Pediatric Trials Network:
Best Pharmaceuticals for Children Act

Pharmacokinetics, pharmacodynamics, and safety of a single dose intravenous methadone in healthy adult volunteers (MTH02)

Phase: 1/2

Funding Sponsor:
The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD)

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IND Sponsor/PTN Principal Investigator:	Kanecia Zimmerman, MD, PhD, MPH Associate Professor of Pediatrics Duke Clinical Research Institute [redacted]

STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Council for Harmonisation (ICH) E6(R2) guideline for Good Clinical Practice (GCP), and the applicable regulatory requirements from the United States (U.S.) Code of Federal Regulations (CFR), including 45 CFR 46 (Human Subjects Protection); 21 CFR 312 (Investigational New Drug); 21 CFR 50 (Informed Consent), 21 CFR Part 54 (Financial Disclosure), and 21 CFR 56 (Institutional Review Board [IRB]); as well as international regulatory requirements, if applicable.

All individuals who are responsible for the conduct, management, or oversight of this study have completed Human Subjects Protection and ICH GCP Training.

SITE PRINCIPAL INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and the investigator brochure or package insert/product label, and I agree that the protocol and associated documents contain all necessary details for my staff and me to conduct this study as described. I will personally oversee the conduct of this study as outlined herein and will make a reasonable effort to complete the study within the time designated. I agree to make all reasonable efforts to adhere to the attached protocol.

I will provide all study personnel under my supervision with copies of the protocol and access to all information provided by the sponsor or the sponsor's representative. I will discuss this material with study personnel to ensure that they are fully informed about the efficacy and safety parameters and the conduct of the study in general. I am aware that, before beginning this study, the Institutional Review Board (IRB) responsible for such matters must approve this protocol in the clinical facility where it will be conducted.

I agree to either obtain legally effective informed consent from participants as required by the IRB of record and according to government regulations and ICH guidelines. I further agree to report to the sponsor or its representative any adverse events in accordance with the terms of this protocol and the U.S. CFRs, Title 21, part 312.64, ICH GCP 4.11, as well as international regulatory requirements, if applicable. I further agree to ensure the study is conducted in accordance with the provisions as stated and will comply with the prevailing local laws and customs.

_____
Site Principal Investigator Name (Print)

_____ _____
Site Principal Investigator Signature Date

PTN PRINCIPAL INVESTIGATOR AND IND SPONSOR SIGNATURES

The signature below documents the review and approval of this protocol and associated study materials (e.g., Manual of Procedures, package inserts), and provides the necessary assurances that this clinical study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality and according to local legal and regulatory requirements and to the principles outlined in applicable U.S. and international regulations and ICH guidelines.

Kanecia Zimmerman, MD, PhD, MPH

PTN Principal Investigator/IND Sponsor Name (Print or Type)

PTN PI/IND Sponsor Signature **Date**

IND Sponsor (if different from above)

IND Sponsor's Signature **Date**

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
AUC	Area Under the Concentration Time Curve
BP	Blood Pressure
BPCA	Best Pharmaceuticals for Children Act
BUN	Blood Urea Nitrogen
BMP	Basic Metabolic Panel
CFR	Code of Federal Regulations
C_L	Clearance
C_{max}	Maximum Concentration
C_{min}	Minimum Concentration
CMP	Complete Metabolic Panel
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
DCF	Data Collection Form
DCRI	Duke Clinical Research Institute
DEPRU	Duke Early Phase Research Unit
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ESI	Events of Special Interest
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IDS	Investigational Drug Services
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
Ke	Elimination Rate Constant
Kg	Kilogram
MedDRA ®	Medical Dictionary for Regulatory Activities
Mg	Milligram
mL	Milliliter
MM	Medical Monitor
Mg	Microgram
MOAA/S	Modified Observer's Alertness Sedation Scale
MOP	Manual of Procedures
N	Number (typically refers to participants)
NIH	National Institutes of Health
NICHD	National Institute of Child Health and Human Development
NONMEM	Non-linear Mixed Effects Modeling

PD	Pharmacodynamics
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PKAP	Pharmacokinetic analysis plan
PTN	Pediatric Trials Network
RR	Respiratory Rate
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SM	Safety Monitor
SOP	Standard Operating Procedure
SpO_2	Oxygen Saturation
SUSAR	Serious, Unexpected, Suspected Adverse Reaction
TDS	Technology and Data Solutions
T_{max}	Time to Research Maximum Concentration
$t_{1/2}$	Half-life
VAS	Visual Analog Scale
V_z	Volume of Distribution at the Terminal Phase

PROTOCOL HISTORY OF CHANGES

Version	Date	Summary of Changes
v1.03	20JUL2022	N/A, original protocol
v2.0	01DEC2022	• Changed title from “Methadone pharmacokinetics and pharmacodynamics in adults” to “Pharmacokinetics, pharmacodynamics, and safety of a single dose intravenous methadone in healthy adult volunteers (MTH02)” • Changed principal investigator from Evan Kharasch to Kanecia Zimmerman • Made background information more concise and added reference tables – Table 1 and Table 2 (Section **2**) • Changed or more clearly defined objectives (Section **3**) ○ Primary objective changed from "Methadone enantiomers plasma concentrations vs time; primary and secondary pharmacokinetic parameters" to "Characterize the PK of methadone hydrochloride injection" ○ Secondary objective changed from "Methadone enantiomers hepatic clearance, hepatic extraction; EDDP enantiomers plasma AUC, Cmax, elimination half-life, EDDP/methadone AUC ratio; analgesia (maximum tolerated temperature threshold and AUC0-120), miosis (maximum, AUC0-120), minimum respiratory rate; maximum end-expired carbon dioxide concentration, maximum sedation" to (1) "Characterize the PD effect of methadone hydrochloride injection" and (2) "Characterize the safety profile of methadone hydrochloride injection" ○ Exploratory objective changed from "Methadone enantiomers renal clearance, EDDP enantiomers formation clearance; miosis, analgesia and respiratory Emax, EC50, t1/2ke0, subjective effects magnitude, influence of CYP2B6 genotype on primary and secondary outcomes, influence of age on primary and secondary outcomes" to "Characterize exposure-response relationships" • Changed/refined outcome measures and endpoints for relevance and clarity to match objectives (Section **3**) • Reduced timeline from 120 to 96 hours (Section **4**) • Made QTc a safety event of special interest (Section **4.6.1**) • Added efficacy events of special interest: miosis, ventilation, analgesia (Section **4.6.2**) • Expanded inclusion age max from 39 to < 40 (Section **5.2**) • Edited exclusion criteria (Section **5.2**) ○ Expanded exclusion criteria, based on further research, to include the following: ▪ History of cardiac dysfunction ▪ History of or current QTc prolongation, defined as > 470 ms in males and > 480 ms in females

		▪ Known hypersensitivity to methadone hydrochloride or any other ingredient in the methadone hydrochloride injection ▪ Known acute bronchial asthma or hypercarbia ▪ Receipt of a serotonergic drug or bupropion within 7 days prior to study enrollment ▪ Receipt of benzodiazepines, muscle relaxants, or other opioids within 7 days prior to study enrollment ▪ CYP2B6 inhibitors: macrolide antibiotics, azole-antifungal agents, fluconazole, *Alstonia boonei*, *Mangifera indica*, and *Picralima nitida* ▪ CYP2B6 inducers: abacavir, amprenavir, nevirapine, telaprevir ▪ Receipt of zidovudine, desipramine, or other drugs that may increase serum concentration when combined with methadone ▪ Known or suspected gastrointestinal obstruction, including paralytic ileus ▪ Significant respiratory depression ○ Qualified CYPB26 exclusion to be for moderate or strong CYP2B inhibitors and inducers ○ Added herbal exclusions, so removed statement expressing none are known ○ To CYP2B6 exclusion, added "in last 30 days" ○ Removed rationale for obesity (BMI > 33) exclusion • Expanded language regarding participant discontinuation/withdrawal (Section **5.3**) • Defined early withdrawal as a participant who has had < 5 specimens obtained (Section **5.3.4**) • Updated study procedures (Section **6**) ○ Updated the table of procedures and assessments (Table 3) ▪ More inclusive of all procedures ▪ Replaced shaded boxes with Xs ▪ Time is across the top columns instead of down rows ▪ Separated PK sampling schedule into separate table (Table 4) ▪ Separated PD measures, study-specific procedures, into separate table (Table 5) ○ Edited time series for PK sampling schedule and study-specific procedures ○ Added COVID-19 test ○ Added urine drug screen and breathalyzer ○ Changed ECG time points to study drug administration and 24 hours after study drug administration ○ Reduced study drug follow-up from 120 to 96 hours ○ Added an early study withdrawal visit (Section **6.6**) ○ Added to clinical laboratory evaluations CMP (complete metabolic panel) and AAG (alpha-1-acid glycoprotein) (Section **6.7**)

		• Reduced the number of PK blood draws from 30 to 18 (Section **6.7.4**) • Reduced the volume of blood collected from 5 cc/sample to 3.0 cc/sample (Section **6.8.3**) • Reduced the max number of blood draws to 20 (Section **6.8.3**) • Made study-specific procedure text more precise (Section **6.8**) • Reduced the number of pharmacodynamic assessment time points and the number of study-specific procedures (Table 5) • Added formulation, packaging, and labeling information (Section **7.1.2**) • Added replacement dose information (Section **7.2.1**) • Changed non-study drug abstinence prior to study drug administration from 2 to 30 days (Section **7.3**) • Expanded text regarding ethics (Section **15**) • Expanded text regarding data handling (Section **16**) • Added text for publication policy (Section **17**) • Removed use of arterial lines • Removed use of safety labs • Removed target for 25% minority population • Removed statement about document translation for non-English speakers. • Minor administrative changes including, but not limited to, formatting, punctuation, grammar, abbreviations
v3.0	20FEB2023	• Changed ECG options from 3 or 5 to 5 or 12 • Edited halting rule language (Section **9.2**) • Changed that methadone hydrochloride will be prepared in IDS, not DEPRU (Section **7.2**) • Removed 10-minute duration for methadone injection (Section **7.1.4**) • Changed term of 'site PI' and 'study investigator' to 'Study PI' for consistency • Minor administrative changes including punctuation
v4.0	03MAY2023	• Eliminated Alpha-1 acid glycoprotein (AAG) • Changed the PK schedule- Updated elapsed time after dose from 36.00 hh:mm to 30.00 hh:mm for PK sample #15. • Inpatient visit time frame was changed from (Day0, Hour 0-24) to (Day 0, Hour 0-30) (Section **6.3)** • Follow-Up time frame was changed from (36 to 72 hours post-drug administration) to (48 to 72 hours post-drug administration) (Section **6.4**) • Inpatient COVID-19 tests to POC antigen rather than PCR • Increased the screening window from 0-10 to 0-14 days • Changed Safety Monitor from Dr. Mike Montana to Dr. Karan R. Kumar

