SUPPLEMENTARY MATERIAL

Use of Physiologically-Based Pharmacokinetic (PBPK) Modeling to Inform Dosing of the Opioid Analgesics Fentanyl and Methadone in Children with Obesity

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PBPK, physiologically-based pharmacokinetic modeling
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Points represent values for individual virtual children (n = 1,000 for each subgroup). Lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; CL, clearance; IV, intravenous; V_d, volume of distribution
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Points represent values for individual virtual children (n = 1,000 for each subgroup). Lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; CL, clearance; IV, intravenous; $V_d$, volume of distribution
Table S1.1. Summary of Clinical Studies used for Pediatric PBPK Modeling.

|                | FENTANYL                        |
|----------------|---------------------------------|
| **POP01 Study**| Clinical study originally described in Maharaj et al. |

|                | METHADONE                       |
|----------------|---------------------------------|
| **MTH01 Study**| Clinical study described herein |
| **POP01 Study**| Clinical study described herein |

MTH01, Pharmacokinetics of Multiple Dose Methadone in Children Treated for Opiate Withdrawal (ClinicalTrials.gov #NCT01945736) study; PBPK, physiologically-based pharmacokinetic; POP01, Pharmacokinetics of Understudied Drugs Administered to Children Per Standard of Care (ClinicalTrials.gov #NCT01431326) study
Table S1.2. Population Demographics for Virtual Subjects with and without Obesity who were used in Dosing Simulations for Each Drug.

| Demographics | Children without Obesity | Children with Obesity |
|--------------|--------------------------|-----------------------|
| Age, years   | 8.9 (2.0, 18.0)         | 9.0 (2.0, 18.0)       |
| Age group    |                          |                       |
| 2-<6 years   | 1,000 (33.3%)           | 1,000 (33.3%)         |
| 6-<12 years  | 1,000 (33.3%)           | 1,000 (33.3%)         |
| 12-21 years  | 1,000 (33.3%)           | 1,000 (33.3%)         |
| Male         | 1,500 (50.0%)           | 1,500 (50.0%)         |
| Weight, kg   | 32.1 (9.5, 102.5)       | 45.1 (10.6, 179.1)    |
| Height, cm   | 135.8 (76.8, 200.2)     | 135.6 (74.4, 200.1)   |
| BMI, kg/m²   | 17.6 (11.5, 29.7)       | 24.9 (17.9, 65.8)     |
| BMI percentile, % | 68.3 (0, < 95.0) | 98.1 (95.0 100.0)     |
| Extended BMI Percentile, % | 83.3 (53.0, < 100.0) | 110.5 (100.0, 236.4) |

Values are medians (range) for continuous variables and counts (%) for categorical variables. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese.

BMI, body mass index
2 FENTANYL

2.1. Fentanyl Background

Fentanyl is a lipophilic, high-potency synthetic opioid extensively used intravenously both in adults and pediatric patients for analgesia, sedation and anesthesia.² It has been also used for the treatment of breakthrough cancer pain and chronic pain through transmucosal and transdermal administration respectively.³ The analgesic effects of fentanyl are mediated through interaction with µ- and κ-opioid receptors in the central nervous system.⁴ Due to a greater extent of drug distributed to adipose tissue and slow redistribution into plasma for elimination, fentanyl half-life is prolonged when administered as continuous infusion by approximately one-fold for every hour increase in the duration of the infusion.⁵

Fentanyl is a high extraction ratio drug with systemic clearance that approaches hepatic blood flow.⁶ It is mainly metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5 to inactive metabolites and shows dose-independent pharmacokinetics (PKs).⁷ In plasma, fentanyl is primary bound to albumin (~ 85% bound).⁸ Fentanyl is U.S. Food and Drug Administration (FDA) approved in children >2 years of age for inducing and maintaining general anesthesia, intra- and post-operative analgesia, and sedation/analgesia in critical care settings.⁹ Despite the extensive use of fentanyl in children, only limited pharmacokinetic (PK) data in pediatric patients are available.

Although opioid dosage regimens are often based on total body weight, the influence of body weight on the PKs of fentanyl is still not fully understood. In a pilot study conducted in adolescents with morbid obesity (mean body mass index [BMI] of 49.6 kg/m²), fentanyl was administered intravenously for intraoperative analgesia based on ideal body weight per standard of care.¹⁰ Results showed enhanced absolute clearance, while absolute volume of distribution was comparable to previously reported lean adult values, suggesting that a loading dose of fentanyl may be based on total body weight followed by maintenance doses based on ideal body weight.¹⁰ However, patients with obesity are more at risk of significant adverse respiratory side effects of opioids.¹⁰

2.2. Fentanyl Clinical Data Used
A literature search was performed to leverage articles reporting fentanyl PK profiles and parameters for adults and pediatric patients. Fentanyl plasma concentration versus time data from six previously published clinical studies of healthy subjects and abdominal and dental surgery patients were digitized and used to evaluate the adult fentanyl physiologically-based pharmacokinetic (PBPK) model (Table S2.1). One pediatric study was also identified in the literature and used to evaluate the pediatric PBPK model.

In addition, data from children with and without obesity who received fentanyl per standard of care collected from the ‘Pharmacokinetics of Understudied Drugs Administered to Children Per Standard of Care’ clinical trial (POP01, ClinicalTrials.gov #NCT01431326). Fentanyl plasma concentrations were quantified using a validated liquid chromatography-tandem mass spectrometry assay developed by OpAns, LLC (Durham, NC, USA) as previously described. There were 3 concentrations from 2 children without obesity and 50 concentration measurements from 30 children with obesity. See Table S2.2, for observed pediatric subject demographics.

