

Impact of Pregnancy on Buprenorphine Pharmacokinetics and Pharmacodynamics

Version: June 5, 2019

NCT 02863601

**Obstetric-Fetal Pharmacology Research Centers (OPRC) Network
The Eunice Kennedy Shriver National Institute of Child Health and Human Development
(NICHD)**

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Revision History:

Version/Protocol Date	Revision Description
11 July 2016	Original version approved by UPITT IRB
14 Sept 2016	<ul style="list-style-type: none"> - Added a pre-dose blood draw during PK visits to allow sufficient volume to analyze BUP and required chemistries - Added a pre-dose saliva collection during a PK study visit, in addition to the pre-dose collection for pH - Removed temperature measurement from the screening procedures - Removed urinalysis from the screening procedures
9 Feb 2017	- Addition of the measurement of QID dosing of buprenorphine
28 Apr 2017	- Additional changes to the consent form and measurement of dosing schedules for patients
16 Nov 2017	- Revision to the timing of the postpartum PK visit to 4-6 weeks after delivery
01 Mar 2018	<ul style="list-style-type: none"> - Section D.5.vi: Clarify that multiple venipunctures may be necessary if blood cannot be obtained from the IV - A screening visit will occur within 1 week prior to each PK visit. Subjects must continue to meet the criteria listed in section D.4, with the exception of AST, ALT and creatinine levels. - Subjects must complete PK visits 1 and 3 (postpartum) to remain enrolled in the study - Urine drug screens will be performed during the screening visits but not during the PK visits - Patients testing positive for non-prescribed opiates/opioids may not proceed with the PK visit. They may be rescreened if still within the gestational window, but subjects will be dismissed from the study after two positive drug screens.
14 Mar 2018	<ul style="list-style-type: none"> - Added the option of 5 times a day buprenorphine dosing. The duration of the PK studies and specific time intervals that biological specimens are collected were added for subjects taking a five time daily dose. - Included content regarding the specific parts of the study subjects may choose to participate in. Subjects are no longer required to participate in the PK studies and may choose from any of the five parts of the study described in the consent form.
25 June 2018	<ul style="list-style-type: none"> - Included an additional sample collection at each PK visit and delivery (for PK study patients only) for peripheral blood mononuclear cells (PBMC). - Revised sections pertaining to the collection of breast milk samples and heel stick blood samples. Trough and peak level heel stick samples will be collected during the PP PK visit and during the optional ancillary study. A total of 8 heel stick samples may be collected. - Revised the section pertaining to the collection of postpartum blood samples. - Addition of blood samples for BUP and DNA analysis at the screening visit for subjects not participating in the PK study. - Changed QID study visit to 7 hour duration - Inclusion Criteria: gestation age changed to < 19 6/7 - Exclusion Criteria changed: <ul style="list-style-type: none"> PK Study – Delivery at another institution where outcome data cannot be obtained on mother and baby Procedures other than the PK Study – Delivery at another institution where samples cannot be obtained on mother and baby - Added vital signs to Baseline Screening Visit for PK Study patients - Added sample processing instructions for PBMC analysis and DNA analysis

6 August 2018	<ul style="list-style-type: none"> - Added clarification that questionnaires for alcohol, tobacco, illicit drug use, and depression will be completed at all screening visits - Study Design, Part A: Clarified that subjects must complete the first PK visit and the postpartum PK visit in order to remain in the PK study. - Added a BUP and DNA sample to be collected at the Baseline Screening Visit for PK study patients to avoid having to rescreen if they switch to a different part of the study. If the DNA sample is unable to be obtained at the screening visit, it may be collected anytime during the study when blood is being drawn. - Section D.5.vi: AST/SGOT, ALT/SGPT, creatinine are collected at PK Visits 2 & 3 - Added additional information for clarity regarding samples collected at delivery and sample processing instructions
15-November 2018	<ul style="list-style-type: none"> - Removed the list of Steering Committee members from the cover page - Subjects are only required to complete one PK study - Revised labs performed at Screening Visit - Created one PK Screening Visit performed prior to all PK study visits - Added clarification that Labor and Delivery biological specimens will be collected when possible.
24-January 2019	<ul style="list-style-type: none"> - Section D.5.iii: Subjects in any part of the study who are dismissed from their MAT clinic due to non-compliance will be dismissed from the study and replaced. - Section D.5.ix: A urine sample will be collected for a comprehensive drug screen within 48 hours of admission for delivery. Samples from subjects with a positive drug screen at delivery will not be used for certain specific aims of the study. - Added section D.9 regarding sample analysis and the collection of negative samples.
5-June-2019	<ul style="list-style-type: none"> - Revised Part D of the study to allow the collection of breast milk and infant heel stick samples during a routine postpartum PRC visit, as long as the mother is still taking Subutex or Suboxone.

OPRC Protocol: Impact of Pregnancy on Buprenorphine Pharmacokinetics and Pharmacodynamics (Version Date: June 5, 2019)