		• Removed COVID-19 test from Summary of Procedures under screening and enrollment (Section **6.1**) • Table 5 schedule of study specific procedures was updated for the following timepoints: 30, 48, 72, 96 to add Vital Signs, BP, SpO2, Observed Sedation, MOAA/S, Thermal Pain Threshold, Subjective Self-Assessment, VAS. • Removed COVID-19 test from screening and enrollment time point from Summary or Procedures. • Revised COVID-19 footnote to indicate it should only be collected during the inpatient visit. • Preparation and Administration was clarified adding IV bolus (Section **7.1.4**) • Applied DUHS policy of re-opening guidelines to COVID-19 testing for inpatient visit. • Updated time point from 24 hours to 30 hours (Sections **4.1** and **4.3**, and **6.9**) • Updated Maximum Blood Volumes-PK blood total from 60 mL to 54 mL per day. In addition, included the total amount of blood collected throughout the study (Section **6.8.3**). • Added time limits for data collection timepoints 0:02 to 18:00 hours in Table 5. • Specified drug infusion time. Changed from 10 minutes to IV push over one to two minutes
v5.0	31MAY2023	• Created a new section which includes the risk of Intravenous (IV) administration (Section **2.4.2**) • Added mild sedation and nausea/vomiting, Muscle spasms to known potential risk. Added risk for Ondansetron, ECG and metoclopramide (Section **2.4**) • Added study procedures to study design (Section **4**) • Maximum Blood Volumes-PK blood total from 54 mL to 47 mL per day (Section **6.8.3**) • Removed Section 6.7 Clinical Laboratory Evaluations • Added Section **6.7.2** Screen Laboratory Assessments • Added the following acronyms to the list of abbreviations table: COVID-19, MOAA/S, SpO_2, TDS, and SPT • Removed CBC from Screen Laboratory Assessments (Section **6.7.2**) • PK blood sample was updated from 3 mL to 2 mL (Section **6.7.5**) • Removed Section 6.92 Blood Collection Labs, added • Section **6.7.1** Urine Collection and Section • **6.7.2** Screening laboratory assessments to provide clarification

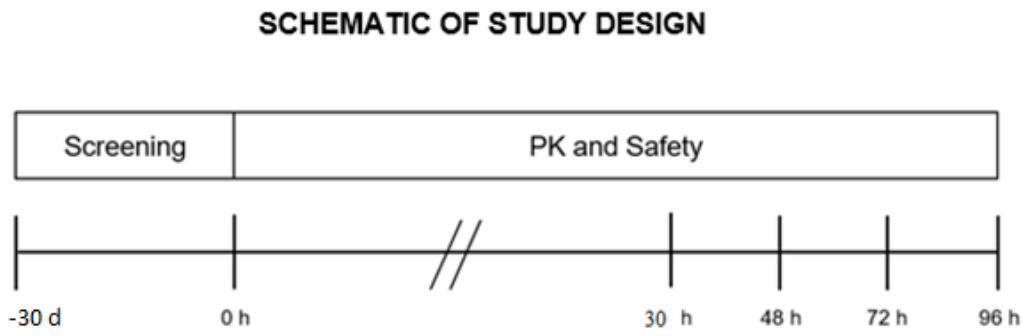
		• Added clarity about ondansetron and administration and added secondary nausea medication intravenous metoclopramide (Section **7.3**) • Changed Data Monitoring Committee to Data Safety Monitoring Board (Section **8.9**) • Clinical Monitoring changed DCC to STP (Section **10**) • Section **2.4.2** was added to describe food and drug interactions. • Updated the protocol synopsis Duration of Participant Participation from 96 hours to 18 days to include screening period.
V6.0	27NOV2023	• Increased screening window from 14 days to 30 days throughout the protocol, including schematic of study design • Increased total duration of participant participation from 18 days to 34 days in the protocol synopsis to accommodate the updated screening period. • Based on DSMB review changed secondary outcome measure from “characterize the safety profile” to “characterize the tolerability” (Protocol Synopsis and Section **3**) • Updated information on where screen failures should be recorded (Section **4.8**) • Removed Small Trials Program from List of Abbreviations and replaced Small Trials Program with DCRI monitoring team in Section **10**. • Based on DSMB review, added IDS to List of Abbreviations • Based on DSMB review, included a lower limit BMI ≤17 in the exclusion criteria (Section **5.2)** • Added parameters to exclusion criteria, specifically for hypercarbia and respiratory depression (Section **5.2**) • Added height to the list of physical examination parameters collected at Screening/Enrollment visit (Section **6.2**) • Removed urine drug screen abbreviations (Section **6.2**) • PK samples storage temperature was changed from -80 to -70°C (Section **6.7.4)** • Updated PK Sample Timing (**Table 4**) • Clarified length of time pupilometer googles should be worn by the participant (Section **6.8.2**) • Added vital sign parameters to the screening visit and specified that CO2 will not be collected at this visit, but all other vital signs (RR, HR, BP, SpO_2) will be. • Clarified instructions for the thermal pain tolerance threshold (Section **6.8.5**)

		• Added +- 8 hours for the following timepoints: 48,72,96 (Section **6.8.7**) • Provided additional details to study drug Methadone administration method and documentation (Section **7.1.4**) • Adjusted fasting period from midnight day before study-drug administration to food 8 hours before and liquids 4 hours before study drug administration (Section **7.3**) • Minor administrative changes including, but not limited to, formatting, punctuation, grammar, abbreviations, and updates to table of contents

PROTOCOL SYNOPSIS

Protocol Title:	Pharmacokinetics, pharmacodynamics, and safety of a single dose intravenous methadone in healthy adult volunteers
Phase:	Phase ½
Study Product:	Methadone hydrochloride
Objectives:	Primary: 1. Characterize the pharmacokinetics (PK) of methadone hydrochloride injection Secondary: 1. Characterize the pharmacodynamic effect of methadone hydrochloride 2. Characterize the tolerability of methadone hydrochloride injection Exploratory: 1. Characterize exposure-response relationships
Study Design:	Prospective, single-center, open-label, single-dose PK study
Study Population:	Healthy adult volunteers
Number of Participants:	Approximately 20
Number of Sites:	Single site
Duration of Participant Participation:	Up to 34 days

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Section 409I of the Public Health Service Act, also known as The BPCA mandates the National Institutes of Health (NIH) to prioritize therapeutic areas in critical need for pediatric labeling; to sponsor pediatric clinical trials; and to submit these data to the Food and Drug Administration (FDA) for consideration for labeling changes. This study will be conducted in accordance with Section 409I of the Public Health Service Act; as such, the results from this research may be submitted to the FDA for review and use in negotiated labeling changes. This research study is contractually supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). The NICHD awarded a contract to Duke University (Durham, NC), which established a Pediatric Trials Network (PTN) Clinical Coordinating Center through its Duke Clinical Research Institute (DCRI) to facilitate trial design for studies supported by the NIH. Duke University's Technology and Data Solutions (TDS) will oversee data management for the Adult Methadone project

See ICH E6(R2) GCP, Section 6.2 (https://www.fda.gov/files/drugs/published/E6%28R2%29-Good-Clinical-Practice--Integrated-Addendum-to-ICH-E6%28R1%29.pdf)

2.1.1 Background of Methadone Hydrochloride

Poorly controlled pain has been associated with notable morbidity and poor quality of life for both children and adults.[1]

In the acute setting, such as in the post-operative period, poorly controlled pain is associated with delayed time to recovery, prolonged duration of opioid use, and higher healthcare costs.[2] Over the long-term, persistent pain is associated with increased risk for depression or anxiety and difficulty working when compared to absence of pain.[3] Further, pain is rated as the single most common reason for consulting a physician in the U.S. and may contribute to more than $55 billion in lost productivity for full-time workers alone.[4,5]

Multiple strategies have been used to treat pain, particularly in the post-operative or other acute settings. Opioids with fast elimination and short duration are becoming the drugs of choice for treatment of acute pain, often administered intravenously through patient-controlled analgesia (repeated and frequent doses of short- and ultrashort-duration opioids), given its association with analgesia and patient satisfaction.[6-8] However, patient controlled analgesia is generally not more effective than conventional opioid therapy and is associated with errors and safety issues.[9,10]

More recent strategies have also included drugs such as methadone. In particular, methadone administration is increasingly common as the first-line therapy for treatment of cancer and neuropathic pain as well as acute post-operative pain.[11,12] A single dose of methadone can provide analgesia for 1-2 days and provides better analgesia than conventional opioids.[6,13,14] Moreover, methadone reduces further opioid requirements.[6,13,14] Methadone may also prevent the need for hospital admissions for pain therapy, such as in vaso-occlusive crises in children and adults with sickle cell disease.[15] Thus, methadone is an "opioid-sparing opioid." Methadone's clinical features provide a highly favorable way of minimizing opioid prescribing and usage to avoid addiction and overdose as well as diversion and misuse.

Methadone is an ideal agent for treatment of acute pain. It is an opioid analgesic, has repeatedly demonstrated long-acting efficacy, and is associated with reduced postoperative opioid requirements, less postoperative pain, and more rapid onset of effect compared to regimens including short-acting opioids.[16,17](**Table 1**) Methadone also has a long elimination half-life, so a single dose may have long-lasting effects. Administration of the methadone is cost-effective, especially compared to the costs of pumps, disposables, and adverse events (AEs) associated with patient-controlled analgesia.[6] Further, methadone has been associated with similar incidence of side effects compared to shorter-acting opioids.

Table 1. Documented use of methadone in the acute setting

Study	Population (age)	Comparator	Surgery	Results
Murphy *et al. 2017*[18]	120 Adults (18 – 80) - 63 methadone - 57 hydromorphone	hydromorphone	Spinal fusion	Intraoperative methadone reduced postoperative opioid requirements, decreased pain scores, and improved patient satisfaction.
Komen *et al. 2019*[14]	39 Adults (18 – 65) - 18 methadone - 21 short duration opioid	fentanyl, hydromorphone	Elective same day surgery	Decreased intraoperative and postoperative opioid requirements and postoperative pain resulted.
Simoni *et al. 2009*[19]	126 Adults (18 – 65) - 42 methadone - 42 clonidine - 42 placebo	clonidine, placebo	Laparoscopic surgery	Postoperative pain scores were significantly lower in the methadone group compared to the clonidine and placebo groups.
Singhal *et al. 2016*[20]	125 Children (mean age of 15)	multimodal anesthesia, epidural with general anesthesia	Nuss procedure	Multimodal anesthesia was associated with decreased postoperative pain compared to general anesthesia with an epidural, with the group receiving methadone having the lowest total opioid use and length of stay.
Carvalho *et al. 2018*[21]	100 Adults (≥ 18) - 50 methadone - 50 morphine	Morphine	Coronary artery bypass graft	Time to first request for analgesics was longer in the methadone group.
Udelsmann *et al. 2011*[22]	55 Adults (14 – 80) - 18 methadone - 19 morphine - 18 placebo	morphine, placebo	Cardiac surgery	The methadone group had a longer time until first request for analgesia, lower use of analgesia, and higher quality of analgesia compared to morphine and placebo without difference in time to extubated.
Russell *et al. 2013*[23]	75 Adult women - 25 methadone - 50 control	fentanyl, morphine, fentanyl and morphine	Caesarean delivery	The methadone group had lower pain scores and decreased opioid requirements immediately postoperatively, as well as decreased opioid use over 48 hours.
Gottschalk *et al. 2011*[24]	30 Adults (18 – 75) - 13 methadone	Sufentanil	Spinal fusion	The methadone group had decreased pain scores at 48 hours

	- 16 sufentanil			and decreased opioid usage at 48 hours compared to the sufentanil group .