2.3. Fentanyl PBPK Model Development

A whole-body fentanyl adult PBPK model was developed by incorporating literature information on physicochemical properties, metabolism and excretion processes of fentanyl (Table S2.3) using PK-Sim® (version 8, Open Systems Pharmacology Suite, open-systems-pharmacology.com). Lipophlicity was optimized using pooled digitized adult intravenous (IV) data and the Levenberg-Marquardt algorithm implemented in PK-Sim®. Tissue expression distribution of metabolizing enzymes in all model compartments was implemented according to the PK-Sim® RT-PCR database. Organ to plasma partition coefficients were calculated using the Rodgers and Rowland method and rates of permeation across the cell membranes (interstitial-cell barrier) were calculated with the method PK-Sim® Standard.

Metabolism of fentanyl via CYP3A4 was described using a literature fentanyl in vitro maximum rate of metabolism (Vmax) value obtained from a microsomal assay and scaled to an in vivo Vmax value using the content of CYP3A4 protein in liver microsomes. The parameter kcat was used in model simulations to describe fentanyl’s clearance via CYP3A4, and it is defined as the ratio of the in vitro Vmax for liver microsomes and the content of CYP3A4 protein in liver microsomes. Specific clearance to CYP3A5 concentration ratio (CLspec/[E]) was optimized using
pooled adult digitized data from adults receiving IV doses of fentanyl and the Levenberg-Marquardt algorithm.\textsuperscript{6,11–15,17} Renal clearance of fentanyl was fixed based on literature data.\textsuperscript{21}

The adult PBPK model was scaled to pediatric population from 2-18 years of age by changing the physiological and anatomic parameters describing the human body.\textsuperscript{22} The remaining drug-dependent parameters, including molecular weight, pKa, lipophilicity, and solubility, were fixed to the values of the adult PBPK model. Clearance via CYP metabolism using Equations 2.1.-2.2.:

\[
OSF_{\text{CYP}3A4} = \frac{PMA^{6.543}}{(72.533^{6.543} + PMA^{6.543})} \quad (2.1)
\]

\[
OSF_{\text{CYP}3A5} = \frac{PMA^{6.543}}{(72.533^{6.543} + PMA^{6.543})} \quad (2.2)
\]

where \(OSF_{\text{CYP}}\) is the ontogeny scaling factor for the corresponding CYP enzyme, and \(PMA\) is postmenstrual age (a composite of gestational age [GA] and postnatal age [PNA]).\textsuperscript{23} Clearance was then scaled by adjusting adult unbound hepatic intrinsic clearance by age- and enzyme-specific percent of adult activity (ontogeny) using Equation 2.3.:

\[
CL'_{\text{CYP}}(\text{child g liver}) = OSF_{\text{CYP}} \times CL'_{\text{CYP}}(\text{adult g liver}) \quad (2.3)
\]

where \(CL'_{\text{CYP}}(\text{child g liver})\) is the scaled unbound hepatic intrinsic clearance due to the corresponding CYP per gram of liver, and \(CL'_{\text{CYP}}(\text{adult g liver})\) is the adult unbound hepatic intrinsic clearance due to the corresponding CYP.

### 2.4. Fentanyl PBPK Model Evaluation

The fentanyl PBPK model was able to capture the digitized literature data, with an overall average fold error (AFE) of 1.01 (range: 0.63 – 1.26 across six previously published clinical studies of adults and one pediatric study) (Figure S2.1., Table S2.1.).

The AFE for two observed children with out obesity was 0.68, where AFE for those with obesity was 0.72. The PBPK model was able to capture 52.0% of observed concentration from children without obesity within the 90% model prediction interval (32.0% above, 16.0% below) (Figures S2.2. – S2.4.).
2.5. Fentanyl Dosing Simulations

A virtual population of 1,000 pediatric subjects with ages 2–<6, 6–<12, and 12–18 years were simulated with a fentanyl dosing regimen of 1 µg/kg over 240 h. The primary PK parameters clearance and volume of distribution were simulated across increasing body size for each age group to better understand how they change with obesity (Figures S2.5. – S2.7.).

In addition, fentanyl dosing of 1, 2, and 3 µg/kg/h infusions over 240 hours was simulated for each of the above age groups. Simulated exposure was compared to a previously described target steady-state plasma concentration (C$_{ss}$) range of 1-3 ng/mL (Figures 3, S2.8.-2.9.).

24–27
Figure S2.1. Population simulations (n = 500) of fentanyl concentrations digitized from six adult studies (a-h) and one pediatric study (i).\textsuperscript{6,11–16} Shaded regions represent the 90% model prediction interval, the solid line represents the median simulated concentration, and points are digitized observed fentanyl concentrations with corresponding standard deviation values when available.
**Figure S2.2.** Population simulations (n = 250) of fentanyl concentrations using ‘individualized populations’ for each observed pediatric subject without obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
Figure S2.3. Population simulations (n = 250) of fentanyl concentrations using ‘individualized populations’ for each observed pediatric subject with obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
Figure S2.4. AFE for pediatric subjects without (blue) and with (red) obesity who received fentanyl plotted versus age and body size. Dashed lines represent 2-fold error for reference. AFE was calculated using median simulated concentration. Extended BMI Percentile is calculated as BMI percentile for a subject’s age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese.