A. Significance

Substance abuse costs our nation over \$600 billion annually. Drug addiction treatment reduces drug use and has been shown to reduce the health and societal cost far more than the actual cost of the treatment. Treatment is also much less expensive than other options. Outcomes of drug treatment programs are dependent on adequate treatment length and drop out is one of the major problems in the current treatment program. It is therefore critical to optimize drug dosing regimens in patients to improve adherence and success of the treatment programs. In 2012, an estimated 400,000 adults aged 18 or older were current illicit drug users. Many of these were pregnant women. In the most recent National Survey on Drug Use and Health (2012), 6% of pregnant women reported using illicit substances within the past 30 days¹. Azadi et al found that 3% of women were urine-positive for illicit opioids at the time of delivery². Currently, the standard of care for an opioid-dependent pregnant woman in most institutions is methadone, however buprenorphine (BUP) is also used for this indication as it is FDA-approved for opioid addiction although not specifically for pregnant women. There has been increasing evidence that BUP may have comparable efficacy to methadone, and may have fewer severe neonatal complications especially neonatal abstinence syndrome (NAS). In a recent study, BUP-exposed, as opposed to methadone exposed, neonates required significantly less morphine for treatment of NAS, had significantly shorter duration of treatment for NAS and had significantly shorter hospital stays³. Methadone, a full μ agonist, has significant adverse reactions such as Q-T prolongation, potential for life-threatening respiratory depression and extreme sedation⁴⁻⁶. Conversion from illicit narcotic use to methadone commonly requires hospitalization for 3-4 days as the dose of methadone is titrated upward based on the patient's symptomatology^{4,5}. Upon hospital discharge methadone treated patients receive daily dosing of medication at outpatient clinics that monitor the patients for continued illicit drug use. This requirement is extremely burdensome on the patients since many do not have transportation and they must arrive at the clinics in the morning. BUP, a partial μ agonist, has decreased severity of adverse reactions and therefore physicians are able to convert patients on an outpatient basis. The patient is given a 1-4 week supply of oral medication thus eliminating the burdensome task of attending daily clinics for drug administration. These benefits of BUP are changing the management of drug addiction dramatically and more institutions will turn from in-patient treatment with methadone to the outpatient strategy with BUP. The American College of Obstetricians and Gynecologists in its 2012 Committee Opinion indicates that the literature "supports the use of buprenorphine as first line treatment for pregnant opioid –dependent women"⁷. Similarly the American Society of Addiction Medicine supports the use of buprenorphine in opioid- addicted pregnant women⁸ and the World Health Organization (WHO) which encourages availability of a buprenorphine option has described the worldwide availability of buprenorphine⁹. The dosing of BUP currently is based on studies in men and non-pregnant women and limited animal data that related plasma concentrations of BUP to the degree of saturation of the μ receptor in the brain¹⁰⁻¹³. These studies suggest that a dose of 16 mg daily saturates 95% of the μ receptors. The plasma half-life in non-pregnant subjects is approximately 22 hours and therefore a dose of 16 mg/day appears to be sufficient to saturate the receptors. The standard recommended dosing regimen for sublingual BUP is therefore 8 mg bid or 16 mg QD. Unlike for methadone, where increased dosing is recommended during pregnancy, currently there are no guidelines on dosing BUP in pregnant women. The dosing regimen used in non-pregnant subjects does not appear to be adequate for pregnant women. Dosing is adjusted to the patient's symptomatology using the Clinical Opiate Withdrawal (COW) score, some of which is subjective and some based on objective physiological parameters. The failure rate for BUP is highest (33%) during the induction period i.e. the time from stopping illicit drug use to the point of achieving a stable dose of BUP³. This suggests that the current treatment regimens for BUP are inadequate for many women. Indeed at Magee-Womens Hospital, using COW scores to determine a dosing regimen has resulted in many women requiring doses higher than the 16 mg daily dose used in non-pregnant subjects. The high failure rate of BUP during induction and the experience at Magee are

consistent and point to the urgent need for pregnancy –specific pharmacokinetic studies of BUP. It is expected that dosing would be different in pregnancy given the dramatic physiological changes characteristic of pregnancy. This includes changes in salivary pH (which affects dissolution and absorption of a sublingual BUP), the change in activity of many cytochrome P450 (CYP) and conjugating enzymes [BUP is metabolized by several phase 1 (CYP3A and CYP2C8 and CYP2C19 and phase 2 (UGT1 and UGT2) enzymes] and changes in renal clearance of medications (particularly relevant for conjugated metabolites)⁴. Despite this anticipated need for dosing adjustment during pregnancy, only one clinical case report study has evaluated the plasma concentrations of BUP after administration of sublingual tablets during pregnancy but only three pregnant women were included¹⁴. Obviously, more subjects should be evaluated to define the pharmacokinetic parameters of sublingual BUP and its metabolites and to make proper dosing recommendations for pregnant women. The critical barrier to optimal prenatal care for opioid addicted women is proper dosing of BUP. Drug addicted pregnant women are at great risk of pregnancy complications and they generally avoid prenatal care because of their addiction issues. Obstetric health care providers are not generally knowledgeable in the area of drug abuse and are frustrated by the common demand by these patients for more medication which is commonly felt by the provider as unnecessary or excessive. A treatment program that controls drug craving will enhance patient compliance and improve prenatal care. Treatment strategies such as methadone which requires hospitalization and daily clinic visits are doomed to fail for many pregnant women. BUP offers the patient a treatment option that is not onerous but if that treatment is inadequate, those women will abandon treatment and seek relief elsewhere i.e. ‘the street’. Optimizing the dosing regimen of BUP will overcome a key barrier to the provision of quality health care for these high-risk women. Our expectation is that higher doses of BUP are needed by pregnant women to achieve the desired plasma concentration that suppresses cravings. By establishing a dosing regimen that is based on the pharmacology of BUP in pregnancy, health care providers will be more willing to utilize higher doses which should reduce the mother’s desire to seek illegal drugs elsewhere. This change will keep the women in the health care system and improve maternal and neonatal outcomes such as NAS. Compliant drug-addicted women in a health care system will be less like to have her baby removed from her care by Children and Youth Services organizations. The likelihood that this pregnant woman does not relapse to use of illegal street drugs is enhanced if she has demonstrated compliance during pregnancy and is on a program that provides her BUP after pregnancy is completed. Our study will also evaluate the appropriate dose reduction that may be necessary in patients postpartum and minimize occurrence of side effects in these patients and their breast fed babies if they are maintained on doses used during pregnancy. The impact of these changes on society would be substantial.

B. Innovation

Our proposed project challenges the current clinical practice paradigm that defines a dose of BUP for pregnant women that is based on pharmacokinetics in men and non- pregnant women and relies on the COW score to titrate the dose. We will define the pharmacokinetics and pharmacodynamics of BUP in early and late pregnancy and the postpartum period and determine what contributes to the variation in plasma concentrations and response. We will also relate plasma concentrations of BUP and its major metabolites to physiologic parameters that can be used to gauge the amount of drug in mother’s plasma. Our expectation is that we will demonstrate that higher doses of BUP are needed throughout pregnancy and that the dosing regimen can be adjusted using patient covariates and biophysical measurements in addition to the COW scores. Our exploration of pupillometry and the galvanic skin response to gauge maternal BUP satiety in pregnancy is unique and challenges contemporary practice¹⁵⁻¹⁷. There is a strong relationship between plasma BUP concentrations and the saturation of brain μ receptors¹². If μ receptor occupancy is a major determinant of maternal satiety and drug-seeking behavior, then plasma concentrations of BUP and its metabolites are key in achieving these outcomes. Any variation in dosage requirements cannot be attributed to differences in blood brain barrier permeability but may rather reflect differences in absorption or metabolism or elimination of BUP or its metabolites. Therefore, to optimize therapy a direct or indirect measure of plasma BUP is needed.

