Impact of Pregnancy on Buprenorphine Pharmacokinetics and Pharmacodynamics

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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:

<table>
<thead>
<tr>
<th>Version/Protocol Date</th>
<th>Revision Description</th>
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<tr>
<td>11 July 2016</td>
<td>Original version approved by UPITT IRB</td>
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| 14 Sept 2016          | - 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           | - 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           | - 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.  
|                       | - Removed the requirement for subjects to participate in the PK studies |
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 days. Azadi et al found that 3% of women were urine-positive for illicit opioids at the time of delivery. 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 stays. Methadone, a full μ agonist, has significant adverse reactions such as Q-T prolongation, potential for life-threatening respiratory depression and extreme sedation. 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. 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.” Similarly the American Society of Addiction Medicine supports the use of buprenorphine in opioid-addicted pregnant women and the World Health Organization (WHO) which encourages availability of a buprenorphine option has described the worldwide availability of buprenorphine. 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 brain. 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 BUP. 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 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). 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 included. 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 practice. There is a strong relationship between plasma BUP concentrations and
the saturation of brain μ receptors. 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

For Specific Aims 1, 2 and 3: We will recruit 40 eligible, consenting pregnant women who are receiving sublingual BUP (subutex) in a supervised hospital or outpatient clinical setting because of their opiate addiction. We will obtain patient demographics and medical history. 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. Each subject will undergo an ‘extensive sampling’ pharmacokinetic study 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. Subjects must complete, at a minimum, PK visit 1 (between 8 0/7-20 6/7 weeks) and the postpartum PK visit (4-6 weeks post-delivery) in order to remain in the study.

Each 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. Blood for measurement of buprenorphine and metabolite will be obtained 13 times over a 12 hour period while physiological measurements will be obtained at baseline and at 9 additional times after dosing for BID dosing. Blood for measurement of buprenorphine and metabolite will be obtained 13 times over an 8 hour period while physiological measurements will be obtained at baseline and at 9 additional times after dosing for TID dosing. Blood for measurement of buprenorphine and metabolite will be obtained 11 times over a 6 hour period while physiological measurements will be obtained at baseline and at 7 additional times after dosing for QID dosing. 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.

For Specific Aims 4 and 5: In addition to these pharmacokinetic studies, a blood and hair sample will be obtained from the mother at delivery and a portion of the placenta and umbilical cord will be taken and immediately frozen. While in the hospital we will seek to enroll subjects for an elective ancillary study to determine how rapidly plasma concentrations increase after delivery. For those agreeing, a predose (trough) maternal blood sample will be drawn once on the day prior to discharge and when feasible every week for 4 weeks at the routine postpartum visits to the buprenorphine clinic. 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. In addition when possible a small quantity of hair will be removed from the baby’s scalp as close to delivery as possible for determination of buprenorphine and metabolites. 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 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 4 hrs. after BUP administration, breast milk will be pumped using a breast pump and total volume measured. An aliquot of 5 ml will be saved for analysis of BUP and its metabolites, the rest of the milk will be returned to the mother for feeding the baby. In those agreeing, a heel stick blood sample will be obtained once in the neonate 3-4 hours after the mother has taken her morning BUP dose, just prior to the next feeding.

We will also perform another elective ancillary study to evaluate the time of appearance of buprenorphine in breast milk. We will approach postpartum women who are breast feeding and will likely be hospitalized with their baby for at least 5 days. Generally this means women delivered by cesarean section or mothers whose infants have NAS and who are receiving breast milk while in the NICU. We will ask for a 5ml sample of the mother’s milk and if feasible will obtain a baby heel stick after one of the breast milk feedings on day 3 and 5 of hospitalization.

Ideally, each center would perform all the component projects of the study to address all of the specific aims. 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 recruitment and enable all centers to participate in this study, recruitment can target one or more specific aims as summarized below.

Specific Aims 1, 2 and 3

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.**

These three specific aims require either an intensive PK study or a POPPK analysis. *Either can be performed on subjects not delivering at your center.* The intensive PK analysis will require the patient to be studied twice during pregnancy and once in the postpartum period. Each study is 13 hours in length for BID dosing, 9 hours for TID dosing, 7.5 hours for QID dosing, and 6 hours for five times daily dosing. The details are outlined in sections D.1. Study Overview. The POPPK analysis requires random blood samples during pregnancy and post-delivery. As few as 3 blood samples/subject will be useful but the more often the blood is obtained, the better. For the POPPK study all that is required beside demographic information and biochemical parameters (kidney and liver) is time of blood draw from the time of dose administration and the dosing history. Subjects recruited to the POPPK analysis will not reduce the sample size requirements (n=40) for the parent trial but will be used to more precisely model BUP pharmacokinetics.