AFE, average fold error; BMI, body mass index; Ext., extended
Figure S2.5. Changes in simulated weight-normalized and absolute clearance (a-b) and volume of distribution (c-d) with increasing body size, or Extended BMI Percentile for virtual children aged 2–<6 years with and without obesity. Extended BMI Percentile is calculated as BMI percentile for a subject’s age and sex divided by 95%, where children with an Extended BMI Percentile $\geq 100\%$ are considered obese. Virtual children received a single 1 µg/kg/h IV dose continuously infused over 240 h. Gray points represent simulated values for virtual children. Solid blue lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; IV, intravenous; $V_d$, volume of distribution
Figure S2.6. Changes in simulated weight-normalized and absolute clearance (a-b) and volume of distribution (c-d) with increasing body size, or Extended BMI Percentile for virtual children aged 6–<12 years with and without obesity. Extended BMI Percentile is calculated as BMI percentile for a subject’s age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese. Virtual children received a single 1 µg/kg/h IV dose continuously infused over 240 h. Gray points represent simulated values for virtual children. Solid blue lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; IV, intravenous; V_d, volume of distribution
Figure S2.7. Changes in simulated weight-normalized and absolute clearance (a-b) and volume of distribution (c-d) with increasing body size, or Extended BMI Percentile for virtual children aged 12–18 years with and without obesity. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese. Virtual children received a single 1 µg/kg/h IV dose continuously infused over 240 h. Gray points represent simulated values for virtual children. Solid blue lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; IV, intravenous; V<sub>d</sub>, volume of distribution
**Figure S2.8.** Probability of being above, within, or below target fentanyl concentrations with 1-3 ng/mL with increasing Extended BMI Percentile.\textsuperscript{24–27} Panels show probabilities for virtual children (n = 1,000 for each subgroup) receiving a 2 μg/kg/h IV fentanyl infusion over 240 h broken down by both age and obesity status. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese.

BMI, body mass index; IV, intravenous
Figure S2.9. Probability of being above, within, or below target fentanyl concentrations with 1-3 ng/mL with increasing Extended BMI Percentile.24–27 Panels show probabilities for virtual children (n = 1,000 for each subgroup) receiving a 3 μg/kg/h IV fentanyl infusion over 240 h broken down by both age and obesity status. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese.

BMI, body mass index; IV, intravenous
Tables S2.1. Population Demographics and PBPK Model Simulation Results for Digitized Fentanyl Studies

| Demographics | Value |
|--------------|-------|
| **Bentley *et al* (1982)\textsuperscript{11}** | |
| Patient population | Adults, abdominal surgery |
| N | 5 |
| Age, y | 36 ± 4 |
| Weight, kg | 64 ± 3 |
| Male | 0 (0%) |
| Dose | 10 µg/kg IV bolus |
| AFE | 0.70 |
| **Bjorkman *et al* (2003)\textsuperscript{6}** | |
| Patient population | Adults, abdominal surgery |
| N | 10 |
| Age, y | 55 ± 8 [20-50] |
| Weight, kg | 65.1 [55-78] |
| Male | 9 (90%) |
| Dose | 100 µg/kg 2 min IVinf |
| AFE | 1.17; 1.26 |
| **Gupta *et al* (1995)\textsuperscript{12}** | |
| Health status | Adults, healthy volunteers |
| N | 28 |
| Age, y | NR |
| Weight, kg | NR |
| Male | 28 (100%) |
| Dose | 50 µg/kg; 20 min IVinf |
| AFE | 0.72; 1.13 |
| Study                        | Patient population                           | N  | Age, y  | BMI, kg/m² | Male | Dose              | AFE  |
|------------------------------|----------------------------------------------|----|---------|------------|------|-------------------|------|
| Saari *et al* (2008)¹³      | Adults, healthy volunteers                    | 12 | NR      | NR         |      | 5 µg/kg IV bolus  | 0.96 |
| Ziesenitz *et al* (2013 & 2015)¹⁴,¹⁵ | Adults, healthy volunteers                    | 16 | 32 ± 8  | 23.2 ± 2.2 | 12   | 5 µg/kg IV bolus  | 1.10; 1.19 |
| Singleton *et al* (1987)¹⁶  | Elective surgery pediatric patients           | 7  | 2.7 ± 2.8 | NR         |      | 30.8 ± 0.6 2 min IVinf | 0.63 |

Values shown as mean ± standard deviation [min-max].

AFE, average fold error; BMI, body mass index; IV, intravenous; IVinf, intravenous infusion; NR, not reported; PBPK, physiologically-based pharmacokinetic
Table S2.2. Population Demographics for Pediatric Subjects Receiving Fentanyl in the POP01 Study

| Demographics            | Children without Obesity (N = 2) | Children with Obesity (N = 30) | Combined (N = 32) |
|-------------------------|---------------------------------|--------------------------------|-------------------|
| n, samples              | 3                               | 50                             | 53                |
| Age, years              | 15.6 (13.7, 17.6)               | 11.4 (2.1, 19.8)               | 13.4 (2.1, 19.7)  |
| Age groups              |                                  |                                |                   |
| 2-<6 years              | 0 (0%)                          | 6 (20.0%)                      | 6 (18.5%)         |
| 6-<12 years             | 0 (0%)                          | 9 (30.0%)                      | 9 (28.1%)         |
| 12-21 years             | 2 (100%)                        | 15 (50.0%)                     | 17 (53.1%)        |
| Male                    | 1 (50.0%)                       | 16 (53.3%)                     | 17 (53.1%)        |
| Weight, kg              | 50.9 (48.6, 53.1)               | 49.5 (15.3, 164.4)             | 50.3 (15.1, 164.4) |
| Height, cm              | 148.0 (143.0, 153.0)            | 138.5 (81.0, 180.3)            | 141.1 (81.0, 180.3) |
| BMI, kg/m²              | 23.9 (21.8, 26.0)               | 26.8 (18.4, 51.9)              | 26.2 (18.4, 51.9) |
| BMI percentile, %       | 82.9 (77.9, 87.8)               | 99.2 (95.1, 100.0)             | 99.2 (77.9, 100.0) |
| Extended BMI Percentile, % | 85.9 (81.1, 90.6)        | 124.0 (100.4, 175.1)           | 123.0 (81.1, 175.1) |
| Weight classificationa  |                                  |                                |                   |
| Without obesity         | 2 (100%)                        | 0 (0%)                         | 2 (6.3%)          |
| With obesity, Class I   | 0 (0%)                          | 13 (43.3%)                     | 13 (40.6%)        |
| With obesity, Class II  | 0 (0%)                          | 9 (30.0%)                      | 9 (28.1%)         |
| With obesity, Class III | 0 (0%)                          | 8 (26.7%)                      | 8 (25.0%)         |
| Race                    |                                  |                                |                   |
| White                   | 1 (50%)                         | 20 (66.7%)                     | 21 (65.6%)        |
| Black or African American | 1 (50%)                        | 7 (23.3%)                      | 8 (25.0%)         |
| Asian                   | 0 (0%)                          | 0 (0%)                         | 0 (0%)            |
| American Indian/Alaskan Native | 0 (0%)                      | 0 (0%)                         | 0 (0%)            |
| Native Hawaiian/Pacific Islander | 0 (0%)                    | 0 (0%)                         | 0 (0%)            |
| Other                   | 0 (0%)                          | 0 (0%)                         | 0 (0%)            |
| Multiple races          | 0 (0%)                          | 1 (3.3%)                       | 1 (3.1%)          |
| Ethnicity                | Unknown/Not reported | Hispanic/Latino | Not Hispanic/Latino |
|-------------------------|---------------------|-----------------|---------------------|
|                         | 0 (0%)              | 2 (6.7%)        | 2 (6.3%)            |
| Hispanic/Latino         | 0 (0%)              | 4 (13.3%)       | 4 (12.5%)           |
| Not Hispanic/Latino     | 2 (100.0%)          | 26 (86.7%)      | 28 (87.5%)          |