Measurement of BUP concentrations in plasma requires high sensitivity equipment and therefore not likely to be useful in clinical practice. An indirect non-invasive measure of BUP plasma concentrations is appealing due to convenience, low cost and rapidly available results. COW scores are now the standard by which buprenorphine is dosed but they are flawed as half the parameters are subjective and can be manipulated by the patient. A purely physiological endpoint is less likely to be controlled by the patient. We will evaluate several maternal and newborn variables that might be predictive of NAS. Our approach is unique in that we will have full pharmacokinetic characterization of the mother as well as hair samples and placental P-gp status to enable a much more complete assessment of predictive factors in combination that have previously been reported singly.

C. Specific Aims

We will define the pharmacokinetics of buprenorphine and determine if there is a better way to gauge dosing based on objective, physiological parameters of satiety. We will also determine which maternal, placental or fetal factors impact the risk of Neonatal Abstinence Syndrome (NAS) and will define neonatal exposure to buprenorphine through breast milk.

Our specific aims are:

1. To determine the impact of pregnancy on the pharmacokinetics of buprenorphine (BUP) and its metabolites after sublingual administration.
2. To determine the impact of patient covariates such as age, race, co-medications and single nucleotide polymorphisms of BUP metabolizing enzymes (CYP3A4/5; UGT) and transporters (P-glycoprotein) on the variability in exposure of BUP and its metabolites in pregnant women.
3. To evaluate potential pharmacodynamic endpoints for dosing sublingual BUP during pregnancy and the non-pregnant state.
4. To evaluate the relationship between maternal and umbilical cord BUP and its metabolite concentrations.
5. To evaluate the relationship between maternal, placental and neonatal covariates such as concentrations of BUP and its metabolites in maternal and umbilical cord blood and maternal and newborn hair to NAS severity and duration.
6. To evaluate potential infant exposure to BUP and metabolites through breast milk.

We anticipate that the currently recommended dose of buprenorphine will be insufficient in many women to satisfy their drug cravings. Although some part of this craving may be psychological rather than physiologic, we anticipate that alterations in physiology due to pregnancy may result in more rapid metabolism and elimination of this medication and thus provide a level of medication that is sub-therapeutic. We also anticipate that the poor relationship between NAS and maternal dosage of buprenorphine may in part be explained by maternal or placental factors which impact fetal exposure.

D. Approach - Overall Strategy Methodology and Analysis

D.1. Study Overview

We will enroll eligible, consenting pregnant women who are receiving sublingual BUP (subutex) in a supervised hospital or outpatient clinical setting because of their opiate addiction. Due to the possibility of limited subject availability at individual centers, recruitment can target subjects receiving pregnancy care at other institutions. Likewise, since buprenorphine is commonly not dispensed in a hospital setting, subjects may need to be recruited from clinics where buprenorphine is provided to pregnant women as part of a structured maintenance program. To facilitate enrollment, recruitment can target one or more specific aims as summarized below.

For Specific Aims 1 and 3:

We will recruit 40 eligible, consenting pregnant women to an 'extensive sampling' pharmacokinetic (PK) study in which they will be studied three times: once between 8 0/7-20 6/7, once between 21 0/7-35 6/7 and once at 4-6 weeks post-delivery. Studies done during pregnancy should be at least 12 weeks apart. We will also obtain patient demographics and medical history.

We will encourage subjects to complete as many PK studies as possible; however, subjects are only required to complete one PK study in order to participate in this portion of the study. Each study is 13 hours in length for BID dosing, 9 hours for TID dosing, 7 hours for QID dosing, and 6 hours for five times daily dosing.

Each PK study will be performed after the subject has been on a stable dose of BUP for a minimum of 7 days. Physiologic testing (pupillometry, galvanic skin response, and heart rate), COW and craving scores will be performed before the daily dose of BUP is taken.

Measurement Type	Dosing	Length of Study	No. of Measurements
Blood (BUP/metabolite)	BID	13 hrs	Baseline + 12 additional
Physiologic			Baseline + 9 additional
Blood (BUP/metabolite)	TID	9 hrs	Baseline + 12 additional
Physiologic			Baseline + 9 additional
Blood (BUP/metabolite)	QID	7 hrs	Baseline + 10 additional
Physiologic			Baseline + 7 additional
Blood (BUP/metabolite)	5 times/daily	6 hrs	Baseline + 10 additional
Physiologic			Baseline + 7 additional

Maternal cotinine and catecholamines will also be measured in the blood and related to the physiologic variables and to maternal craving scores. A maternal hair sample will be obtained around the time of delivery when possible for determination of buprenorphine and metabolite content. Pharmacokinetic modeling will be performed for both the parent compound and its major metabolites. We will determine the impact of the CYP and UGT SNPs on plasma and umbilical cord blood concentrations of BUP and its metabolites.

We will also seek to enroll subjects in an elective ancillary study to determine how rapidly the metabolizing enzyme activity changes after delivery, For those agreeing, we will collect 1-2 maternal blood samples at least 2 days apart while the patient is in the hospital, We will continue to collect weekly maternal blood samples during the subject's routine visits to the MWH BUP clinic for 4 weeks.

For Specific Aims 2, 4 and 5:

Blood will be obtained and genotyping of enzymes responsible for the metabolism of BUP (CYP3A and UGT) will be performed. Given that norbuprenorphine is a substrate for P-glycoprotein (P-gp), genotyping for Pgp will also be performed. We will also evaluate peripheral blood mononuclear cells (PBMCs) as a surrogate marker of metabolic enzymes and transporters. The expression of drug metabolizing enzymes and transporters in the PBMCs will be correlated with the plasma concentrations of BUP and its major metabolites.

Those women who do not participate in the PK study described in the section above (for specific aims 1 and 3) will be consented to participate in some or all of the other components of the study. In those women, we will obtain a blood specimen at the time of consent and record the time of blood draw, the time of the last dose administration, and the dosing history. Subjects recruited to the other components of the study will not reduce the sample size requirements (n=40) for the parent trial but will be used to more precisely model BUP pharmacokinetics.

The following applies to all enrolled women whether or not they are participating in the PK study.