Methadone was, however, developed and approved long before the regulatory requirements for pharmacokinetic (PK) and pharmacodynamic (PD) characterizations and numerous *in vitro* and *in vivo* preclinical and clinical studies, which are required today for drug approvals. While some investigations in the past few years have discovered missing information regarding methadone biotransformation, drug interactions, and genetics,[25-27] much fundamental information about methadone PK and PD remains missing. In addition, far more is known about the pharmacology of oral methadone used in high doses for the treatment of opioid addiction (80-160 mg daily) than about smaller intravenous doses used for acute pain (5-10 mg).

Based on known information, methadone, is clinically used as a racemic mixture, with R-methadone acting on the mu receptor, while both R- and S-methadone act to antagonize N-methyl-D-aspartate receptors.[28] The drug is lipophilic and has linear PK with a steady state volume of distribution ranging from 2-6 L/kg. Methadone is also 85-90% protein bound in plasma, primarily to alpha1-acid glycoprotein. Metabolism of methadone primarily occurs via CYP2B6 N-demethylation to inactive metabolites, primarily ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene (EDDP).[25] Methadone has a highly variable clearance, ranging from 3-10 L/h, and a terminal half-life of 8-59 hours. However, methadone persists in the liver and other tissues, with slow release sometimes resulting in prolonged duration of action.[29]

Because of its potential prolonged duration of action, labeled dosing of intravenous (IV) methadone for use in opioid non-tolerant patients is 2.5-10 mg every 8-12 hours, slowly titrated to effect. Available data on methadone PK during treatment of acute pain are listed in **Table 2**.

Table 2. Methadone PK during treatment of acute pain

Study	Population (age)	Route	Dose	Substrate	Results
Horst *et al.* 2016[15]	24 Adolescents (7 – 17) - 12 methadone - 12 control 23 Adults (18 – 55) - 11 methadone - 12 control	IV	0.1 – 0.125 mg/kg	Venous blood	Methadone and EDDP concentrations and PK in children with sickle cell disease was comparable to those in children and adults receiving surgery.
Sharma *et al.* *2011*[30]	31 Children (5 – 18)	IV	0.1mg/kg (10 children) 0.2mg/kg (10 children) 0.3mg/kg (11 children)	Venous blood	Methadone did not affect postoperative pain scores and did not decrease daily or total postoperative opioid consumption.
Stemland *et al.* 2012[31]	17 Adolescents (12 – 19)	IV	0.25 mg/kg	Arterial blood	PK of methadone in adolescents is similar to those reported in adults.
Meissner *et al.* *2014*[32]	16 Adults (18 – 40)	IV and Oral	IV: 5 mg/kg over 2 hours	Venous plasma, and urine	Cyclosporine did not affect methadone PD.

			Oral: 4.5 mg/kg twice daily for 4 days		
Kharasch *et al.* 2015[25]	64 Adults (18 – 50)	IV and Oral	IV: 6 mg/kg Oral: 11 mg/kg	Venous plasma, and urine	Methadone plasma concentrations were influenced by CYP2B6 polymorphisms, oral was affected more than intravenous, and S- more than R-methadone.

The influence of age on methadone PK is unknown. Also, no label information exists on the PK or PD of methadone in children (< 18 yr). Nonetheless, methadone is widely used in children, such as to treat neonatal abstinence syndrome and as an intraoperative and postoperative analgesic. From a perioperative perspective, more than 500,000 inpatient operations are performed on children, and several times that many outpatient operations. Thus, a significant opportunity exists to improve the clinical evidence informing the use of methadone, and specifically, the unmet need for a better understanding of methadone PK and PD across all age groups.

2.1.2 Adult Labeling of Methadone Hydrochloride

Methadone currently has the following labeled indications:

- For the treatment of moderate-to-severe pain not responsive to non-narcotic analgesics.
- For use in temporary treatment of opioid dependence in patients unable to take oral medication.

Methadone PKs have not been evaluated in children, nor is methadone approved for use in children.

2.2 Scientific Rationale

Identifying the PK/PD relationship for IV methadone in adults is essential to determining optimal dosing and understanding variation in drug disposition. Further, establishing this relationship will lay the foundation for labeling methadone in children with acute pain.

2.3 Potential Benefits

There is no expected benefit to the healthy volunteers who participate in this study. However, data from this study will provide benefits to adults and children in the future.

2.4 Known Potential Risks

Methadone administration is associated with the following risks:

- Mild sedation (being in a relaxed, calm state)
- Nausea and/or vomiting
- Addiction, Abuse, and Misuse
- Life Threatening Respiratory Depression
- QTc Prolongation

- Interactions with central nervous system Depressants
- Serotonin Syndrome
- Adrenal Insufficiency
- Severe Hypotension
- Gastrointestinal Adverse Reactions
- Seizures
- Withdrawal
- Spasm of muscles

The major hazards of methadone are respiratory depression and systemic hypotension, though to a lesser degree. Additionally, respiratory arrest, shock, cardiac arrest, and death have occurred. The most frequently observed adverse reactions include lightheadedness, dizziness, sedation, nausea, vomiting, and sweating.[33]

Risks will be minimized using standard procedures of the DEPRU. Briefly, participants will be monitored by trained nursing personnel and have direct access to emergency services throughout the study duration. Additionally, DEPRU has a crash cart, each participant room is equipped with oxygen, and naloxone can be requested to be at the bedside.

Ondansetron will be administered to help prevent nausea and vomiting. Ondansetron can cause headache and in extremely rare cases severe allergic reaction.

Metoclopramide will be administered if ondansetron is not effective in preventing nausea and vomiting. Metoclopramide can cause tardive dyskinesia, neurological effects, gastrointestinal effects, cardiovascular effects and mental health effects. Metoclopramide may have reduced efficacy in the presence of methadone.

Electrocardiogram (ECG) will be administered to detect possible QTc Prolongation. The ECG may cause skin irritation, itching, and redness from the ECG electrode pads.
Thermal stimulation, which will be used to assess methadone PD, includes a slight risk of a burn, but this is minimized by (a) positive lockout of stimulus parameters above 53°C and (b) a built-in shut-down system to prevent the delivery of prolonged or high-intensity stimuli.

Biological specimens will not be collected if, in the opinion of the investigator, collection is determined potentially harmful to the participant.

Any condition that would make the participant, in the opinion of the investigator, unsuitable for the study, will preclude the participant from being enrolled into this study.

2.4.1 Risks of Blood Drawing

The participant may feel discomfort, dizzy, or light-headed during the blood draw. Where the needle enters the skin, bruising, swelling, or bleeding are possible. Every effort will be made to use existing indwelling lines for blood sampling.

The total participant blood volume drawn will not exceed the maximum blood volume allowed for the study period (see Section **6.7.5**).

2.4.2 Risk of Drug and Food Interactions

The participant must tell the study doctor or nurse about all the prescribed medical foods and drugs, herbal products, over-the-counter (OTC) drugs, vitamins, natural remedies, and alcohol they are taking before starting the study. In addition, participants should let the study staff know of any changes in medications or new products you start taking during the study.

2.4.3 Risk of Intravenous (IV) Infusion and Taking Blood from IV

The study drug is administered through an IV catheter which is a small, flexible hollow tube inserted into a vein in the arm. Inserting an IV requires a needle and can cause localized discomfort. The vein the catheter is inserted in may become inflamed with signs of redness and warmth at or near the IV insertion site. Inflammation in a vein due to a blood clot is also a potential risk. In some instances, the vein may develop a small rupture causing the drug to leak out of the vein. This is generally not dangerous, but can cause discomfort and bruising. There is a risk of infection; however, this is a small risk as aseptic technique will be used. Blood samples will be drawn from an IV. The potential risk related to this are: risk of infection into the bloodstream during blood draw procedure, pain or discomfort, inflammation of the vein known as phlebitis, and bruising at injection site.

2.4.4 Potential Risk of Loss of Confidentiality

Loss of confidentiality is a potential risk. Every effort will be made to protect the participant's confidential medical information, but this cannot be guaranteed.

2.4.5 Pregnancy Risks

Pregnancy is an exclusion for this study. Risks for pregnant women can be found in the methadone hydrochloride product label.[33]

2.4.6 Unforeseen Risks

This research may present other risks to the participant that are not known or foreseeable at this time.

3 OBJECTIVES AND OUTCOME MEASURES

	Objective	Measures	Endpoints
Primary	Characterize the PK of methadone hydrochloride injection	Plasma concentrations	1. Clearance (CL) 2. Volume of distribution (Vz) 3. Half-life (t1/2) 4. Area under the curve (AUC) 5. Maximum concentration (Cmax) 6. Time to maximum concentration (Tmax) 7. Minimum concentration (Cmin) 8. Elimination rate constant (ke)
Secondary	Characterize the PD effect of methadone hydrochloride injection	1. Dark-adapted pupillometry 2. Observed sedation 3. Thermal pain tolerance thresholds 4. Subjective self-assessment of methadone effects using visual analog scale (VAS)	Change in pupillometry, observed sedation, thermal pain tolerance thresholds, and VAS from pre-dose to each post-dose time point
Secondary	Characterize the tolerability of methadone hydrochloride injection	1. Adverse events (AEs) 2. Serious adverse events (SAEs) 3. Suspected Unexpected Serious Adverse Reactions (SUSARs) 4. Safety events of special interest (QTc)	For AEs, SAEs, SUSARs: Proportion of participants from baseline to the end of safety follow-up For SESI: Change in QTc from baseline to the end of safety follow-up
Exploratory	Characterize exposure-response relationships	Plasma concentrations and VAS	Correlation of plasma concentrations to VAS score at each post-dose VAS time point

4 STUDY DESIGN

4.1 Study Design

The trial aims to investigate the pharmacokinetics (PK), pharmacodynamics (PD), and safety of intravenous (IV) methadone hydrochloride in approximately 20 healthy adults. Eligible participants who have followed fasting and dietary requirements will be admitted to the Duke Early Phase Research Unit (DEPRU) for a single study-drug/methadone infusion and PK blood draws. The inpatient stay duration will be approximately 30 hours. Various assessments will be conducted from the inpatient visit to the final study visit, including: pupil diameter measurement, vital sign collection, Modified Observer's Assessment of Alertness and Sedation (MOAA/S), which measures sedation level evaluation, thermal pain tolerance assessment, subjective self-assessment using the Visual Analog Scale (VAS), Electrocardiogram (ECG) and safety evaluations. After discharge, participants will be asked to return to clinic at various time points over the subsequent 96 hours to provide blood samples for PK analysis and complete the various study procedures.