Values are medians (range) for continuous variables and counts (%) for categorical variables. Demographics recorded at the time of the first study dose were used to calculate descriptive statistics.

aWeight classifications are assigned using conventional definitions: Extended BMI Percentile < 100% is without obesity, 100% - < 120% is Class I obesity, 120% - < 140% is Class II obesity, and ≥ 140% is Class III obesity.

BMI, body mass index; POP01, Pharmacokinetics of Understudied Drugs Administered to Children per Standard of Care study
### Table S2.3. Parameters used in Fentanyl PBPK Model Development

| Parameter                                           | Fentanyl      | Source                  |
|-----------------------------------------------------|---------------|-------------------------|
| **PHYSICOCHEMICAL PROPERTIES**                      |               |                         |
| Molecular weight, g/mol                             | 336.47        | Drug Bank               |
| Effective molecular weight, g/mol                   | 336.47        | ---                     |
| pKa value                                           | 8.99          | Roy & Flynn             |
| Lipophilicity                                       | 3.21          | Optimized               |
| Protein binding partner                             | albumin       | Bista et al             |
| Fraction unbound                                    | 0.11          | Bista et al             |
| Solubility, mg/L                                   | 280           | Roy & Flynn             |
| Solubility reference pH                             | 7.6           | Roy & Flynn             |
| Solubility gain per charge                          | 1000          | Roy & Flynn             |
| Blood to plasma ratio                               | 1.59          | Calculated value\(^a\) |
| **DISTRIBUTION**                                    |               |                         |
| Partition coefficients                              | Rodgers & Rowland | Rodgers et al |\(^b\) |
| Cellular permeabilities                             | PK-Sim\(^c\) Standard | Willmann et al |\(^d\) |
| **METABOLISM**                                      |               |                         |
| CYP3A4                                              |               |                         |
| \textit{In vitro} \(V_{\text{max}}\) for liver microsomes (nmol/min/mg microsomal protein) | 3.60          | Guitton et al           |
| \textit{In vitro} \(K_{\text{m}}\) (µmol/L)        | 35.50         | Guitton et al           |
| Content of CYP3A4 protein in liver microsomes (nmol/mg microsomal protein) | 0.108         | Rodrigues et al         |
| \(k_{\text{cat}}\) (min\(^{-1}\))                   | 33.33         | Calculated value\(^b\) |
| CYP3A5                                              |               |                         |
| \(CL_{\text{spec}}/[E]\), first order (L/µmol/min)  | 24.67         | Optimized               |
| **EXCRETION**                                       |               |                         |
| Plasma clearance (mL/min/kg)                        | 0.48          | Tanaka et al            |
\[ \left( f_{\text{water}_{\text{rbc}}} + f_{\text{lipids}_{\text{rbc}}} \cdot 10^{\log P} + f_{\text{proteins}_{\text{rbc}}} \cdot K_{\text{Prot}} \right) \cdot f_u \cdot HCT \] - HCT + 1; where \( f_{\text{water}_{\text{rbc}}} \) is the fractional volume content of water in blood cells, \( f_{\text{lipids}_{\text{rbc}}} \) is the fractional volume content of lipid in blood cells, \( \log P \) is the lipophilicity measure, \( f_{\text{proteins}_{\text{rbc}}} \) is the fractional volume content of protein in blood cells, \( K_{\text{Prot}} \) is partition coefficient of water to protein, \( f_u \) is the fraction unbound, and \( HCT \) is the hematocrit.

\[ \text{b} \text{ In vitro } V_{\text{max}} \text{ for liver microsomes / Content of CYP3A4 protein in liver microsomes} \]

CL_{\text{spec}}/\text{[E]}, specific clearance to enzyme concentration ratio; CYP, cytochrome P450; \( k_{\text{cat}} \), catalytic activity; \( K_m \), concentration of half-maximal metabolism; PBPK, physiologically-based pharmacokinetic; pKa, negative log of the acid dissociation constant; \( V_{\text{max}} \), maximal rate of metabolism
3 METHADONE

3.1. Methadone Background

Methadone is a commonly used opioid in adults for pain management and treatment of opioid addiction. Methadone is administered as a racemic mixture of R- and S-enantiomers in a variety of intravenous and enteral formulations. The R-enantiomer is responsible for methadone’s opioid effects, acting as an agonist on the μ-opioid receptor, whereas the S-enantiomer is largely inactive but associated with serious adverse events, including QT prolongation and arrhythmias. In children, methadone is commonly used for neonatal abstinence syndrome, opioid weaning process, and severe pain, despite the fact that the FDA product label states that the PKs, safety, and efficacy of methadone has not been established in children.