- A maternal blood sample will be obtained from the mother prior to delivery. A portion of the placenta, umbilical cord, and umbilical cord blood will be collected at delivery and immediately frozen.
- When possible, a small quantity of maternal and newborn hair will be removed from the scalp as close to delivery as possible for determination of buprenorphine and metabolites.
- In the nursery, the infant will undergo standard newborn testing for signs of neonatal withdrawal (Neonatal Abstinence Syndrome, NAS). The duration and intensity of treatment will be recorded.

Maternal and umbilical cord blood and maternal and infant hair concentrations of BUP and its major metabolites will be determined by mass spectrometry. We will determine maternal cord blood concentration ratios and hair concentrations of buprenorphine and related concentrations to the severity of neonatal withdrawal symptoms.

For Specific Aim 6: Subjects who are breastfeeding and undergoing the postpartum pharmacokinetic study will also be asked to provide breast milk samples for estimation of total amount of BUP and its metabolites secreted. On this PK study day, subjects will be asked to pump the breast just prior to taking the study dose of BUP. Subjects will then take the BUP dose. Approximately every 2-4 hrs. after BUP administration, breast milk will be pumped using a breast pump and total volume measured. An aliquot of at least 1 ml but no more than 5 ml will be saved for analysis of BUP and its metabolites, and the rest of the milk will be returned to the mother for feeding the baby. In those women consenting, we will also obtain one heel stick blood sample prior to a feeding and another sample 1 hour after the feeding. Optimally, this will be done during the feeding that is as close as possible to the mother's BUP dose.

We will also perform another elective ancillary study while the baby is hospitalized or during routine postpartum PRC appointments to evaluate the time of appearance of BUP in breast milk and to assess how much drug gets into the baby's blood. We will study postpartum women who are

breast feeding and will request 1-3 samples (at least 1 ml but no more than 5 ml each) of the mother's milk for analysis of BUP. We will request 2 infant heel stick samples with each breast milk collection. One heel stick sample will be collected prior to a breast feeding (trough level) and another one 1 hour after the feeding (peak level).

D.2. Primary Research Question

The primary research questions are whether BUP and metabolite exposure (reflected as the dose-adjusted AUC) differs during pregnancy and between pregnancy and the postpartum state.

D.3. Secondary Research Questions

This study will also attempt to address the following questions recognizing that an inadequate sample size may be available for some of these outcomes:

1. How quickly do buprenorphine trough concentrations decrease after delivery?
2. What maternal covariates impact the plasma concentration of BUP and its metabolites?
3. Is there a pharmacodynamic parameter that reflects maternal satiety and withdrawal?
4. What is the relationship between maternal BUP and metabolite concentrations and cord blood concentrations and NAS risk?
5. What is the infant's exposure to BUP through breast milk?
6. How quickly in the postpartum period does BUP appear in breast milk?
7. How well does cotinine concentration relate to smoking reported by the subject on the smoking questionnaire?
8. Does smoking modify the relationship between physiologic measures and plasma buprenorphine concentrations?
9. Does smoking impact the risk of NAS?
10. How well do catecholamine concentrations reflect physiologic parameters and satiety?

D.4. Study Design

This will be a multicenter pharmacokinetic (PK) and pharmacodynamic (PD) study of sublingual BUP in pregnant women on the medication for their drug addiction. There are several parts to this study and subjects may participate in one or more parts, as described below.

For subjects that choose to participate in procedures other than the PK studies (Parts B-E), only minimal testing is required prior to coming to the hospital for delivery. Blood samples for BUP determination and DNA (deoxyribonucleic acid) analysis will be obtained at the time of consent.

Part A: Subjects will participate in PK studies that will occur at 2-3 different times during the study: 1) between 8 and 20 weeks of pregnancy; 2) between 21 and 35 weeks of pregnancy; and 3) between 4 to 6 weeks postpartum. There will be a minimum of 12 weeks between PK visits during pregnancy. Subjects must complete at least one PK visit in order to participate in this portion of the study.

Part B: Prior to delivery, we will collect maternal blood samples. At the time of delivery, we will collect a cord blood sample, a 3 x 3 cm placental sample, and a 3 inch umbilical cord sample.

Part C: Hair samples from the mother and baby collected within 3 days after delivery

Part D: While the baby is hospitalized, we will request 1-3 samples (at least 1 ml but no more than 5 ml each) of the mother's breast milk for analysis of BUP. We will collect 2 infant heel stick samples with each breast milk collection. One heel stick sample will be collected prior to a breast feeding (trough level) and another one 1 hour after the feeding (peak level).

Alternatively, subjects may also elect to participate in Part D during 1-3 routine postpartum PRC appointments, as long as they are still taking Buprenorphine (Subutex or Suboxone). The research staff will work with the patient to coordinate the sample collection with the timing of the patient's last BUP dose.

During each visit, we will request an infant heel stick sample prior to a breast feeding (trough level). The subject will then collect a breast milk sample. We will reserve a sample of at least 1 ml but not more than 5 ml for analysis of BUP and return the remainder to the mother for her infant. We will then collect a second infant heel stick sample 1 hour after the feeding (peak level). Ideally, we will collect both breast milk and heel stick samples during each collection; however, it is acceptable to collect only breast milk samples if the mother does not consent to the collection of heel stick samples.

Part E: We will collect 1-2 maternal blood samples while the patient is in the hospital, at least 2 days apart. We will continue to collect maternal blood samples during the subject's routine visits to the MWH BUP clinic weekly for the next 4 weeks. The time of the blood draw, the time of the last dose administration, and the dosing history will also be recorded.