Study intervention: Single-dose trial of IV methadone hydrochloride

Study procedures: Pupillometry, vital sign collection, MOAA/S, thermal pain tolerance, ECG, VAS

Duration of participant participation: Up to 4 days after study enrollment, including up to 30 days for screening.

Biological specimen collection: Blood for PK, blood for pregnancy testing, urine for drug screening, breathalyzer, urine for pregnancy testing

Biological specimen retention plan: See Section **12**

4.2 Study Product or Intervention

4.2.1 Rationale for Dose Selection

The administered dose of 0.1 mg/kg methadone hydrochloride is the labeled dose for the treatment of pain in adults. The goal of this study is to better understand the PK of this labeled dose.

4.3 Duration of Participant Participation

The screening visit will occur 0-30 days prior to study start. From time of consent, participation may be up to 96 ± 8 hours after study drug administration, wherein the first 30 hours is an inpatient visit, including study drug administration and PK blood draws, followed by a 48 hour, and 72-hour follow-up. The final visit will occur at 96 hours.

4.4 Biological Specimen Collection

Blood samples will be collected from participants for PK analysis.

4.5 DOI-specific Testing

Blood samples obtained during the study will be analyzed for plasma concentrations of methadone and a methadone metabolite, EDDP, using a validated liquid chromatography with tandem mass spectrometry assay under Good Laboratory Practice conditions.

4.6 Events of Special Interest

4.6.1 Safety ESIs

Safety ESI includes QTc interval (ECG).

4.6.2 Efficacy ESIs

Efficacy ESIs include the following:

- miosis (dark-adapted pupillometry)
- ventilation (respiratory rate (RR), end-tidal expired CO_2)
- analgesia (thermal pain tolerance thresholds, VAS responses to methadone, self-reported assessments)

4.7 Study Definition of Enrollment

4.7.1 Study Definition of Enrollment into Active Study

Study enrollment is defined as a participant providing informed consent and receiving IV methadone.

4.8 Screen Failures

A participant will be considered a screen failure if he or she provided informed consent but did not receive IV methadone. This screen failure will be recorded on the disposition form in the EDC under “Did Participant Receive Study Drug?

4.9 Study Definition of Completion

4.9.1 Participant Completion*

A participant will be considered complete if he or she received the single dose of IV methadone and was followed for safety through the last follow-up visit.

4.9.2 Study Completion

The study will be considered complete when all participants have completed the final visit and all required study and follow-up assessments.

*Participants not satisfying the above criteria for study completion will be reported as early terminators in the data system.

5 STUDY POPULATION

5.1 Selection of the Study Population/Sample Size

Approximately 20 evaluable healthy volunteers 18 to < 40 years old will be enrolled. Sex will be approximately equally distributed. Participants will be recruited from the greater Durham and Triangle community and a healthy volunteer registry in the DEPRU. Participants will be compensated.

5.2 Inclusion/Exclusion Criteria

Inclusion Criteria	1. 18 to < 40 years of age at the time of enrollment 2. Provides informed consent
Exclusion Criteria	1. History of cardiac dysfunction 2. History of or current QTc prolongation, defined as > 470 ms in males and > 480 ms in females 3. Known hypersensitivity to methadone hydrochloride or any other ingredient in the methadone hydrochloride injection 4. Known acute bronchial asthma or hypercarbia (history of known $PaCO_2$ above 45 mm HG) 5. Receipt of a serotonergic drug or bupropion within 7 days prior to study enrollment 6. Receipt of benzodiazepines, muscle relaxants, or other opioids within 7 days prior to study enrollment 7. Receipt of a moderate or strong CYP2B6 inhibitor or inducer – either prescription or non-prescription medications, herbals,[34] or foods known to be metabolized by or affecting CYP2B6 within 30 days prior to study enrollment a) CYP2B6 inhibitors include clopidogrel, prasugrel, thioTEPA, ticlopidine, voriconazole, macrolide antibiotics, azole-antifungal agents, fluconazole, *Alstonia boonei*, *Mangifera indica*, and *Picralima nitida* b) CYP2B6 inducers include artemisinin antimalarials, barbiturates, carbamazepine, cyclophosphamide, efavirenz, lopinavir, methimazole, nelfinavir, phenobarbital, phenytoin, primidone, rifampicin/rifampin, ritonavir, abacavir, amprenavir, nevirapine, telaprevir 8. Receipt of zidovudine, desipramine, or other drugs that may increase serum concentration when combined with methadone within 30 days prior to study enrollment 9. Known or suspected gastrointestinal obstruction, including paralytic ileus 10. Significant respiratory depression (respiratory rate less than 8 breaths/min or oxygen saturation (SpO_2) <95%) 11. BMI ≥ 33 and BMI ≤ 17 12. Known history of moderate-to-severe liver (Child Class B or C) or kidney disease (serum creatinine > 1.5) 13. Known history of drug or alcohol addiction (prior or present addiction or treatment for addiction) 14. Females who are pregnant or nursing

5.3 Participant Discontinuation/Withdrawal

5.3.1 Participant Decides to Withdraw Consent

A participant may voluntarily withdraw consent to participate in the study at any time. No additional study procedures or data will be collected after consent has been withdrawn; however, all data and specimens collected up to the point of consent withdrawal will be maintained. The participant will be marked as early terminated from the study. The reasons for withdrawal, or failure to provide a reason, must be documented by the investigator. The participant is not obligated to state the reason for withdrawal.

5.3.2 Participant Decides to Withdraw from Study Intervention

A participant may choose to withdraw from the study intervention during or after receiving methadone for any reason but continue to be followed for all study assessments. The reasons for withdrawal from study intervention, or failure to provide a reason, must be documented by the investigator. The participant is not obligated to state the reason for withdrawal.

5.3.3 Study Investigator/Sponsor Decides to Withdraw Participant

The Study Principal Investigator (PI) or IND sponsor (i.e., a study authority) may withdraw a participant without consent if the following occurs:

- The participant's condition changes or a safety event occurs while on study, and the study is no longer in his or her best interest;
- The participant is non-compliant with the study protocol; or
- The entire study is stopped by the FDA, the sponsor, or other applicable party.

A participant who is discontinued from methadone due to an AE, whether serious or non-serious, must be followed by the investigator until the AE is resolved or considered stable. The medical monitor (MM) or Study PI must be notified if the AE possibly relates to methadone overdose. The package insert should be consulted for details of any specific actions to be taken (link to package insert).[29]

If any of the above occurs, the participant will be informed of the withdrawal, and the investigator will discuss other options.

5.3.4 Replacements

In cases of early withdrawal from study, study dosing, or procedures – defined as a participant who has had < 5 specimens obtained – either by the participant or study authority, a replacement participant may be enrolled.

6 STUDY PROCEDURES

6.1 Summary of Procedures

Table 3. Schedule of study procedures and assessments

	Screening/ Enrollment	Inpatient Visit	Follow Up Visits		Final Study Visit	Early Withdrawal Visit
PROCEDURE	**Day -30 to 0**	**Day 0, Hour 0 - 30**	**Hour 48 ± 8**	**Hour 72 ± 8**	**Hour 96 ± 8**	
Informed consent	X					
Confirm eligibility criteria	X					
Demographics	X					
Physical examination	X					
Medical history	X					
Concomitant medications	X	X	X	X	X	
Pregnancy test	X	X[1]				
COVID-19 test[2]		X[2]				
Blood collection[3]	X	X[3]	X	X	X	X
Drug and alcohol screen	X	X				
Electrocardiogram	X	X			X	
Pupillometry		X[5]	X	X	X	
Vital signs[4]	X	X[5]	X	X	X	
Observed sedation, MOAA/S		X[5]	X	X	X	
Thermal pain tolerance		X[5]	X	X	X	
Subjective self-assessment, VAS		X[5]	X	X	X	
Study drug administration		X				
Safety events		X	X	X	X	X

[1] Serum quantitative testing will occur at screening and urine qualitative (point of care) testing will occur at the start of the in-patient visit.
[2] Per DUHS policy, COVID-19 testing may occur if the participant presents with new onset symptoms of COVID-19 or has had COVID-19 exposure within the last ten days.
[3] See Sections 6.7.2.(labs at Screening/Enrollment) and 6.7.4 (PK at Day 0) for details regarding blood sample collection times.
[4] Vital signs collected at enrollment: respiration rate (RR), heart rate (HR), blood pressure (BP), and oxygen saturation (SpO_2). Vital signs collected at 30,48,72 and 96 hours: RR, carbon dioxide (CO_2), HR, BP, and SpO_2. See Section 6.8.3 for details.
[5] See Table 5 for details regarding collection times.

6.2 Screening/Enrollment (Day -30 to 0)

The investigator will screen participants according to the eligibility criteria (Section **5.2**). Screening must remain impartial (no selectivity) to prevent bias.

Potential candidates' eligibility must be confirmed, or re-confirmed, and documented in the source records by the Study PI (or designee), prior to the conduct of any study procedures. Potential candidates will be educated on the study procedures, benefits, and potential risks as part of the applicable informed consent. Following consent, the enrolled participant's demographic information will be recorded, and the participant will undergo the following:

- Physical examination, including capturing weight and height
- Vital signs (heart rate, blood pressure, respiratory rate and oxygen saturation)
- Medical history
- ECG to determine QTc
- Review of concomitant medications
- Blood collection for clinical laboratory assessments (Section **6.7.2)**
- Serum quantitative pregnancy test
- Urine drug screen to test for illegal and prescription drugs
    - Methamphetamine
    - Morphine-a drug in the opiate class
    - Marijuana
    - Amphetamine
    - Barbiturates
    - Benzodiazepines
    - Cocaine
    - Phencyclidine
    - Methadone
    - Ecstasy/MDMA
- Breathalyzer

For alcohol and substance abuse, the study team will query the potential participant about past and current drug and alcohol use or treatment of use, then document said testimony, which will be validated by the urine drug screen and breathalyzer.

This visit will occur up to 30 days before the inpatient visit to allow adequate time for the return of clinical laboratory results.

Refer to MOP for details.