Specific Aim 4. **To evaluate the relationship between maternal and umbilical cord BUP and its metabolite concentrations.**

This specific aim only requires a maternal and a cord blood sample at the time of delivery. Specimens can be collected at other institutions. Knowledge of mother’s dosing regimen, last dose
Specific Aim 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. This specific aim requires at a minimum a maternal blood and an umbilical cord blood and data on NAS from your or another nursery. When possible obtain a 3x3 cm piece of placenta that is to be frozen immediately and a small portion of hair from the mother’s and baby’s scalp.

Specific Aim 6. To evaluate potential infant exposure to BUP and metabolites through breast milk.
This specific aim requires recruitment of a woman on buprenorphine who will consent to a 13 hour CRC study for BID dosing, a 9 hour CRC study for TID dosing, and a 7.5 hour study for QID dosing in which blood is drawn and breast milk is collected periodically over the 13 hours, 9 hours, or 7.5 hours of study. The milk can be pumped from the breast at each time point, volume is measured, a sample taken for analysis and the rest given back for feeding the baby.

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. How quickly in the postpartum period does buprenorphine appear in breast milk
3. How well does cotinine concentration relate to smoking reported by the subject on the smoking questionnaire
4. Does smoking modify the relationship between physiologic measures and plasma buprenorphine concentrations
5. Does smoking impact the risk of NAS
6. 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:

Part 1: PK studies intended to measure the PK properties of buprenorphine during pregnancy and after delivery. These visits will occur at 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.

**Part 2:** At the time of delivery, we will collect maternal blood samples, cord blood sample, a 3 x 3 cm placental sample, and a 3 inch umbilical cord sample.

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

**Part 4:** If the baby has Neonatal Abstinence Syndrome (NAS) and is being breast fed, we will collect a sample of breast milk from the mother and heel stick blood samples from the baby on days 3 and 5 after delivery.

**Part 5:** Blood samples will be collected from mothers at the time of discharge and each week hereafter during their visits to the Pregnancy Recovery Center.

For subjects that choose to not participate in the PK studies, no special testing is required prior to coming to the hospital for delivery.

*D.4.1. 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
3. Gestational age < 19 0/7 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 2 PK/PD studies during pregnancy and 1 PK Study after pregnancy *

*Requirement applies only to those subjects in the PK study

*D.4.2. 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-see MOO)
4. Taking medication known to interfere with buprenorphine metabolism (see MOO)
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. Delivery at other institution where outcome data 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.4.3. 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 for Subjects Participating in the PK Study

A. Baseline Screening Visit:

The following information will be obtained for randomized patients within 1 week prior to scheduling the first PK visit (PK1):

1. Demographic information: age, race, weight, height
2. Medical history: medical disorders, HIV, mental health disorders
3. Obstetrical history including outcome of all prior pregnancies
4. Social history: alcohol use, tobacco use and illicit drug use
5. Concomitant medications
6. Project gestational age and estimated date of delivery.
7. 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], hematocrit [Hct], platelet, albumin).
   a. Hct tested only if not available from the subject's medical record within the past 4 weeks.
8. Urine sample will be collected and analyzed for drugs of abuse.
9. Review procedures for the upcoming study visit.

B. PK 2 and PK3 Screening Visits:

The following information will be obtained for randomized patients within 1 week prior to scheduling PK visit 2 (21-35 weeks of pregnancy) and PK visit 3 (4-6 weeks postpartum):

1. Measure and record vital signs: blood pressure, heart rate, respiratory rate
2. Obstetrical history updates: hospitalizations, etc.
4. Concomitant medication updates
5. Screening blood draw: hematocrit [Hct] – tested only if not available from the subject’s medical record within the past 4 weeks.
6. Urine sample will be collected and analyzed for drugs of abuse.
7. Review procedures for the upcoming study visit.

Questionnaires (depression, smoking, alcohol and drug use) will be completed by the subject at each Screening Visit as part of their social history.