D.4.i. Entry criteria include:

1. Age between 18 – 45 years
2. Currently on a stable two, three, four, or five times daily dose of sublingual BUP. Subjects participating in Part D during postpartum PRC visits must be taking Subutex or Suboxone.
3. Gestational age < 19 6/7 weeks *
4. Singleton gestation
5. Able to give informed consent and undergo study procedures
6. Willing to have urine samples screened for the presence of alcohol, barbiturates, opiates, cocaine (or metabolites), benzodiazepines, synthetic opioids and PCP *
7. Willing to participate in at least one PK study either during pregnancy or in postpartum *

*Requirement applies only to those subjects in the PK study

D.4.ii. Exclusion Criteria include:

1. Major fetal anomalies or malformations
2. HIV or AIDS
3. Comorbid dependence on benzodiazepines or other central nervous system depressants (including anti-seizure medications)
4. Taking medication known to interfere with buprenorphine metabolism
5. Active or chronic suicidal or homicidal ideation or attempts
6. Elevated liver enzymes (AST, ALT > 2 times normal) *
7. Creatinine > 1.5 mg/dl *
8. Part A (PK Studies): Delivery at other institution where outcome data cannot be obtained on mother and baby
Parts B-E (Procedures other than the PK Study): Delivery at another institution where samples cannot be obtained on mother and baby

9. Active use of non-prescribed opiates/opioids detected during the urine drug screen performed within 1 week prior to each PK visit *
10. Hematocrit <28 *

* Requirement applies only to those subjects in the PK study

D.4.iii. Gestational Age Determination:

A project gestational age will be established for all subjects prior to study initiation. We will use criteria used by the Maternal-Fetal Medicine Units for their clinical trials (see MOO).

D.4.iv. Informed Consent Criteria:

Written informed consent will be obtained before entry into the trial. Full disclosure of the nature and potential risks of participating in the trial will be made. Each center will develop its own consent form according to the requirements of its Institutional Review Board. Women who are not fluent in English will be enrolled by a person fluent in their language and both verbal and written informed consent obtained in that language; if such are not available, they will not be included.

D.5. Study Procedures

D.5.i. Screening for Eligibility:

The inclusion/exclusion criteria will be reviewed with the patient's chart. Any woman who appears to be eligible will be informed about the study and asked to sign a medical records release so that the medical records may be obtained. If a patient appears to meet the criteria for enrollment and expresses interest in the study, she will be told about the study and asked to sign the informed consent form. A copy of the signed consent form will be provided to the patient.

D.5.ii. Screening Visit Procedures

1. PK Studies:

The information listed below will be obtained for consented patients within 1-2 weeks prior to scheduling a PK study:

- a. Demographic information: age, race, weight, height
- b. Medical history: medical disorders, HIV, mental health disorders
- c. Obstetrical history including outcome of all prior pregnancies
- d. Social history: questionnaires regarding alcohol use, tobacco use, illicit drug use and depression
- e. Concomitant medications
- f. Project gestational age and estimated date of delivery.
- g. Vital signs (blood pressure, heart rate, respiratory rate)
- h. Screening blood chemistries to confirm eligibility include: chemistry (blood urea nitrogen [BUN], creatinine, total protein, alanine aminotransferase [ALT/SGPT], aspartate aminotransferase [AST/SGOT], hemoglobin [Hgb], platelet, albumin).*
- i. A hematocrit [Hct] is required at the first screening visit, unless a result is available from the subject's medical record within the past 4 weeks. If the Hct is within normal limits at the first screening visit, it will not be repeated during the remainder of the study.
- j. Urine sample will be collected and analyzed for drugs of abuse.

- k. Blood sample for BUP analysis - record the time of the blood draw, the time of the last dose administration, and the dosing history *
- l. Blood sample for DNA analysis - If the DNA sample is unable to be obtained at the screening visit, it may be collected anytime during the study when blood is being drawn. *
- m. Review procedures for the upcoming study visit.

**Performed at the subject's first PK study only*

If information was collected as part of a previous Screening Visit, some items above (a, b, c, e and f) will require updating only, if applicable.

2. Procedures other than the PK Studies:

The information listed below will be obtained at the time of consent from women agreeing to other parts of the study but not participating in the PK study.

- a. Demographic information: age, race, weight, height
- b. Medical history: medical disorders, HIV, mental health disorders
- c. Obstetrical history including outcome of all prior pregnancies
- d. Social history: questionnaires regarding alcohol use, tobacco use, illicit drug use, and depression
- e. Concomitant medications
- f. Project gestational age and estimated date of delivery.
- g. Blood sample for BUP analysis (record the time of the blood draw, the time of the last dose administration, and the dosing history).
- h. Blood sample for DNA analysis (If the DNA sample is unable to be obtained at the screening visit, it may be collected anytime during the study when blood is being drawn.)

D.5.iii. Patient Management:

No attempt will be made to mandate clinical management of the subjects. If complications arise, the patient will still be eligible for additional PK studies unless the complication affects the pharmacology of BUP i.e. significant liver or renal disease.

If a patient who consented to any part of this study (Parts A-E) is dismissed from her Medication Assisted Treatment (MAT) clinic due to non-compliance, she will be dismissed from the study and another subject will be recruited to replace her.

If non-prescribed opiates/opioids are detected during any PK screening visits, the subject will be ineligible to complete the pending PK visit. Subjects may be re-screened at a later date as long as they are within the gestational window for the PK visit. If a subject in the PK study has two positive drug screens for non-prescribed opiates/opioids during any screening visits, she will be dismissed from the study and replaced with another study subject.

At the time of enrollment, patients will consent to those components of the study that they wish to participate in. Subjects are required to complete at least one PK study in order to participate in this portion of the study. If a subject consents to portion of the study and they are unable to complete it within the acceptable timeline, they will still be eligible to participate in the remaining

parts of the study they consented to.

D.5.iv. Study PK Visits:

Once the subject has signed the consent form, completed the Screening Visit, and is deemed eligible for the study, the research staff will establish a schedule for the subject to undergo their first PK study. Subjects participating in the PK study must complete at least one PK study.

Screening Visits will be conducted within 1-2 weeks prior to each PK study and subjects must continue to meet the criteria listed in section D.4, with the exception of exclusion criteria items 6 and 7. A urine drug screen (Immunoassay) will be performed on the day of the PK study.

Each PK study, both during and after pregnancy, will be performed after the subject has been on a consistent two, three, four, or five time daily dose of BUP for a minimum of 7 days.

D.5.v. BUP Administration During PK Visit:

Each subject will bring her own prescribed medication to the Clinical Research Center (CRC). The subject will be on a stable dose of BUP two, three, four, or five doses daily which will be supplied by her health care provider or a Drug Addiction clinic. She will use her own medication during the entire study and the research staff will not provide any BUP at any time during the study. We are only studying women on two, three, four, or five times daily medication to minimize time in the CRC and to reduce the number of blood samples to be drawn.