6.3 Inpatient Visit (Day 0, Hour 0 - 30)

The study day will consist of an approximately 30-hour stay for participants in the DEPRU for methadone administration, blood sampling, and PD assessments. Abstinence requirements are listed in Section **7.3**. The following will occur at the inpatient visit:

- Rapid COVID-19 test will occur per the DUHS policy if the participant presents with new onset symptoms of COVID-19, or reports COVID exposure within the last ten days,
- Urine qualitative pregnancy test
- Review of concomitant medications
- Urine drug screen and breathalyzer
- Blood collection for PK analysis (see Section **6.7.4** for timing of blood draw)
- Electrocardiogram for QTc, prior to study drug administration and 30 hours after study drug administration
- Pupillometry, goggles for dark-adapted pupil diameter
- Vital signs, including RR, end-expired CO_2, HR, BP, and SpO_2
- Observed sedation, MOAA/S
- Thermal pain tolerance threshold
- Subjective self-assessment, VAS
- Methadone hydrochloride administration
- Safety event assessment

Participants will be free to move around and be given a standard meal 4 hours after completion of methadone hydrochloride administration. Thereafter, participants will have free access to food and water while in the DEPRU. After the inpatient visit, participants should be transported home by a person previously arranged by the participant.

6.4 Follow-Up (48 to 72 hours post-drug administration)

At approximately 48, and 72 ± 8 hours after methadone administration, participants will return to the DEPRU for the following procedures:

- Review of concomitant medications
- Blood collection for PK analysis
- Pupillometry, goggles for dark-adapted pupil diameter
- Vital signs, including RR, end-expired CO_2, HR, BP, and SpO_2
- Observed sedation, MOAA/S
- Thermal pain tolerance threshold
- Subjective self-assessment, VAS
- Safety event assessment

6.5 Final Study Visit (96 hours post-drug administration)

At approximately 96 ± 8 hours after methadone administration, the final study visit will be in-person at the DEPRU and include the following:

- Review of concomitant medications
- Blood collection for PK analysis
- Electrocardiogram for QTc
- Pupillometry, goggles for dark-adapted pupil diameter
- Vital signs, including RR, end-expired CO_2, HR, BP, and SpO_2
- Observed sedation, MOAA/S
- Thermal pain tolerance threshold
- Subjective self-assessment, VAS
- Safety event assessment

6.6 Early Study Withdrawal Visit

In the event the participant chooses to terminate the study early, an early study withdrawal visit will include the following:

- Blood collection for PK analysis

- Safety event assessment

6.7 Specimen Collection for Study-Specific Tests

Refer to MOP for details.

6.7.1 Urine Collection

Urine will be collected for all participants at the screening visit to assess for illegal drugs. Urine will be collected from females of child-bearing potential at the inpatient visit for pregnancy status.

6.7.2 Screening laboratory assessments

Blood will be collected at the screening visit for the following assessments: serum pregnancy test (for females of child-bearing potential and CMP (complete metabolic panel). Fasting is not required. The CMP will be used to assess for exclusion criteria for those for known history of moderate-to-severe liver (Child Class B or C) or kidney disease (serum creatinine > 1.5).

6.7.3 Pharmacokinetic Tests

Blood specimens for methadone PK will be collected per the schedule in **Table 4**.

6.7.4 PK Sampling Schedule

The total number of PK blood samples will be 18, 2 ml/sample, 36 ml (less than one-quarter cup). Blood will be centrifuged and plasma removed and stored at -70°C until shipment to a laboratory for analysis. The time of each sample collection should be recorded.

Table 4. Optimal PK sampling schedule relative to single dose of methadone

PK Time Point Sample Number	Elapsed Time After Dose (hh:mm)	
1	Baseline draw	
	Start IV dose	**methadone HCl 0.1 mg/kg bw**
2	0:00 + 0:01[1]	IV push over 1 -2 minutes (including 3ml 0.9NS flush immediately following study medication administration
3	0:05 ± 0:01	
4	0:10 ± 0:01	
5	0:15 ± 0:01	
6	0:30 ± 0:05	
7	1:00 ± 0:10	
8	1:30 ± 0:10	
9	2:00 ± 0:10	
10	4:00 ± 0:30[2]	
11	6:00 ± 0:30	
12	12:00 ± 0:30	
13	18:00 ± 0:30	
14	24:00 ± 0:30	

15	30:00 ± 1:00
16	48:00 ± 8:00
17	72:00 ± 8:00
18	96:00 ± 8:00

[1]Immediately after starting IV dose
[2]Food can be served after the 4:00 sample collection.

6.7.5 Maximum Blood Volumes

The maximum volume of fresh/non-scavenged whole blood to be collected is approximately 47 mL (equivalent to 3.2 tablespoons). A maximum of 18 blood samples of 2 mLs each can be obtained for each PK sample. A total of 8 mLs will be collected for on-site laboratory test: HCG (3.5 mLs and CMP 4.5 mLs). This total volume also includes a small amount of additional blood (3 mL) that is discarded prior to collecting each PK sample.

6.8 Study-specific Procedures

6.8.1 Electrocardiogram

A 5- or 12-lead ECG will acquire QTc prior to study drug administration screening**,** 30 and 96 hours after study drug administration.

Refer to MOP for details.

6.8.2 Pupillometry, Dark-Adapted

Dark-adapted pupillometry will measure pupil diameter using a goggle-based, camera-like device (I-Portal® Falcon, https://www.neurolign.com/products/#hardware). A goggle-based system effectively allows the participant to be in the dark while room lights are on for research staff. The participant will wear the goggles continuously for the first hour of PK sampling. Thereafter, the goggles can be removed. See **Table 5** for details when this measurement should be completed.

Refer to MOP for details.

6.8.3 Vital Signs

Vital signs collected at screening include: RR, HR, BP, and SpO_2. Vital signs collected at 30,48,72 and 96 hours include RR, CO_2, HR, BP, SpO_2. Respiratory rate and end-expired CO_2 concentration are recorded using Medtronic Capnostream monitor with FilterLine sampling lines (https://www.medtronic.com/covidien/en-us/products/capnography/capnostream-20p-bedside-patient-monitor.html (https://www.medtronic.com/covidien/en-us/products/capnography/filterline-etco2-sampling-lines.html).

Refer to MOP for details.

6.8.4 Observed Sedation, MOAA/S

Sedation will be observed and recorded by the study team using the Modified Observer's Assessment of Alertness/Sedation (MOAA/S).

Modified Observer's Assessment of Alertness/Sedation [MOAA/S]	
Responsiveness	Score
Responds readily to name spoken in normal tone	5 (alert)
Lethargic response to name spoken in normal tone	4
Responds only after name is called loudly and/ or repeatedly	3
Responds only after mild prodding or shaking	2
Responds only after painful trapezius squeeze	1
Does not respond to painful trapezius squeeze	0

Refer to MOP for details.

6.8.5 Thermal Pain Tolerance Threshold

Thermal pain tolerance will be measured by a 3 cm^2 computer-controlled Peltier-type thermal stimulator (Pathway; Medoc Advanced Medical Systems, https://medoc-web.com/products/pathway-model-ats/ or similar thermal stimulator). The stimulator delivers painful heat stimuli to the volar side of the forearm. Before any stimulus is applied, participants are familiarized with the test procedure and instructed on how to evaluate their pain. Pain is evaluated using a standard verbal scale from 0 (no pain) to 100 (worst possible pain).

The thermode system's baseline temperature is set at 32°C. The computer-controlled thermode system is programmed to gradually increase the stimulus (0.8°C/sec) until participants press a stop button, which indicates maximum tolerable temperature was reached and initiates immediate thermode cooling. The maximum tolerable temperature is recorded.

The thermode system's maximum temperature is set to 52°C to prevent thermal injury. If the thermode reaches 52°C without the participant pressing the button, then temperature increase ceases and immediate thermode cooling is initiated, and 52°C is recorded as the maximum tolerable temperature.

The maximum temperature testing is repeated three times for each time event, as the probe is moved and cooled between each stimuli, the averages of the three maximum temperatures and pain ratings are recorded,. The primary outcome measure is the average maximum tolerable temperature.

Refer to MOP for details.

6.8.6 Subjective Self-assessment

Each participant will use a VAS to indicate levels of alertness/sedation (almost asleep to wide awake), energy level (no energy to full of energy), confusion (confused to clear headed), clumsiness (extremely clumsy to well-coordinated), anxiety (calm/relaxed to extremely nervous), and nausea (no nausea to worst nausea), each on a score of 0 to 100. Alternatively, plastic sliders may be used (http://www.custompromotionalrulers.com/visual-analog-scale-vas-rulers/vas-pain-scale-rulers-0-10-cm-w/buckle-slider/ or similar), which are then scored from 0 to 100.

1. Please rate how awake you feel: 0 (asleep) - 100 (wide awake)

2. Please rate your level of energy: 0 (no energy) - 100 (full of energy)
3. Please rate how confused you feel: 0 (clear headed) - 100 (extremely confused)
4. Please rate how clumsy you feel: 0 (well-coordinated) - 100 (extremely clumsy)
5. Please rate how anxious you feel: 0 (totally relaxed) - 100 (extremely nervous)
6. Please rate if you feel nauseated: 0 (no nausea) - 100 (worst nausea)

6.8.7 Summary of Study-specific Procedures

Table 5. Schedule of study-specific procedures

Elapsed Time After Dose (hh:mm)	Pupillometry, Dark-Adapted	Vital Signs RR, CO_2, HR	Vital Signs BP, SpO_2	Observed Sedation, MOAA/S	Thermal Pain Threshold	Subjective Self Assessment, VAS
Baseline draw	X	X	X	X	X	X
Start IV dose						
0:02 ± 0:01	X	X			X	
0:04 ± 0:01	X	X		X		X
0:06 ± 0:01	X	X			X	
0:08 ± 0:01	X	X				
0:10 ± 0:01	X	X	X	X	X	X
0:12 ± 0:01	X	X				
0:15 ± 0:01	X	X				
0:20 ± 0:01	X	X				
0:30 ± 0:02	X	X				
0:40 ± 0:02	X	X		X	X	X
0:50 ± 0:02	X	X				
1:00 ± 0:05	X	X	X	X	X	X
1:30 ± 0:05		X		X		X
2:00 ± 0:10	X	X	X	X	X	X
3:00 ± 0:10		X		X		X
4:00 ± 0:10	X	X	X	X	X	X
6:00 ± 0:30		X	X	X		X
8:00 ± 0:30	X	X		X	X	X
10:00 ± 0:30		X		X		X
12:00 ± 0:30	X	X	X	X	X	X
18:00 ± 0:30						
24:00 ± 1:00	X	X		X	X	X
30:00 ± 1:00	X	X	X	X	X	X
48:00 ± 8:00	X	X	X	X	X	X
72:00 ± 8:00	X	X	X	X	X	X
96:00 ± 8:00	X	X	X	X	X	X

Some timepoints have multiple tasks scheduled. It is suggested that the tasks be prioritized in order of: obtaining the PK sample, pupillometry, vital signs, observed sedation. thermal pain threshold, and subjective self-assessment. These tasks are not all performed during all the timepoints, please follow Tables 4 and 5 above. The research staff does have the discretion to perform the task order according to their trained judgement as long as they are within the timepoint parameters.