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 the patient is dismissed from her clinic due to non-compliance she will be dismissed from the study and another subject will be recruited to replace her. If a subject 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.

D.5.iv. Study PK Visits:

Once the subject has signed the consent form, completed the Baseline Screening Visit, and is deemed eligible for the study, the research RN will establish a schedule for the subject to undergo the first pharmacokinetic/ pharmacodynamic study (PK1 visit). Subjects participating in the PK study must complete, at a minimum, PK visit 1 (between 8 0/7-20 6/7 weeks) and the postpartum PK visit (4-6 weeks post-delivery) in order to remain in the study.

Screening Visits will also be conducted within 1 week prior to the second and third PK visits and patients must continue to meet the criteria listed in section D.4, with the exception of exclusion criteria items 6 and 7. AST, ALT and creatinine levels are drawn during the Baseline Screening visit only. Once patients are enrolled, we will continue to monitor these laboratory results during PK2 and PK3 visits, but it is not a criteria for proceeding with the next PK visit.

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. Subjects will be dismissed from the study after two positive drug screens for non-prescribed opiates/opioids and replaced with another study subject.

Each PK study 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. In the postpartum period, the subject will be taking BUP (subutex) two, three, four, or five times daily and likewise will be on a consistent dose 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 chemistries (collected only at PK Visits 2 and 3, see screening blood chemistries above), saliva and urine will be collected. These biological samples will be analyzed for buprenorphine and metabolites. Blood will also be utilized for determination of catecholamines (epinephrine and nor epinephrine) and cotinine. Physiologic measurements will be taken and the subject will subsequently take her medication as prescribed. Blood (7 mL each) and saliva (1-2 mL each) samples will be obtained at 12 additional times: 10, 20, 30, 60, 90,120, 180, 240, 360, 480, 600 and 720 minutes hours post-dose for those on BID dosing. Those on TID dosing will have blood and saliva taken at 10, 20, 30, 60, 90,120, 180, 240, 300, 360, 420, and 480 minutes after dosing. Those on QID dosing will have blood and saliva taken at 10 additional times: 10, 20, 30, 60, 90, 120, 180, 240, 300, and 360 minutes after dosing. Those on 5 times a day dosing will have blood and saliva taken at 12 additional times: 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 minutes after dosing. The maximum amount of blood drawn from a subject on a study day is 98 mL. Blood samples will be collected in 10 mL heparinized vacutainer tubes that will be centrifuged to recover plasma for each of the time points listed above. All saliva samples will be collected using a Sarstedt Salivette collection device (see directions in Manual of Operations). The salivary collection sponge can be centrifuged later in the day. Salivary pH will be determined. Plasma and oral fluid samples will then be transferred into polypropylene tubes and frozen at -80 degrees centigrade until analysis.

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 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, we will obtain infant blood by heel stick just once prior to the second feeding after the BUP dose in the postpartum PK study. In total no baby will have more than 3 heel sticks, 2 while in the NICU as part of the postpartum ancillary study and 1 in the postpartum PK 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 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: for those on BID dosing - 0 (baseline), 30, 60, 90, 120, 240, 360, 480, 600 and 720 minutes post-dose; for those on TID dosing 0 (baseline), 30, 60, 90, 120, 240, 300, 360, 420 and 480 minutes post-dose; for those on QID dosing 0 (baseline), 30, 60, 90, 120, 240, 300, and 360 minutes post-dose; and for those on five times daily dosing 0 (baseline), 30, 60, 90, 120, 180, 240, and 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):

At the time of delivery, maternal and cord blood specimen, a placental sample (3x3 cm), and an umbilical cord sample (3 inches) will be collected and immediately frozen at -80 degrees.

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.

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 (see MOO). 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. The scoring system and treatment plan at Magee is documented in the MOO.

When possible, 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. 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:**

1. Other maternal outcomes to be measured are: 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.xii. 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 (α = 0.05, β = 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 al\textsuperscript{13} 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\textsubscript{max}, T\textsubscript{max}, AUC(0-\textsubscript{t}), AUC(0-\infty), k\textsubscript{e}, λ\textsubscript{Z}, CL/F, CLR, Vd/F, V\textsubscript{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.6. 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 clinical very relevant and the lack of PK/PD information to provide clinical guidance begs for such studies to be done.