After all baseline procedures, including performance of the physiologic testing and collection of biological specimens, the patient will be instructed to take her usual prescribed dose of BUP. Prior to taking her medication, the subject will fill her mouth with saliva and then swallow it twice to help ensure that the saliva flows well. Then, the subject will place some saliva onto pH paper and the pH of the saliva will be recorded. A saliva (1-2 mL) sample also will be obtained analyzed for buprenorphine and metabolites. The subject will then take her prescribed morning dose of BUP sublingual tablets. BUP tablets are supplied in two dosage strengths (2mg and 8mg). The drug will be taken sublingually and patients will be asked to hold the tablet(s) under the tongue for at least five minutes without swallowing to allow for full disintegration of the tablet(s). The sublingual area will be examined at the end of five minutes, and any premature swallowing will be recorded, along with the time it occurred following administration. If pieces or residues of the tablet(s) are still present after five minutes, the subject will be asked to hold the tablet(s) for an additional five minutes without swallowing. Subjects will be asked to abstain from smoking, drinking (except for water), and eating for two hours following drug administration to ensure maximum absorption from the sublingual site and the gastrointestinal tract. At two hours post-dose, the subject will be provided with a light meal. Smoking is then allowed.

D.5.vi. Venous Blood, Urine, Saliva and Postpartum Breast Milk Collection (Applicable to Subjects in the PK Study):

On the day of the PK study, a venous catheter will be inserted and used for frequent blood draws, whenever possible. Multiple venipunctures may be necessary if the patient has poor venous access and the blood sample(s) cannot be obtained through the IV.

Baseline blood samples (12 mL), saliva (1-2 mL) and urine will be collected and analyzed for buprenorphine and metabolites. We will perform a urine drug screen by immunoassay for drugs of abuse. Blood will also be utilized for determination of catecholamines (epinephrine and nor epinephrine), cotinine, and PBMC analysis. Screening blood chemistries and hematology labs

(AST/SGOT, ALT/SGPT, creatinine, BUN, total protein, albumin, hemoglobin, and platelets) are collected at the first Screening Visit only to establish eligibility. A hematocrit is also required at the first screening visit, unless a result is available from the subject's medical record within the past 4 weeks. If the hematocrit is within normal limits at the first screening visit, it will not be repeated during the remainder of the study. Baseline physiologic measurements (pupillometry, galvanic skin response, blood pressure, and heart rate) will be taken and the subject will subsequently take her medication as prescribed.

Additional blood and saliva samples will be collected at the following time intervals. The maximum amount of blood drawn from a subject on a study day is 103 mL.

Sample/Measurement Type	Dosing	Time Interval
blood & saliva	BID	Baseline and 10, 20, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720 minutes post dose
blood & saliva	TID	Baseline and 10, 20, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480 minutes post dose
blood & saliva	QID	Baseline and 10, 20, 30, 60, 90, 120, 180, 240, 300, 360 minutes post dose
blood & saliva	5 times/day	Baseline and 10, 20, 30, 60, 90, 120, 150, 180, 240, and 300 minutes post dose

Refer to section D.5.xi for blood and saliva collection and processing instructions.

All urine voided from 0-12 hrs. (subject will be asked to empty bladder at 12 hrs for BID dosing, at 8 hours for TID dosing, at 6 hours for QID dosing, and at 5 hours for five times daily dosing) will be collected, total volume recorded and aliquots will be frozen immediately.

For those women who are breast-feeding during the postpartum PK visit and wishing to participate in the breast feeding component, and if the CRC allows infants to come to the unit with their mothers, we will obtain breast milk at baseline prior to the morning BUP dose and approximately every 2-4 hours post dosing using an automated breast pump to empty the breasts. We will use at least 1 mL but not more than 5 ml of the milk for analysis of drug and metabolite concentrations and allow the mother to use the remaining milk for her infant. If these women participated in the post delivery ancillary study of breast milk, they can still participate in the postpartum PK breast milk study.

For those women consenting to the postpartum PK study, we will obtain infant blood by heel stick once prior to a feeding and again 1 hour after the feeding. Optimally this will be done during the feeding that is as close to the BUP dose as possible. In total, no baby will have more than 8 heel sticks, 6 while in the hospital or during a postpartum PRC visit as part of the postpartum ancillary study and 2 in the postpartum PK study. If the subject is not participating in the BUP PK study, the baby will have up to a maximum of 6 heel sticks (trough and peak levels on 3 occasions) as part of the postpartum ancillary study.

Upon completion of all biological fluids collection, the venous catheter will be removed and the subject will be discharged to home.

D.5.vii. Physiological Testing (Applicable to Subjects in the PK Study):

Pupillary diameter will be determined using a hand held pupillometer device (PLR-200™ Pupillometer- Neuroptics Irvine Cal.), galvanic skin response will be obtained using the Mind Ware Mobile Impedance Device, heart rate and blood pressure will be recorded by standard means. The Cravings and COW scores will be performed in conjunction with physiologic assessments at the following times:

Sample/Measurement Type	Dosing	Time Interval
physiologic	BID	Baseline and 30, 60, 90, 120, 240, 360, 480, 600, 720 minutes post dose
physiologic	TID	Baseline and 30, 60, 90, 120, 240, 300, 360, 420, 480 minutes post dose
physiologic	QID	Baseline and 30, 60, 90, 120, 240, 300, 360 minutes post dose
physiologic	5 times/day	Baseline and 30, 60, 90, 120, 180, 240, 300, minutes post dose

D.5.viii. Dietary Restrictions (Applicable to Subjects in the PK Study):

Subjects will be required to fast (other than water) after midnight prior to the study visit, and for two hours after the study drug is administered. Participants may not consume grapefruit juice within 3 days prior to the study visit. Two to three hours after the subject takes her morning BUP dose, subjects may receive a meal, and then will continue to receive meals in the CTRC or MWH for the duration of the study visit with snacks as requested. Subjects will be encouraged to consume at least 100 mL of water per hour in order to maintain adequate urine flow.

D.5.ix. Delivery Specimens (Applicable to all Subjects):

A urine sample will be collected for a comprehensive drug screen within 48 hours of admission for delivery. Samples from subjects with a positive drug screen at delivery will not be used for specific aim 5 of the study.

Prior to delivery, maternal blood specimens (for BUP and PBMC analysis) will be collected, when possible.

At the time of delivery, a cord blood specimen, a placenta sample (3x3 cm), and an umbilical cord sample (3 inches) will be collected, when possible. The umbilical cord and placenta samples are immediately frozen at -80 degrees. Refer to section D.5.xi below for maternal and cord blood sample collection and processing instructions.