Methadone is mainly metabolized via demethylation to the inactive metabolite 2-ethylidene-1,5-dimethyl-3,3,3-diphenylpyrrolidine (EDDP), a reaction primarily conducted via CYP2B6 with additional contribution by CYP3A4 and CYP2C19. Methadone PKs exhibit high variability, with reports of a wide range of clearance (1.4 – 126 L/h), volume of distribution (1-8 L/kg) and bioavailability (36-100%) in adults. High variability in PKs is also observed in children, with weight-normalized clearance deceasing (0.32 – 0.13 L/h/kg) and weight-normalized volume of distribution increasing (7.2 - 8.2 L/kg) in children from 2-18 years. Previous population PK models identified CYP2B6 activity and genotype (included as a power categorical covariate on clearance), α1-acid glycoprotein (AAG) concentration, age, and ethnicity as significant covariates on clearance, with different magnitudes of effect per enantiomer.

3.2. Methadone Clinical Data Used

For the adult methadone PBPK model, several previously published studies from healthy subjects and from sickle cell, chronic pain, cancer pain, and methadone maintenance treatment patients were used for model evaluation. Concentration data for racemate, R-methadone, S-methadone, and EDDP following both IV and oral dosing was digitized. In addition to observed data form the clinical studies described next, pediatric concentration versus
time data was digitized from a previously published study that included adolescent healthy volunteers.\textsuperscript{40}

For the pediatric methadone PBPK model, clinical data from both the POP01 and the ‘Pharmacokinetics of Multiple Dose Methadone in Children Treated for Opiate Withdrawal’ (MTH01, ClinicalTrials.gov NCT01945736) studies were used. The POP01 study enrolled children <21 years administered methadone both IV and/or orally and measured R-methadone, S-methadone, and EDDP. The MTH01 study enrolled children ≥90 days and <18 years, administered methadone orally and measured racemic methadone and EDDP. Across both studies, 217 samples (49, 40, 38, and 90 racemate, R-methadone, S-methadone, and EDDP samples, respectively) were available from 33 subjects without obesity, and 130 samples (25, 27, 26, and 52 racemate, R-methadone, S-methadone, and EDDP samples, respectively) from 22 subjects with obesity were available (Table S3.2).

For both the POP01 and MTH01 studies, a validated high performance liquid chromatography-tandem mass spectrometry method was used for quantifying methadone and EDDP concentrations. Both studies were analyzed via a method developed by a central laboratory (OpAns, LLC, Durham, NC, USA). Samples were extracted from plasma by addition of internal standard extraction solution (methadone-d9 in methyl tertiary butyl ether), followed by centrifugation and addition of 20 mM ammonium acetate pH 5.5. For the POP01 study, the R- and S-methadone enantiomers were separated on a ChiralPak AGP column before injection. Samples were injected onto the instrument using mobile phases of water containing 0.2% and methanol containing 0.1% (v/v) acetic acid. The validation range was 0.2 – 200 ng/mL of methadone, with a lower limit of quantitation of 0.1 ng/mL. Accuracy and precision levels were within the FDA bioanalytical assay validation criteria (e.g., ±15%).

Genomic DNA samples were extracted from leftover frozen whole blood samples to genotype two single nucleotide polymorphisms (SNPs) for the CYP2B6 gene, CYP2B6 *4 and CYP2B6 *9. For the POP01 study, genomic DNA samples were genotyped by ACGT, Inc. (Wheeling, IL, USA) using TaqMan SNP genotyping assays on the QuantStudioTM 6 Flex Real-Time polymerase chain reaction (PCR) system using TaqPathTM ProAmpTM Master Mix and the corresponding genotyping assay. For the MTH01 study, samples were genotyped by ILS Genomics, LLC (Morrisville, NC, USA) using validated sequencing and restriction fragment length polymorphism assays, with resulting PCR product assessed by gel electrophoresis imaged
by ultraviolet light. Positive controls were included for both studies, and the CYP2B6 *6 haplotype status was determined based on the presence of the CYP2B6 *4 and *9 SNP analysis. For the 37 (67%) subjects successfully genotyped, 16 were wildtype (*1/*1), 14 were *1/*6, 4 were *6/*6, 2 were *4/X, and 1 was *1/*9 (Table S3.2).

3.3. Methadone PBPK Model Development

A whole-body methadone adult PBPK model was developed for R- and S-methadone and EDDP by incorporating known physicochemical, clearance, and absorption properties (Table S3.3). Organ to plasma partition coefficients were calculated using the Rodgers and Rowland method. Each enantiomer was modeled separately, incorporating enantiomeric-specific physicochemical and elimination properties for each. Clearance for each enantiomer was modeled as a combination of renal clearance plus metabolism to EDDP via CYP2B6, CYP3A4, and CYP2C19. For CYP enzymes, relative organ contributions were determined using previously reported RT-PCR values, and reference concentrations were taken from the literature. Renal clearance was fixed, whereas absolute CYP clearances were optimized as described in the following paragraph while retaining reported fraction metabolized values. Methadone autoinduction of CYP2B6 and CYP3A4 was incorporated using reported maximum effect values. Renal clearance of EDDP was modeled via renal transport. The parameter $k_{cat}$ was used in model simulations to describe renal transport into the kidney, and it is defined as the ratio of the $V_{max}$ and transporter concentration.

The methadone adult model included a number of optimization steps, all of which involved digitized adult data and the Levenberg-Marquardt algorithm. The first steps involved optimization using digitized data from adults receiving a single IV dose of methadone. First, lipophilicity was optimized. Then, the specific clearance of CYP2B6, CYP2C19, and CYP3A4 were optimized simultaneously whilst holding the ratio of the three constant in order to fix the fraction metabolized to reported values. Both of these steps were performed for R- and S-methadone independently. Next, EDDP digitized data from the same study was used to optimize the $k_{cat}$ value describing EDDP transport into the kidney for renal elimination.