6.9 Study-specific PK Specimen Preparation, Handling, Storage, and Shipping

Biological samples collected for the purposes of this research will be labeled only with a unique accession number (via study-provided barcode label) without protected health information (PHI) that can directly identify the study participant. These samples will be sent to the PTN-designated laboratory for analysis (i.e., methadone concentration measurements). Once samples are analyzed, data will be entered into the study records. After all study-related testing is complete, any remaining samples will be shipped to an NIH-designated biorepository for future unspecified use, unless prohibited by local ethics committee or regulatory agencies (see Section **12**).

Refer to the MOP for instructions for the collection, labeling, preparation, handling, and storage of specimens.

7 STUDY PRODUCT DESCRIPTION

7.1 Study Product Information

Methadone hydrochloride is a mu-agonist; a synthetic opioid with multiple actions qualitatively similar to those of morphine. The most prominent action involves the central nervous system and organs composed of smooth muscle. Methadone's principal therapeutic uses are for analgesia and for detoxification or maintenance in opioid addiction. Specific PK and PD qualities of methadone are in the FDA drug label.[29]

7.1.1 Dosage and Dose Timing

Each participant will be administered a single dose of IV methadone at 0.1 mg/kg per measured body weight. The single dose will be calculated to the closest 0.1 mg.

7.1.2 Formulation, Packaging, and Labeling

Methadone Hydrochloride Injection USP, 10 mg/mL from Mylan Institutional LLC will be used for this study.[33] It is supplied as 20-mL multi-dose vials. Each mL of Methadone Hydrochloride Injection USP contains 10 mg (0.029 mmol) of methadone hydrochloride. The injectable solution contains the following inactive ingredients: chlorobutanol, 0.5%, as a preservative, and sodium chloride.

Refer to MOP for details.

7.1.3 Product Storage and Stability

Methadone Hydrochloride Injection USP should be stored at 20 to 25°C (68 to 77°F), with excursions permitted between 15 to 30°C (59 to 86°F). It should be protected from light and stored in carton until contents have been used.

Refer to MOP for details.

7.1.4 Preparation and Administration

Methadone will be prepared by Duke Investigation Drug Services (IDS). Each participant will receive 0.1 mg of methadone per kg of body weight from the 10 mg/mL stock concentration administered via IV push. Methadone (0.1mg/kg bw) will be administered via IV push followed by 3mL flush over 2 minutes. The time IV push begins and the time flush ends will be documented on the source.

Refer to MOP for details.

7.2 Accountability Procedures for the Study Product

Methadone hydrochloride will be prepared by Duke Investigational Drug Services and administered intravenously to participants by study nurses.

Refer to MOP for details.

7.2.1 Replacement Doses

If the product dose is broken or unusable, study personnel will prepare study product from a new vial. Replacement doses may be requested by contacting the Duke Investigational Drug Services.

See MOP for contact information.

7.2.2 Disposition of Study Products Upon Study Completion or Expiration

Upon completion of the study, or upon notice of the study products' expiration, the study products should be disposed of at the site, pursuant to the ICH/GCP guidelines and the Duke Investigational Drug Services policy for drug destruction.

See MOP for details.

7.3 Concomitant Medications/Treatment Restrictions

Participants should not receive serotonergic drugs within 7 days prior to drug administration or during treatment or follow-up period (up to 96 hours). Participants should not receive inhibitors or inducers of CYP2B6 within 30 days prior to enrollment or during the study period. See Section **5.2** for a listing.

Participants will be required to abstain from the following:

- *30 days before study-drug administration*: non-study medications (including over the counter and/or herbal), without prior PI approval – non-study medications that are given per standard of care and are not exclusionary will receive approval, at the discretion of the Study PI.
- *24 hours before study-drug administration and during study days*: alcohol
- *8 hours before study-drug administration*: food
- *4 hours before study-drug administration*: liquids
- *Day of administration*: alcohol and caffeine

Current substance or drug dependence will be confirmed by positive urine drug screen at the screening/enrollment visit. Current alcohol abuse will be confirmed by positive breathalyzer at the screening/enrollment visit.

Ondansetron (4 mg IV) will be administered 15 minutes prior to methadone administration to decrease or prevent nausea. If initial dose is not efficacious, and nausea persists, intravenous metoclopramide (10 mg) will be provided.

The date, time, route, and dose of all concomitant medications of interest will be recorded.

8 ASSESSMENT OF SAFETY

8.1 Specification of Safety Parameters

Studies performed under IND FDA regulations require reporting based on the definition included in 21 CFR 312.32 (a), regardless of the definition of AE used in the protocol.

An **adverse event (AE)** is any untoward medical occurrence in humans, whether or not considered intervention-related, which occurs during the conduct of a clinical trial. (Any change in clinical status, ECGs, routine labs, x-rays, physical examinations, etc., that is considered clinically significant by the Study PI is considered an AE).

An **unexpected adverse event** is any AE, of which the nature, specificity, or severity is not consistent with the applicable product information (e.g., package insert/approved label) or investigational plan.

A **suspected adverse reaction (SAR)** is any AE for which there is a reasonable possibility that the intervention caused the AE. A reasonable possibility implies that there is evidence that the intervention caused the event.

An **adverse reaction (AR)** is any AE caused by the intervention.

A **serious, unexpected, suspected adverse reaction (SUSAR)** is a SAR that is both serious and unexpected.

All AEs related to methadone administration will be reported. Safety will be assessed by frequency and incidence of reported AEs, SAEs, and SUSARs. A safety Data Monitoring Safety Monitoring Board (DSMB) convened by NICHD will review data and safety information from study participants.

All AEs related to the study procedures or assessments (e.g., blood draws) and SUSARs related to study product will be reported. Safety ESIs will be recorded.

8.2 Guidelines for Assessing Association of an Adverse Event

A **serious adverse event (SAE)** or **serious suspected adverse reaction** or **serious adverse reactions** determined by the investigator or the sponsor is an AE or SAR that results in any of the following outcomes:

- Death
- Life-threatening AE (“life-threatening” means that the study participant was, in the opinion of the investigator or sponsor, at immediate risk of death from the reaction as it occurred and required immediate intervention)
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Inpatient hospitalization or prolongation of existing hospitalization
- Congenital abnormality or birth defect
- Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event.

8.3 Guidelines for Assessing Intensity of an Adverse Event

Safety events will be assessed by the study clinician using a protocol defined grading system.

The investigator/delegate (defined as a clinician licensed to make a diagnosis) should use the following definitions when assessing intensity of an adverse event**:**

- **Mild -** Participant is aware of symptoms or has minor findings but tolerates them well, and no or minimal intervention required
- **Moderate** - Participant experiences enough symptoms or findings to require intervention
- **Severe -** Participant experiences symptoms or findings that require significant intervention

8.4 Guidelines for Assessing Causality

The investigator/delegate (defined as a clinician licensed to make a diagnosis) will use the following question when assessing causality of an AE to study intervention: Is there a reasonable possibility that the intervention caused the event? “Reasonable possibility” means that there is evidence to suggest a causal relationship between the study intervention and the AE. An affirmative answer designates the event as a suspected adverse reaction.

The investigator/delegate (defined as a clinician licensed to make a diagnosis) must document his or her assessment of intensity, causality, and association in the participant records in a timely manner and submit safety reports as required by his or her IRB.

8.4.1 Safety Events of Special Interest

A safety event of special interest (SESI) is a select safety event that could be related to the study intervention. Safety ESIs will be solicited for their occurrence (No or Yes) and assessed by the investigator/delegate (defined as a clinician licensed to make a diagnosis) for intensity, causality, and association to provide uniform data collection of all events.

Safety ESIs are expected, pre-specified events, so they will not be reported in an expedited manner to the FDA but will be reviewed by the MM and the Data Safety Monitoring Board (DSMB) convened by NICHD and reported to the FDA in the annual report.

The only SESI for this study will be the QTc interval obtained by ECG prior to study drug administration), 30 hours after study drug administration, and at the final study visit (96 hours post-administration).

8.5 Collection Period and Reporting Procedures

Adverse Event information will be gained from direct monitoring of the study participants as well as from clinician observation and self-reporting by the study participants. The investigator/delegate (defined as a clinician licensed to make a diagnosis) must document his or her assessment of severity and causality in the participant records in a timely manner and submit safety reports as required by his or her IRB.

Death irrespective of causality, identified as either an unsolicited or solicited event (i.e., SESI), at any time through end of study will be reported.

Serious Adverse Events/SUSARs will be collected for all participants, following the execution of informed consent through 7 days post last sample collection, and reported on the AE/SAE electronic case report form (eCRF).

Safety ESIs (Section **4.6.1** and **8.4.1**) will be collected for all participants following the execution of informed consent through 7 days post last sample collection, and assessed for intensity, causality, and association, and reported in the data system. To determine if SESIs are dose related, additional data around solicited safety events will be recorded.

Adverse Events will be recorded from the time of methadone administration until 96 hours (the final study visit) and reported in the data system.

8.6 Safety Event Follow-up and Sponsor Reporting

Adverse Events, SAEs, and SESIs **directly related** to study intervention/procedures will be followed until resolution, even if this period extends beyond the study-reporting period.

Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that resolution will remain chronic. If the event resolves during the study or follow-up period, a resolution date should be documented in the data system.

Adverse Events **directly related** to study intervention/procedures and SESIs (without a causal relationship to study intervention, that are not defined as an SAE) will be reported in the data system within 7 days of identification/site awareness.

Adverse Events considered related to study intervention/procedures and all SAEs/SESIs ongoing at the time of the last dose of study intervention will be followed for as long as necessary to adequately evaluate the participant's safety or until the event stabilizes. If the event resolves during the study or follow-up period, a resolution date should be documented in the data system.

Serious Adverse Events, SUSARs, and SESIs that are associated with any of the criteria that define the event as an SAE will be reported in the data system within 24 hours of identification/site awareness. Upon entry in the Electronic Data Capture (EDC) system, these events will generate an automatic email notification to the MM and the sponsor. The investigator/delegate (defined as a clinician licensed to make a diagnosis) must document his or her assessment of intensity, causality, and association in the participant records in a timely manner and submit safety reports as required by his or her IRB.

The MM will review all safety events at the time they are reported. Any event that requires expedited reporting based on federal regulations (21 CFR 312.32) will be forwarded to the IND sponsor. The IND sponsor or its representative will submit expedited safety reports (e.g., IND safety reports) to the regulatory agencies, as necessary.