Additionally, a sample of maternal hair will be collected by research staff from an inconspicuous part of the scalp, as close to delivery as possible, but not to exceed 3 days post-delivery.

Labor and Delivery biological specimens (including the urine drug screen) will be collected when possible.

D.5.x. Neonatal Evaluation and Care (Applicable to all Subjects):

All infants of subjects in this trial will be evaluated for Neonate Abstinence Syndrome according to study criteria. Treatment of withdrawal will be standardized to the degree possible. At Magee the assessment for symptoms of withdrawal is performed every 2-4 hours with the Finnegan Scoring system. Morphine is initiated as the drug of choice for infants with opioid exposure when the averages of three consecutive scores are ≥ 8 , according to the scoring system and treatment plan at Magee.

When possible, a sample of newborn scalp hair will be obtained, as close to delivery as possible, but not to exceed 3 days post-delivery.

D.5.xi. Blood and Saliva Collection and Processing:

The following collection and processing instructions apply to all blood and saliva samples collected during this study. The samples collected will be dependent on which parts of the study the subject participates in.

Blood samples will be collected as follows:

- Maternal and Cord Blood Samples for BUP Analysis: One 10 mL heparinized vacutainer tube will be drawn at the time points described in sections D.5.vi and D.5.ix. The tube should be centrifuged at 1500 g at 4°C for 15 minutes. Plasma samples will then be transferred into polypropylene tubes and frozen at -80 degrees centigrade until analysis.
- PBMC analysis: One 5 mL EDTA vacutainer tube will be drawn with the first sample (pre-dose) at each PK study visit and at delivery (applicable to all subjects), when possible. The sample should be stored at room temperature. Notify Dr. Venkataramanan's lab in advance at 412-400-7027 / 412-706-0085 or via email (rv@pitt.edu) to allow for pick up and processing within 4 hours of collection.
- DNA Analysis: One 10 mL EDTA vacutainer tube will be drawn at the Baseline Screening Visit. If the DNA sample is unable to be obtained at the screening visit, it may be collected anytime during the study when blood is being drawn. The sample should be frozen at -80 degrees centigrade until analysis.

Saliva samples will be collected using a Sarstedt Salivette collection device. The salivary collection sponge can be centrifuged later in the day. Salivary pH will be determined. Oral fluid samples will then be transferred into polypropylene tubes and frozen at -80 degrees centigrade until analysis.

D.5.xii. Study Outcome Measures and Ascertainment (Applicable to all Subjects):

Mothers and infants will be followed until they are discharged from the hospital, at which time relevant data from the labor, delivery and nursery records will be recorded.

a. Primary Outcome:

The primary outcome is the exposure to BUP (AUC) during pregnancy and the post-partum state.

b. Secondary Outcomes Maternal:

Other maternal outcomes to be measured are:

1. The relationship between COW and Craving scores, pupillometry, heart rate, blood pressure

- and GSR scores to the maternal plasma concentration of BUP and its major metabolites
2. The impact of polymorphisms of CYP 450 and conjugating enzymes of buprenorphine on plasma concentration of BUP and its major metabolites
 3. The relationship between the umbilical cord and maternal concentration of BUP and its major metabolites
 4. The relationship between maternal dose and concentration of buprenorphine and metabolites in umbilical cord, maternal and infant hair
 5. The relationship between maternal plasma and breast milk concentrations of BUP and major metabolites
 6. Cumulative amount of BUP and its metabolites excreted in breast milk.

c. Secondary Outcomes Neonatal:

Neonatal outcomes to be measured include the following:

1. Relationship between concentrations of BUP and its metabolites in maternal and umbilical cord plasma and maternal and newborn hair and maternal dose and parameters of neonatal withdrawal
2. Infant hospital days
3. Birthweight
4. Gestational age
5. Neonatal Complications including: Respiratory distress syndrome (RDS), stillbirth, neonatal death, intraventricular hemorrhage as determined by cranial ultrasound, bronchopulmonary dysplasia, necrotizing enterocolitis, early onset of neonatal sepsis, seizures, retinopathy of prematurity (ROP), and hyperbilirubinemia.

D.5.xiii. Statistical Considerations

Data Analysis Plan:

The primary outcomes will be the area under the plasma concentration x time curve (AUC) of BUP during early pregnancy compared to the AUC in late pregnancy. We will also compare the AUC of BUP and metabolites during early and late pregnancy and the post-partum period. Mixed-effect models, repeated measures ANOVA or paired t-testing, will be used to compare early to late-pregnancy and overall pregnancy values to the postpartum values. Secondary outcomes such as metabolite concentrations will be analyzed in the same fashion. Additional analysis will also include multiple regressions to evaluate the relationships between maternal and fetal plasma concentrations of BUP and its metabolites, placental p-glycoprotein, physiologic parameters and CYP3A4/5 expression. We will also use population pharmacokinetic analysis to evaluate the contribution of various covariates (i.e. maternal genotype, BMI, etc.) to the observed variability in the trough plasma concentrations of BUP.

Primary Hypothesis

Based on data from our recently completed study, the dose-adjusted AUC was 2.34 ± 1.82 (ng/ml).hr in pregnant women at a mean gestational age of 34 weeks. In postpartum women, the dose adjusted AUC was nearly 100% higher at 4.0 ± 2.8 (ng/ml).hr. We anticipate that the AUC in the first half of pregnancy will be somewhere between values of non-pregnant women and those of pregnant women in the latter part of pregnancy. The sample size required to demonstrate a 50% difference between the dose adjusted AUC in the first half of pregnancy and the second half of pregnancy as well as a 50% difference between the dose adjusted AUC in the first half of pregnancy and the postpartum state is 37 ($\alpha = 0.05$, $\beta = 0.80$). We will recruit an additional 3 subjects to

account for dropouts or noncompliance. The secondary physiological outcomes will also be addressable with this sample size as the variance in pupil measurements is small (4.65 ± 0.4 mm at baseline and 2.64 ± 0.08 mm at 2 hours in the paper of Middleton et al¹³ and each patient will provide data from 2-3 PK studies. Thus there will be 120 studies that relate a physiologic variable to plasma buprenorphine. Specific Aim 5 which relates NAS to various parameters (maternal dose, maternal and cord plasma buprenorphine /metabolite concentrations etc.) can be addressed with the projected sample size as correlations between the dependent variable NAS and the numerous independent variables i.e. maternal dose, maternal concentration at delivery, maternal hair concentration, cord blood concentration, newborn hair concentration etc. can be performed with samples size of 15. We assume that 50 % of these infants will have some degree of NAS so the sample size of 40 should be sufficient. If the prevalence of NAS is lower, we can recruit additional subjects. Since the extensive PK studies will not be required to test this hypothesis, recruitment should be easier and can be done at secondary sites if needed.