Once these parameters were optimized using adult IV data, only then was the model expanded to describe oral administration of methadone in adults. Digitized data from an adult
study of orally administrated methadone was used to develop the absorption parameters in the model. A Weibull function was found to best fit the absorption profile. A previously reported lag time was implemented, and the dissolution time and shape were optimized using the digitized adult data owing to a lack of reported values for these parameters. Dissolution time and shape were optimized to ensure that the maximum concentration and the time to maximum concentration were adequately captured. No enantiomeric differences were incorporated.

Following development and evaluation of the adult PBPK model, the model was expanded to include pharmacogenomic effects of the \textit{CYP2B6} *6 and *4 alleles using digitized data from a previously reported clinical trial that included 62 healthy adult volunteers with wildtype, *1/*6, *6/*6, and *4/X (representing subjects with a *4 variant plus any other allele) genotypes receiving IV and oral methadone. To account for changes in clearance by \textit{CYP2B6} genotype, the specific clearance to \textit{CYP2B6} concentration ratio (CL\text{spec}/[E]) was optimized for each of the non-wildtype genotypes using the Levenberg-Marquardt algorithm.

Methadone clearance was scaled from adults to children by scaling glomerular filtration (renal clearance) and CYP concentration (hepatic clearance). Renal clearance was scaled as the percentage of adult glomerular filtration rate (GFR) corrected by fraction unbound as shown in \textbf{Equation 3.1}:

\[ CL_{GFR,child} = \frac{CL_{GFR,adult}}{GFR_{child}} \times f_{u,child} \times CL_{GFR,adult} \]  
\( (3.1) \)

where \( CL_{GFR,child} \) is the child’s clearance as a function of GFR, \( GFR_{child} \) is the estimated infant GFR, \( GFR_{adult} \) is the adult GFR (110 mL/min), \( f_{u,child} \) is the fraction unbound in infants, \( f_{u,adult} \) is the fraction unbound in adults, and \( CL_{GFR,adult} \) is the adult clearance as a function of GFR. \( GFR_{child} \) was estimated using a sigmoidal postmenstrual age (PMA, a composite of gestational age [GA] and postnatal age [PNA]) model using \textbf{Equation 3.2}:

\[ GFR_{child} = GFR_{adult} \times \left( \frac{0.74 + PMA^{15.0}}{(44.4^{15.0} + PMA^{15.0})} + 0.26 \right) \]  
\( (3.2) \)

which also incorporates a small fractional offset of 0.26 to correct for the sigmoidal PMA model’s tendency to slightly underestimate the GFR for preterm infants below 32 weeks GA. Hepatic clearance via CYP metabolism was also scaled as a function of PMA by \textbf{Equations 3.3-3.5}:

\[ OSF_{CYP2B6} = 10^{0.3871 \times \log_{10}(PMA) - 0.5412} \]  
\( (3.3) \)

\[ OSF_{CYP3A4} = \frac{PMA^{6.543}}{(72.533^{6.543} + PMA^{6.543})} \]  
\( (3.4) \)
\[ OSF_{CYP2C19} = \frac{PMA^{9.390}}{(35.447^{9.390} + PMA^{9.390})} \]  \hspace{1cm} (3.5)

where \( OSF_{CYP} \) is the ontogeny scaling factor for the corresponding CYP enzyme.\(^{23,54}\) Clearance was then scaled by adjusting adult unbound hepatic intrinsic clearance by age- and enzyme-specific percent of adult activity (ontogeny) using **Equation 3.6**:

\[ CL'_{CYP(child \text{ } g \text{ } liver)} = OSF_{CYP} \times CL'_{CYP(adult \text{ } g \text{ } liver)} \]  \hspace{1cm} (3.6)

where \( CL'_{CYP(child \text{ } g \text{ } liver)} \) is the scaled unbound hepatic intrinsic clearance due to the corresponding CYP per gram of liver, and \( CL'_{CYP(adult \text{ } g \text{ } liver)} \) is the adult unbound hepatic intrinsic clearance due to the corresponding CYP. EDDP clearance was scaled by multiplying the transporter concentration by the scalar presented in **Equation 3.7**:

\[ OSF_{trans} = 0.59e^{-0.185\cdot PMA} + (1 - e^{-0.185\cdot PMA}) \]  \hspace{1cm} (3.7)

where \( OSF_{trans} \) is the ontogeny scaling factor of the transporter.\(^{55}\) Binding to AAG was scaled using a Hill function increase during the maturation phase.\(^{56}\)

Children in the MTH01 study had observed AAG concentrations available (Table S3.2). The majority (82\%) of these measured AAG values were above the healthy reference range, with values up to 5-fold higher.\(^{57}\) Measured AAG was also highly variable, with an observed CV of 54.5\% and 53.3\% for children without and with obesity, respectively. In order to incorporate this variability in AAG concentration within the PBPK model, 50\% CV was added to fraction unbound. Though methadone exhibits relative low extraction, the additional variability on fraction unbound resulted in comparable clearance estimates (within 10\% of model simulations without added variability).

### 3.4. Methadone Model Evaluation

The adult methadone PBPK model captured reported concentrations well, with nearly all studies, patient populations, routes of administration, and molecules (racemate, R-methadone, S-methadone, and EDDP) achieving an AFE within the two-fold range (Table S3.1). The model tended to predict R-methadone best, with AFEs closest to 1.0, and EDDP the worst, with AFEs farthest both above and below 1.0. There were no trends with the route of administration or patient population. Adult population simulations can be found in **Figure S3.1**.