8.6.1 Discontinuation of a Participant Due to Adverse Events

Any participant who experiences an AE may be withdrawn at any time from the study at the discretion of the investigator. The AE(s) should be reported in the EDC system, and the participant's progress should be followed until the AE is resolved or considered stable. The MM must be notified. If the AE may be related to overdose of study treatment, the package insert should be consulted for details of any specific actions to be taken.

8.7 Pregnancy

Although not considered an AE, pregnancy must be reported on the specific pregnancy report form. If a participant is found to be pregnant during the study, the site must immediately report it. The site must document that they contacted the participant, advised the participant to obtain appropriate prenatal medical care, and referred the participant for such care. In addition, per the consent, researchers will follow the participant for the duration of the pregnancy and to obtain information (via direct examination or medical record review) to determine whether the resulting fetus/baby survived delivery or had any congenital abnormalities. If the fetus/newborn does not survive delivery or any congenital abnormalities are present, these must be reported as an SAE following the usual requirements for SAE reporting. Please note that if a pregnancy is reported, the participant's subsequent weight, vital sign, and laboratory data will not be included in analyses for these variables. If the pregnancy is terminated early within the first 12 weeks of the pregnancy, inclusion of the participant's subsequent weight/height, vital signs, and laboratory data in the analyses will be determined by the PTN study team.

8.8 Regulatory Reporting

Any event that requires expedited reporting based on federal regulations will be forwarded to the IND sponsor via reporting on the study specific eCRF. The IND sponsor or its representative as detailed in the Transfer of Regulatory Obligations will submit expedited safety reports (e.g., IND safety reports) to the FDA and other regulatory agencies as necessary and will inform the investigators of such regulatory reports. Site investigators must submit safety reports as required by their IRB. Documentation of the submission and receipt by the IRB must be retained for each expedited safety report.

Any event that requires reporting to Regulatory Authorities (e.g., SUSARS) based on applicable national regulations will be forwarded to the IND sponsor in time to meet reporting requirements.

8.9 Data and Safety Oversight (Medical Monitor/Data Safety Monitoring Board)

The study will be monitored by the BPCA Data Safety Monitoring Board (DSMB). The NIH charters the DSMB to support all research activities conducted under the BPCA PTN.

In addition, the study has designated a qualified and experienced physician not otherwise associated with this protocol to serve as the Medical Monitor (MM) and review all safety events at the time they are reported and/or updated. The MM may request additional information regarding a site reported SAE; the Study PI is expected to provide requested information or materials to the MM in an expeditious manner to support the writing/submission of study safety reports. The MM will be available to study sites as needed. The study IND sponsor will also review all SAEs to determine regulatory reporting and will be available to sites as needed.

If safety concerns are identified, the MM may request a meeting of the DSMB to review safety data. The MM will also provide an initial report to the PTN PI/IND sponsor and an unbiased written report to the DSMB of the event per the DSMB charter/safety monitoring plan. At a minimum, the MM will comment on the outcomes of the safety event and the relationship of the safety event to the study product/intervention. The MM will also indicate whether they concur with the details of the report provided by the study site investigator. If no safety events prompt review at an earlier time point, the DSMB will review safety events per the DSMB charter.

The DSMB will convene and make recommendations on termination of the study based on review of safety reports and halting rules. The safety data will be compiled by the TDS. Based on the recommendations of the DSMB, PTN, and NIH/NICHD, the IND sponsor will decide to terminate or continue the study.

Ad Hoc Meetings of the DSMB: The DSMB may convene an ad hoc meeting to discuss any issue of data and safety raised by the site investigator, MM, the IND sponsor, NICHD, or a member of the DSMB. At the discretion of the investigators, the IND sponsor, NICHD, and DSMB members, a non-serious safety event that is associated with the product or procedures may be considered as a trigger for an ad hoc DSMB meeting to assess the safety of the product/intervention, without resulting in halting the enrollment of the trial.

9 STUDY TERMINATION / HALTING

9.1 Study or Site Halting Criteria / Termination Criteria

This study may be terminated at any time by NICHD, the IND sponsor, or the DSMB. Reasons for termination include, but are not limited to, if in their judgment, no further benefits are to be achieved from the study or the intervention presents an unreasonable and significant risk to participants. If the study is terminated, notifications will be made to the regulatory authorities (e.g., FDA), investigators, IRBs, and study participants, in accordance with all applicable regulations governing the study and site/investigator.

9.2 Halting Rules

If any participant experiences a study drug-related SAE in the 24 hours following methadone administration, then study dosing administration will be stopped. The DSMB will be notified and will conduct a review, study activities will be allowed to proceed on previously enrolled and new participants.

If 3 or more of participants enrolled to date experience a severe (Grade 3 or higher) reaction related to the study drug, then the study will be halted for DSMB.

Refer to Section **5.3** for requirements of participant safety follow-up following cessation of study product/intervention.

10 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of study participants are protected, that the reported study data are attributable, legible, contemporaneous, original, accurate, and complete, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s), with ICH GCP [E6(R2)], and with applicable regulatory requirement(s) including 21 CFR 312 Subpart D Responsibilities of Sponsors and Investigators.

The Study PI, or as detailed in the Transfer of Regulatory Obligations, or his/her designee will conduct site-monitoring visits. Site visits will be made at standard intervals as defined by the clinical site monitoring plan and may be made more frequently as directed by the PTN Monitoring Team, IND sponsor and/or NICHD. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, data collection forms, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

The study-specific clinical site monitoring plan will supplement the BPCA project-wide clinical monitoring plan based on protocol risk assessments.

11 STATISTICAL CONSIDERATIONS

Pharmacokinetics (PK), Pharmacodynamics (PD), and safety analyses will be conducted. Participant demographics will be summarized. In addition, the number of participants enrolled, completed, discontinued early from study, and the reasons for discontinuation will also be summarized.

Descriptive statistics, such as the number of observations, mean, median, 95% confidence interval, standard deviation, standard error, minimum, and maximum, will be considered for continuous variables (such as age, weight, etc.). Other descriptive statistics, such as counts, proportions, and/or percentages, will be presented to summarize discrete variables (such as sex, race, etc.).

11.1 Study Endpoints

11.1.1 Primary Endpoints

The list of primary endpoints includes the following:

- Clearance (CL)
- Volume of distribution (Vz)
- Half-life ($t_{1/2}$)
- Area under the curve (AUC)
- Maximum concentration (C_{max})
- Time to maximum concentration (T_{max})
- Minimum concentration (C_{min})
- Elimination rate constant (k_e)

11.1.2 Secondary Endpoints

The list of secondary endpoints includes the following:

- Change in pupillometry
- Observed sedation
- Thermal pain tolerance thresholds
- VAS from pre-dose to post-dose
- Proportion of participants reporting AE, SAE, SUSAR from baseline to the end of safety follow-up
- QTc from baseline to the end of safety follow-up

11.1.3 Exploratory Endpoints

The list of exploratory endpoints includes the correlation of plasma concentrations to VAS score at each post-dose VAS time point. Plasma concentrations are listed in the primary endpoints. Post-dose VAS time points are displayed in **Table 5**.

11.2 Analysis Population

PK population: All enrolled participants who receive a dose of IV methadone and have at least one evaluable PK sample will be eligible for the PK analysis population.

Safety population: All participants enrolled who received a dose of IV methadone will be included in the safety analysis.

Efficacy (PK/PD) population: All participants enrolled who received at least one dose of IV methadone and provided at least one PK sample and a corresponding efficacy assessment will be included in the efficacy analysis.

11.3 Analysis Plan

Noncompartmental analysis will be conducted to determine methadone PK. Compartmental modeling may be explored. Additional details are in the statistical analysis plan.

Descriptive statistics, such as number of observations, mean, median, standard deviation, standard error, minimum, and maximum, will be presented for continuous variables (such as age, weight, etc.). Other descriptive statistics, such as counts, proportions, and/or percentages, will be presented to summarize discrete variables (such as race, sex, etc.). All descriptive analyses will be presented overall. A 95% confidence level will be used for confidence intervals. A detailed description of statistical methods and secondary analyses will be prepared and presented in the statistical analysis plan prior to data lock for final analyses.

11.3.1 Primary Analysis

The dosing, sampling, and demographic information recorded on the eCRFs will be merged with bioanalytical information to create a PK dataset for methadone. A noncompartmental PK analysis will be performed in Phoenix WinNonlin (version 6.3 or later) using concentration–time data of methadone in plasma. The PK parameters for methadone will be summarized using descriptive statistics, including arithmetic mean, standard deviation, minimum, median, maximum, and geometric mean.

Following the study dose on Day 0, peak drug concentration (C_{max}), time at peak concentration (T_{max}), and area under the concentration versus time curve (AUC) will be calculated using the observed data for methadone as appropriate and, if possible, depending on actual samples collected. AUC will be calculated using the linear trapezoidal method. At least 3 time points after the last dose with measurable plasma concentrations will be required for the calculation of $AUC_{0\text{-}24}$.

In addition, the following additional PK parameters will be estimated as appropriate and, if possible, depending on actual samples collected: apparent terminal elimination rate constant (k_e), terminal-phase disposition half-life ($t_{1/2}$), total clearance (CL, and volume of distribution during terminal phase (V_z). k_e will be determined as the slope of a log-linear least squares of at least 3 concentration-time points after the last dose judged, by visual inspection, to be in the apparent terminal elimination phase. Half-life will be calculated as $t_{1/2} = \ln 2/k_e$.

Nominal sampling time will be used in PK calculations, unless actual times are needed. If samples are below the quantification limit and occur prior to the time at C_{max}, a value of zero will be assigned; for samples occurring after C_{max}, half the quantification limit will be assigned for a single below-quantifiable-limit sample, or zero for all values in consecutive samples that are below quantifiable limit.

Compartmental methods will also be explored. Please see the PK analysis plan (PKAP) for additional details.

11.3.2 Secondary Analysis

1) The relationship between methadone concentrations/other PK parameters and endpoints of change in pupillometry, observed sedation levels, thermal pain tolerance thresholds, change in VAS from pre to post dose, and change in respiratory rate will be evaluated using standard descriptive statistics. Descriptive summaries of the endpoints predose, postdose, end of follow-up will be presented. The change between time points also will be presented, along with the 95% CI, t-test, and p-value.

2) The following endpoints will be analyzed in the safety analyses:

Incidence of safety related events

A. AE related to study procedure

B. SAE related to study procedure

C. SUSAR related to study drug

D. Safety Events of Special Interest (QTc prolongation)

In addition, changes in QTc interval will be assessed over time.

All AEs recorded during the study period will be coded with Medical Dictionary for Regulatory Activities (MedDRA) Version March 2018. Summaries of AE will be tabulated for the following types:

A. Number (%) of participants with any AE

B. Number (%) of participants with any SAE

C. Number (%) of participants withdrawn from treatment due to SUSAR

All summaries will be reported in total and by study site.