Secondary Hypotheses

Patients will be at steady state when the pharmacokinetic studies are performed. This will be documented by comparing the concentration at time 0 and at 12 hrs. (less than 20% variation). Plasma concentration-time profiles will be analyzed using both non-compartmental and population pharmacokinetic modeling and simulation approaches. Data will be analyzed using WINNONLIN and NONMEM software. We will compare various pharmacokinetic parameters between pregnant and non-pregnant (postpartum) subjects using a paired t-test as described previously. This will include all non-compartmental data (C_{max} , T_{max} , $AUC(0-\tau)$, $AUC(0-\infty)$, k_e , λ_z , CL/F , CL_R , V_d/F , V_{ss} , $AUMC$, and MRT). Population pharmacokinetic model will be developed using nonlinear mixed effects modeling software (NONMEM version 7.1; ICON Development Solutions, Endicott City, MD), GNU Fortran 95 compiler and PLT tools (version 5.1.0). The general linear model under steady state will be constructed using ADVAN5 SS5 subroutines. In order to identify the covariates that influence the pharmacokinetics of BUP, the following covariates will be evaluated: maternal age (years), body weight (kg), race, gestational age, serum albumin, serum creatinine (mg/dL), salivary PH, buprenorphine dose, tablet size and co-medications and SNPs of the various metabolizing enzymes. For covariate selection, univariate analysis with stepwise forward addition ($p < 0.01$) and backward elimination ($p < 0.001$) procedures will be followed. The covariates that significantly influence the pharmacokinetics of BUP and its metabolite will be incorporated in the final model. The predictive performance of the model will be internally evaluated using prediction corrected-visual predictive check, where 1500 data sets will be simulated using the parameter estimates in the final model. The 50th percentile concentration (median) and the 5th and 95th percentile concentrations (90% prediction interval) will be plotted and compared to the observed concentrations. The data obtained will be used to validate the predictions of SIMCYP-PBPK simulation from the basic/translational project.

D.6. Preliminary Data Relevant to the Primary Outcome

We have developed a HPLC-MS-MS assay for BUP and metabolites with a lower limit of quantitation of the assay for BUP (BUP), of 0.05 ng/ml, while that for nor-BUP is 0.5 ng/ml. We have performed an intensive PK study on 12 subjects. The dose-adjusted AUC in the third trimester of pregnancy (2.4 ng/ml).hr was lower than in the postpartum period (4.0 ng/ml).hr while clearance is higher in pregnancy (589 L/hr) than in the postpartum period (288 L/hr). There was a large variation (cv = 50%) in both AUC and clearance values. None of the subjects in this cohort was studied in the first half of pregnancy.

D.7. Potential Problems, Alternative Strategies, Benchmarks

Recruitment is a potential problem in any clinical trial and if other sites in the OPPTB do not have access to patients on BUP in their own institutions they may have difficulty recruiting subjects. These patients have difficulty with compliance to drug treatment regimens so dropout may be higher than in most clinical trials. Postpartum studies are difficult because of childcare issues and this may hamper recruitment. These challenges can be overcome by identifying physicians and clinics that write prescriptions for BUP and care for such patients. The childcare issue can be addressed by financial incentives or by allowing the mother to bring her baby to the CRC during study days.

D.8. Feasibility

The sample size for this study is not large and the number of patients on BUP is increasing. We started a BUP clinic in July 2014 and as of mid-November we have 30 patients in the system. We estimate acceptance rates of 33% given time requirements. The other OPRC sites appear to have limited BUP patients delivering at their institutions which impact their ability to carry out all aspects of this study. Potential subjects however can be recruited at clinics that provide opioid substitution maintenance therapy to pregnant women. The CRC studies do not require the patient to deliver at the OPRC hospital. Likewise cord blood and maternal blood at delivery can be obtained for other institutions where these patients deliver. Follow-up of the infants in non-OPRC hospitals will be a challenge and therefore Hypothesis 5 may require that more infants be studied at Pittsburgh than the other sites. Given our increasing volume of BUP patients we should be able to recruit additional subjects at the Pittsburgh site if necessary. This study contributes to the primary aims of the OPRC as the issue of opioid substitution therapy is clinically very relevant and the lack of PK/PD information to provide clinical guidance begs for such studies to be done.

D.9. Sample Analysis and Collection of Negative Control Samples

Biological specimens will be collected and labeled with the study and subject number only and stored in locked freezers at MWH or the Magee Women Research Institute. Only the study research staff will have access to individually identifiable private information. Biological specimens will intermittently be sent to Dr. Venkataramanan's lab at the University of Pittsburgh for analysis. Samples sent for analysis will not contain any identifiable information. Patient demographics necessary for pharmacokinetic analysis will also be blinded and sent to Dr. Venkataramanan's lab.

Negative Control Samples: Biological samples from women who are not taking BUP are required as negative controls. Subjects potentially eligible as negative controls will be identified by reviewing the medical records of patients presenting for prenatal or postpartum care at private offices or the MFM offices at MWH. Patients will be approached by their care provider or a co-investigator about the study. Healthy volunteers serving as negative controls will be recruited prior to delivery or within 12 months after delivery. Subjects recruited as negative controls will be asked to sign a separate consent allowing for the collection of samples.

The negative control samples will be collected during pregnancy, delivery or postpartum, as requested by Dr. Venkataramanan's lab, and according to study procedures. The biological samples may include any of the following: urine, saliva, placenta, umbilical cord blood, umbilical cord tissue, maternal hair, infant hair, and breast milk.

D.9.i. Eligibility Criteria for Volunteers Serving as Negative Controls

Inclusion Criteria:

1. Age between 18 – 45 years
2. Current pregnancy or delivery within the last 12 months
3. Able to give informed consent and undergo study procedures

Exclusion Criteria:

1. Currently taking buprenorphine