria have been met
Please note, any waiver of consent excludes any record that has been “locked” by the participant. See hospital policy “Lockbox.”

Other comments

Mesorhin collected in Uganda from consented subjects to send here (de-identified) to complete FABT analysis

<table>
<thead>
<tr>
<th>Level of continuing review</th>
<th>Version date of approved data collection form</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Nov 2014</td>
</tr>
</tbody>
</table>

Signature of REB Chair or delegate:

Date of approval: Jan 23, 2015
Approval expires: June 12, 2016
Fetal alcohol exposure during pregnancy in Southwestern Uganda: A Pilot Study

Principal Investigators:
- Dr. Godfrey R Mugenyi
  Mbarara University of Science and Technology
  Mbarara, Uganda
- Dr. Matthew O Wiens
  School of Population and Public Health
  University of British Columbia

Co-Investigators:
- Dr. Stuart MacLeod, University of British Columbia
- Dr. Gideon Koren, University of Toronto
- Dr. Joseph Ngonzi, Mbarara University of Science and Technology
- Dr. Gertrude Kiwanuka, Mbarara University of Science and Technology
- Dr. Brian Grunau, University of British Columbia

Sponsors: The MotherRisk Program, The Hospital for Sick Children, University of Toronto, Canada
WHAT YOU SHOULD KNOW ABOUT THIS RESEARCH STUDY
You are being invited to take part in this research study because you are in active labour or have very recently delivered your baby. Your participation is entirely voluntary, so it is up to you to decide whether or not you wish to take part in this study. This consent form explains the research study and your role in the study. If you wish to participate you will be asked to sign this form. If you do decide to take part in this study you are still free to withdraw at any time without giving any reasons for your decision. If you choose not to participate, or to withdraw your consent you will not lose the benefit of any medical care you or your newborn infant are entitled to. This study is being conducted in partnership with the Hospital for Sick Children in Toronto, Canada and the University of British Columbia in Canada.

PURPOSE OF THIS RESEARCH
The consumption of chemicals and toxins (such as alcohol and tobacco) that may be hazardous to the health of your baby is not well understood in pregnant Ugandan women. This study is focusing on collecting information from you to better understand the consumption patterns of potentially hazardous substances in women who are pregnant in Uganda. We will use this information to help doctors, nurses and public health practitioners make sure that pregnancies in Uganda remain as healthy as possible so as to ensure healthy future generations of Ugandan children. In order to do this we will require you to answer several questions about your consumption of potentially hazardous substances such as alcohol and tobacco during the course of your pregnancy. We will also collect a small sample of your baby’s meconium (the first stool he/she passes) for analysis of your baby’s health while in the womb.

WHY YOU ARE BEING ASKED TO PARTICIPATE
Any woman who delivers a baby during the study period is eligible to participate. You are either in labour or have delivered a baby.

PROCEDURES
This study is being done at the Mbarara Regional Referral Hospital. We will enroll about 500 mother-child pairs over the course of about 3 months. This study involves collecting health-related information following the delivery of your baby. We also require that we be allowed to collect a small sample of your baby’s meconium (this is the first stool that a baby passes after birth). We shall need your permission to store your baby’s meconium for a period of 3 months from the time of collection to allow us complete the necessary analyses, after which it will be destroyed. These analyses are going to be conducted at a research laboratory in Canada.

RISKS / DISCOMFORTS
The primary discomfort you may experience is that we administer the questionnaire shortly after you have delivered your baby, and you may be tired. We will not collect any health information from you until after delivery to make this aspect of the study as easy as possible for you.

**BENEFITS**
This study offers no direct benefits to you or your child. We will provide a package of diapers free of charge to you so as to make the collection of meconium easy for our research assistant and as comfortable as possible for your baby.

**INCENTIVES / REWARDS FOR PARTICIPATING**
There are no incentives or rewards for participating in this study.

**PROTECTING DATA CONFIDENTIALITY**
We will not be collecting any information which identifies you such as your name, address or telephone number (other than this consent form). All data we collect is confidential and will be stored in a locked office and only seen by study investigators and study staff. There will be no way for investigators or staff to link your responses with your identity. Details of the laboratory analysis and the data collected will be shared with investigators in Canada, but no details of your identity will be revealed.

**PROTECTING SUBJECT PRIVACY DURING DATA COLLECTION**
The questionnaire will be conducted as private a location as possible, however we cannot guarantee that no one may overhear some of your responses.

**WHAT HAPPENS IF YOU LEAVE THE STUDY?**
If you choose not to participate, or to withdraw your consent you will not lose the benefit of any medical care you or your newborn infant are entitled to.

**WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?**
If you have any questions or desire further information about this study before or during participation, you can contact Kobugyeniy Solome, study administrator, at 0750375374 / 0773064034.

If you have questions about your rights as a research participant, you may call Mr. Simon Anguma, chairman of the Institutional Ethical Review Committee of Mbarara University of Science and Technology at 04854-33795.
SUBJECT CONSENT TO PARTICIPATE

The parent(s)/guardian(s) and the investigator are satisfied that the information contained in this consent form was explained to the child to the extent that he/she is able to understand it, that all questions have been answered, and that the child assents to participating in the research.

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me.
- I have read this form and I freely consent to participate in this study.
- I will receive a signed copy of this consent form for my own records.

SIGNATURES

Printed name of infant

________________________

Printed name of mother

________________________

Signature Date

Printed name of principal investigator/designated representative

________________________

Signature Date

Printed name of translator (if applicable)

________________________

Signature Date

Leave blank for IRC office only

MUST-IRC Stamp:

IRC OFFICIAL USE ONLY:

Approval date:
Approved consent IRB version number:
PI NAME:
IRB NO:

Version 1.3
September 5, 2013
INFORMED CONSENT FOR STORAGE OF RESEARCH MATERIALS

1) Nature of research materials to be stored.

The meconium from your baby (first stool after birth) will be stored prior to analysis at a laboratory in Toronto Canada.

2) State the reason(s) why you wish to store the research materials.

The meconium needs to be stored prior to analysis since the research team must collected sufficient samples prior to transporting it to Canada. This is due to cost limitations of the project. Once analyzed, the research material will be disposed of immediately.

3) How long will you store the research materials?

The meconium will be stored for up to three months in Mbarara prior to shipping to Canada to ensure sufficient samples have been collected.

4) Where will you store the research materials? (Give the full physical address, telephone contact, and email address of the location)

Storage in Mbarara in preparation for transport to Canada:
Mbarara University of Science and Technology Research Laboratory
PO Box 1410
Contact person: Yona Mbalubulha
Telephone number: +256-485-20228
Email: mustcarl@yahoo.com

Storage in Toronto, Canada in preparation for analysis
The Hospital for Sick Children
555 University Avenue
Toronto, Ontario, Canada
Contact Person: Gideon Koren  
Telephone number: 416-813-5781  
Email: gideon.koren@sickkids.ca

Describe the disposal plan for the research materials after the expiry of the storage period.

Following analysis all research material will be disposed of according the protocols set forth by the Government of Canada for disposal of biological research materials.

I, .......................................................... accept that the research materials obtained from the body of my infant as a research participant can be stored confidentially for future use.

Participant  
Name: ............................................  
Signature: ........................................  
Date: .............................................  

Principal Investigator  
Name: ............................................  
Signature: ........................................  
Date: .............................................