Additional analyses (e.g., Exposure-Response [safety] analysis) and summaries may be derived if deemed appropriate and will be further described in the SAP.

11.3.3 Exploratory Analysis

A tabular or graphical data exploration of the relationship between safety endpoints (i.e., the occurrence of selected AEs) by methadone steady state exposures (e.g., AUC and Cmax) quantiles will be evaluated. The relationship between these steady-state exposures and the probability of experiencing AEs with possible covariates will be evaluated using logistic regression analysis.

11.4 Demographics and Baseline Characteristics

The number of participants who either completed or discontinued early from the study will be summarized. Demographic and baseline characteristics will be summarized.

11.5 Planned Interim Analyses

No planned interim analyses will occur as part of this study.

11.6 Sample Size Considerations

For the primary objective, the sample size is based on the ability to precisely estimate the clearance in adults using population PK techniques. For IV methadone in healthy adult volunteers, we anticipate a CV% of 23 and 38% for R and S-enantiomers, respectively.[15] Based on this range of CV%, 15 participants will be adequate to precisely describe CL with a 95% CI (confidence interval) within 60% and 140% of the geometric mean.

12 FUTURE USE OF STUDY RECORDS AND BIOLOGICAL SPECIMENS

The medical data and study information entered in the participant's medical records will be kept per individual site policies for medical record retention. Other study records held at the study site will be kept until the FDA has completed any necessary review of the study results, or for a minimum of 2 years after the study has ended – whichever is longer. The research data collected in this study, and provided to the sponsor, will be kept indefinitely.

Information about this study, including study results, will be published without further permission from the participant, as detailed in the ICF. Participants will not be identified in any publications or presentations made about the study.

After the study is completed, information about the study, including de-identified study data, will be submitted to an NIH designated storage location, such as the NICHD Data and Specimen Hub or DASH (https://dash.nichd.nih.gov) or the NIH database of Genotypes and Phenotypes or dbGaP (https://www.ncbi.nlm.nih.gov/gap/). Controlled access means that only researchers who apply for and get permission to use the information for a specific research project will be able to access the information.

With NIH approval, the data submitted to NIH-designated repositories may be used by other researchers for future research. The study data submitted to these NIH-designated repositories will be de-identified, meaning it will not include any information that can identify the participant. The study team may also share the de-identified study data with other researchers. When the participant's de-identified study data are provided to other researchers for the purposes of future research, it will be done without obtaining additional permission from the participant.

Biological specimens collected for study specific testing will be labeled at the site with a study-provided barcode label. The specimen labels will only contain a unique code number; it will not include protected health information (PHI) or any other information that could identify the study participant. Specimens will be stored at the site and then shipped to an analytical laboratory for study-specific testing. After analysis, specimens will be stored until the FDA completes review of the final research study report and proposed drug label changes. Once FDA review of the study results is complete, and after consent for future use of these specimens is confirmed, the specimens will be submitted to an NIH storage facility.

De-identified study specimens may be made available to other researchers for future research without obtaining additional permission from the participant. Although whole genome sequencing is not part of this study, study specimens containing genetic materials, may be made available to other researchers who may conduct whole genome sequence in the future.

The participant's study specimens will not be sold to anyone; however, the use of these specimens may result in commercial profit. There is no provision to provide the participant with financial compensation beyond what is described in the ICF. Biological specimens may be stored indefinitely. If a participant decides to withdraw permission to use his or her study data or specimens, he or she will be instructed per the ICF to contact the site investigator. Study data and samples including data collected using the participant's specimen that has been recorded or collected prior to withdrawal will continue to be used, but no new data or specimens will be collected.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 (R2), Section 4.9 and 21 CFR 312.62, and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of the sponsor, its designees, and appropriate regulatory agencies to examine (and, when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, participants' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Data collection forms will be derived from the eCRFs.

14 QUALITY CONTROL AND QUALITY ASSURANCE

The Study PI will provide direct access to all trial-related sites, source data/documents, data collection forms, and reports for the purpose of monitoring and auditing by the sponsor and its designees, and inspection by local and regulatory authorities. The Study PI will ensure that all study personnel are appropriately trained and applicable documentation is maintained on site.

The Study PI will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

The Study PI will implement quality control procedures, beginning with the data entry system, and generate data quality control checks that will be run on the data. Any missing data or data anomalies will be promptly clarified and resolved.

15 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

15.1 Informed Consent Process

Informed consent is a process that is initiated prior to the participant agreeing to participate in the study and continuing throughout the individual's study participation.

15.1.1 Informed Consent of Adult Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant, and written documentation of informed consent is required prior to starting study-specific procedures or intervention.

15.2 Documentation of Permission, Assent, and Consent

Consent must be documented using forms and processes determined by the Duke University Health System (DUHS) IRB.

Prior to enrollment of participants into this trial, the protocol, the applicable informed consent templates, and any materials or advertisements presented to participants will be reviewed and approved by the DUHS IRB and the site's IRB of record. The consent templates approved by DUHS IRB and the site's IRB of record will then be provided to sites and revised as necessary to comply with local regulations and institutional requirements. Sites are required to submit all changes to the templates to the study investigator or designee, which ensures compliance with U.S. and international regulations and sponsor (NIH) policies, prior to submission and approval to the IRB of record for each site. Notification of the IRB's approval, its composition, and the institution's federal-wide assurance number will be provided to the Study PI or designee.

Should amendments to the protocol and consent documents be required, the amendments will be written by the sponsor, submitted by DEPRU, and approved by the DUHS IRB.

Participants may be compensated for their participation in this study. Compensation will be in accordance with the sponsor's maximum compensation limits and institutional policies and procedures, must be documented in the consent form with the local IRB's policies and procedures, requires IRB approval, and must be documented in the consent forms.

Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participant should have the opportunity to discuss the study and to think about it prior to agreeing to participate. Participants must be informed that participation is voluntary and that consent may be withdrawn from the study at any time, without prejudice. A copy of the executed informed consent document will be given to the participant for his or her records.

Site staff may employ recruitment efforts approved by the IRB of record and per institutional policy prior to the participant consenting; however, before any protocol-specific procedures are performed to determine protocol eligibility, informed consent or waiver of informed consent must be obtained. The informed consent process will be conducted, and the form fully executed, i.e., signed and dated, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The IND sponsor or designee will provide the investigator, in writing, with any new information that bears significantly on the participants' risk to receive the investigational product. This new information will be communicated by the site investigator to participants who consent to participate in the trial in accordance with IRB of record's requirements. The informed consent document will be updated, and participants will be re-consented, if necessary.

15.3 Confidentiality and Privacy

This study is covered by a Certificate of Confidentiality (CoC) from the NIH. The CoC prevents U.S. courts and other U.S. agencies from forcing the study team to share information that may identify the participants during a legal or legislative action unless the participant allows this. The CoC does not keep the participants from sharing information about their participation in this study.

All research activities will be conducted in as private a setting as possible.

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological specimens in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The Study PI will ensure that the use and disclosure of PHI obtained during a research study complies with the HIPAA Privacy Rule. Authorization is required from each research participant (i.e., specific permission granted by an individual to a covered entity for the use or disclosure of an individual's PHI). A valid authorization must meet the implementation specifications under the HIPAA Privacy Rule. Authorization may be combined in the informed consent document (if approved by the IRB).

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, and regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements. Both the Study PI and the Institution at which the study is contracted to be conducted, will hold responsibility to maintain custody of all study records until the sponsor permits their destruction.

A unique study identification number will identify individual participants and their research data. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected.

The clinical study site will permit access to all documents and records that may require inspection by the sponsor or its authorized representatives, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study.

16 DATA HANDLING AND RECORD KEEPING

16.1 Data Handling

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Data collection forms will be derived from the eCRFs for recording and maintaining data for each participant enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with the data collection form/source documents, or the discrepancies should be documented. The sponsor and/or its designee will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

16.2 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be assessed for intensity association and causality by a licensed clinician and reviewed by the Study PI or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the Study PI. During the study, the investigator must maintain complete, current, and accurate documentation for the study.

The Study PI will be responsible for data management, quality review, analysis, and reporting of the study data.

16.3 Data Capture Methods

Clinical data (including AEs) will be entered into a 21 CFR Part 11-compliant web-based data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms/source documents.

16.4 Types of Data

Data for this study will include PK, PD, and safety data.

16.5 Timing/Reports

The DSMB convened by NICHD will make recommendations on study continuation based on the safety data collected according to DSMB charter.

The DSMB will convene and make recommendations on study continuation based on the safety data collected periodically.

16.6 Study Records Retention

Study records and source documents will be kept until the FDA has completed any necessary review of the study results, or for a minimum of 2 years after the study has ended – whichever is longer. The research data collected in this study will be kept indefinitely.

The disposition date related to FDA application will be posted on the PTN website for the Investigator's reference.

16.7 Protocol Deviations

A protocol deviation is any noncompliance/unplanned excursion from approved investigational plan (e.g., protocol, MOP), or ICH GCP guidelines. The noncompliance may be on the part of the participant, investigator, or site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

Each investigator must adhere to the investigational plan as detailed in the study protocol and/or associated study materials (e.g., MOPs, Forms Instructions, User Guides etc.). Each investigator will be responsible for the training of delegated staff and enrolling only those participants who have satisfied all protocol eligibility criteria.

All deviations will be recorded. The site is responsible for vigilantly identifying and reporting deviations within 5 working days of identification of the protocol deviation or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to the sponsor via the Electronic Data Capture system.

All deviations from the protocol must be reported in the study records/data system. Protocol deviations must be submitted to the local IRB per their guidelines. The Study PI and study staff are responsible for knowing and adhering to their IRB requirements.

17 PUBLICATION POLICY

Following completion of the study, the PTN PI may publish the results of this research in a scientific journal under the oversight/approval of the Publication Committee of the PTN. The PTN Publication Committee comprises representatives of the network cores, thought leaders, collaborators, and PTN and is responsible for generation and coordination of the publications that report scientific findings of the network. All public presentations (abstracts, manuscripts, slides and text of oral or other presentations, and text of any transmission through any electronic media) by participating investigators, participating institutions, and PTN that use PTN data and are intended to represent the PTN or are supported by the PTN will be reviewed by the PTN Publication Committee per the Publication Committee Charter.

The PTN Publication Committee guarantees that the study results are presented by experts in the field that have working knowledge of the study design, implementation, data synthesis/analysis, and interpretation. The committee goals are to ensure that any confidential or proprietary information is protected, and that all appropriate statistical analyses have been included.

The PTN Publication Committee will adhere to the trials registration policy adopted by the International Committee of Medical Journal Editors member journal. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of the IND sponsor to register this trial in an acceptable registry.

The International Committee of Medical Journal Editors defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials), would be exempt from this policy.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

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