After incorporating changes in clearance with \( CYP2B6 \) genotype, AFE for \( CYP2B6 *1/*1 \) was 1.17, 1.07, and 0.91, for \( CYP2B6 *1/*6 \) was 1.09, 0.71, and 0.85, for \( CYP2B6 *6/*6 \) was
1.03, 0.63, and 1.27, and for CYP2B6 *4/X was 1.88, 1.45, and 2.85 for R-methadone, S-methadone, and EDDP, respectively. When comparing adult PBPK-simulated clearance and volume of distribution to reported values, all reported values were within 1.5-fold except for one reported clearance for the CYP2B6 *4/X genotype, which was within two-fold (Figure S3.2).

The pediatric PBPK model also captured observed concentrations from children without obesity well, with an AFE of 1.32, 0.97, 0.89, 1.93 for racemate, R-methadone, S-methadone, and EDDP, respectively. There were no observable trends in AFE with age or body size (Figure S3.3). Sixty-three percent (12.2% above, 24.5% below), 82.5% (5.0% above, 12.5% below), 94.7% (0% above, 5.3% below), and 42.2% (1.1% above, 56.7% below) of observed concentrations from children without obesity fell within the 90% model prediction interval for racemate, R-methadone, S-methadone, and EDDP, respectively (Figure S3.4-S3.7). Model simulations of digitized data from the pediatric methadone clinical study captured digitized observed data well (Table S3.1, Figure S3.1).

For children with obesity, the pediatric PBPK model had an AFE of 0.58, 1.09, 1.12, and 1.42 for racemate, R-methadone, S-methadone, and EDDP, respectively. There were no observable trends in AFE with age or body size (Figure S3.8). Thirty-two percent (56.0% above, 12.0% below), 85.2% (7.4% above, 7.4% below), 84.6% (7.7% above, 7.7% below), and 59.6% (1.9% above, 38.5% below) of observed concentrations from children with obesity fell within the 90% model prediction interval for racemate, R-methadone, S-methadone, and EDDP, respectively (Figure S3.9-S3.12).

Overall, datasets for both studies fell within the model acceptance criteria, with an AFE of 1.28 and 1.21 for the POP01 and MTH01 studies, respectively. AFE by genotype was within two-fold for wildtype, CYP2B6 *6/*6, and *4/X genotypes (1.31, 0.88, and 2.99, respectively, Figure S3.3, Figure S3.8). There was no observable trend with reported AAG concentration for those subjects with an observed value available.

### 3.5. Methadone Dosing Simulations

Oral dosing of 0.2 mg/kg every 8 hours was simulated for each age group (2-<6 years, 6-<12 years, and 12-18 years; n = 1,000 virtual children each) for children without and with obesity. Steady-state absolute clearance and volume of distribution increased with increasing body size.
for each age group, whereas weight-normalized clearance and volume of distribution decreased (Figure S3.13-S3.15).

Under this recommended dosing, 97.7% and 98.6% of simulated children without and with obesity, respectively, maintained methadone C_{ss,min} above 30 ng/mL, the minimum effective concentration (MEC) reported for adult perioperative and cancer pain patients (Figure S3.16, Table S3.4).^{59,60} Median methadone concentration for children without obesity was within 7.8%, 17.7%, and 16.5% of children with obesity for simulated children 2-<6 years, 6-<12 years, and 12-18 years, respectively, without implementing the recommended dosing cap of 10 mg (Figure S3.17).^{58}

Using this cap, 27.6% (4.4% of children 6-12 years and 78.5% of children >12-18 years) and 45.7% (37.2% of children 6-12 years and 100% of children >12-18 years) of children without and with obesity experienced dose capping. After implementing the dosing cap, 97.7% and 98.1% of simulated children without and with obesity, respectively, maintained methadone concentrations above the MEC (Figure S3.16, Table S3.4). Median methadone concentration for children without obesity was within 7.8%, 9.7%, and 8.6% of children with obesity 2-<6 years, 6-<12 years, and 12-18 years, respectively, with the dosing cap (Figure S3.17).

### 3.6. Methadone Dosing Simulations – Pharmacogenomic Sub-Analysis

Model population simulations were performed to generate hypotheses for methadone dose adjustments necessary for CYP2B6 *6/*6 pediatric patients to achieve exposure similar to wildtype. A virtual population of 1,000 children was created for each of three age groups: 2-<6 years, 6-<12 years, and 12-18 years. Recommended oral dosing of 0.2 mg/kg every 8 hours was simulated for each age group.^{58} Various alternative dosing regimens were simulated using demographic-matched virtual populations with the CYP2B6 *6/*6 genotype to identify which dosing regimen produced a concentration versus time profile similar to wildtype counterparts. For each virtual population representing the different age groups and CYP2B6 genotypes, evaluation of target attainment was assessed as defined in the section above (≥ 90% of virtual children achieving a C_{ss,min} ≥ 30 ng/mL, the MEC reported from adult perioperative and cancer pain patients).^{59,60}

Methadone model simulations in children using recommended oral dosing of 0.2 mg/kg every 8 hours showed that children with the CYP2B6 *6/*6 genotyped had significantly higher
and more variable steady-state methadone exposure (Figure S3.18). Steady-state exposure was approximately matched to that of wildtype subjects when halving the recommended dose for \textit{CYP2B6} *6/*6 subjects to 0.1 mg/kg.

For wildtype and \textit{CYP2B6} *6/*6 genotypes, 97.9\% and 99.8\% of simulated virtual subjects across all age groups (n = 3,000 total) were above the target $C_{ss,min}$ at 80 hours, respectively, using the recommended oral dosing of 0.2 mg/kg every 8 hours for both genotypes. Approximately 96\% of \textit{CYP2B6} *6/*6 virtual subjects of all ages still met the target $C_{ss,min}$ when using the adjusted dosing regimens of half the recommended dose (0.1 mg/kg) in order to better match wildtype exposure.
3.7. Methadone Supplementary Figures

(a) Dale, IV - Methadone

(b) Dale, IV - EDDP

(c) Dale, PO - Methadone

(d) Dale, PO - EDDP

(e) Horst adults, IV - R-Methadone

(f) Horst adults, IV - S-Methadone

(g) Horst adults, IV - EDDP

(h) Inturrisi (1987), IV - Methadone, P1
Figure S3.1. Population simulations (n = 500) of methadone concentrations digitized from six adult studies (a – v) and one pediatric study (w – y).\textsuperscript{40,45–47,61,62} Shaded regions represent the 90% model prediction interval, and points are digitized observed methadone concentrations.

EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; IV, intravenous; P, patient; PO, oral
Figure S3.2. PBPK model simulated clearance (a) and volume of distribution (b) versus reported values from literature for adults receiving methadone. Long and short dashes represent 1.5- and 2-fold error, respectively. The solid line is the line of unity for reference. IV, intravenous; PBPK, physiologically-based pharmacokinetic; PO, oral; \( V_d \), volume of distribution.
**Figure S3.3.** AFE for pediatric subjects without obesity who received methadone plotted versus age, body size, and *CYP2B6* genotype. Dashed lines represent 2-fold error for reference. AFE was calculated using median simulated concentration. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese. Boxes represent the median and IQR, and whiskers extend to 1.5*IQR with further outlying values represented as points.

AFE, average fold error; BMI, body mass index; CYP, cytochrome P450; Ext., extended; IQR, interquartile range; MTH01, Pharmacokinetics of Multiple Dose Methadone in Children Treated for Opiate Withdrawal study; NA, not applicable (not reported); POP01, Pharmacokinetics of Understudied Drugs Administered to Children per Standard of Care study
**Figure S3.4.** Population simulations (n = 250) of racemate methadone concentrations using ‘individualized populations’ for each observed pediatric subject without obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
Figure S3.5. Population simulations (n = 250) of R-methadone methadone concentrations using ‘individualized populations’ for each observed pediatric subject without obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
Figure S3.6. Population simulations (n = 250) of S-methadone methadone concentrations using ‘individualized populations’ for each observed pediatric subject without obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
Figure S3.7. Population simulations (n = 250) of EDDP concentrations using ‘individualized populations’ for each observed pediatric subject without obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.

EDDP, 2-ethyldene-1,5-dimethyl-3,3-diphenylpyrrolidine
Figure S3.8. AFE for pediatric subjects with obesity who received methadone plotted versus age, body size, and CYP2B6 genotype. Dashed lines represent 2-fold error for reference. AFE was calculated using median simulated concentration. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese. Boxes represent the median and IQR, and whiskers extend to 1.5*IQR with further outlying values represented as points.

AFE, average fold error; BMI, body mass index; CYP, cytochrome P450; Ext., extended; IQR, interquartile range; MTH01, Pharmacokinetics of Multiple Dose Methadone in Children Treated for Opiate Withdrawal study; NA, not applicable (not reported); POP01, Pharmacokinetics of Understudied Drugs Administered to Children per Standard of Care study
Figure S3.9. Population simulations (n = 250) of racemate methadone concentrations using ‘individualized populations’ for each observed pediatric subject with obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
Figure S3.10 Population simulations (n = 250) of R-methadone concentrations using ‘individualized populations’ for each observed pediatric subject with obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
Figure S3.11. Population simulations (n = 250) of S-methadone concentrations using ‘individualized populations’ for each observed pediatric subject with obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.
**Figure S3.12.** Population simulations (n = 250) of EDDP concentrations using ‘individualized populations’ for each observed pediatric subject with obesity. The shaded regions are the 90% model prediction interval, which are overlaid with points representing observed plasma concentrations. Solid lines are the median simulated concentration.

EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
Figure S3.13. Changes in simulated weight-normalized and absolute clearance (a-b) and volume of distribution (c-d) with increasing body size, or Extended BMI Percentile for virtual children aged 2–6 years with and without obesity. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese. Virtual children received 0.2 mg/kg PO doses every 8 hours. Gray points represent simulated values for virtual children. Solid blue lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; CL, clearance; PO, oral; V_d, volume of distribution
**Figure S3.14.** Changes in simulated weight-normalized and absolute clearance (a-b) and volume of distribution (c-d) with increasing body size, or Extended BMI Percentile for virtual children aged 6–12 years with and without obesity. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese. Virtual children received 0.2 mg/kg PO doses every 8 hours. Gray points represent simulated values for virtual children. Solid blue lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; CL, clearance; PO, oral; V_d, volume of distribution
Figure S3.15. Changes in simulated weight-normalized and absolute clearance (a-b) and volume of distribution (c-d) with increasing body size, or Extended BMI Percentile for virtual children aged 12-18 years with and without obesity. Extended BMI Percentile is calculated as BMI percentile for a subject's age and sex divided by 95%, where children with an Extended BMI Percentile ≥100% are considered obese. Virtual children received 0.2 mg/kg PO doses every 8 hours. Gray points represent simulated values for virtual children. Solid blue lines represent the central tendency, which is the Loess line as calculated by the generalized additive model.

BMI, body mass index; CL, clearance; PO, oral; V_d, volume of distribution
**Figure S3.16.** Boxplots of simulated methadone $C_{\text{ss,min}}$ in virtual children with and without obesity (n = 1,000 each for each age group). All virtual children received oral dosing of 0.2 mg/kg every 8 hours without (a) and with (b) a dose cap of 10 mg. Boxes represent the median and IQR, and whiskers extend to the minimum and maximum values. Dashed lines represent the target $C_{\text{ss,min}}$ for efficacy (30 ng/mL), the MEC reported for adults with perioperative and cancer pain. $C_{\text{ss,min}}$, steady-state minimum concentration; IQR, interquartile range; MEC, minimum effective concentration.
Figure S3.17. Boxplots of simulated methadone \( \text{AUC}_{\text{ss}} \) in virtual children with and without obesity (\( n = 1,000 \) each for each age group). All virtual children received oral dosing of 0.2 mg/kg every 8 hours without (a) and with (b) a dose cap of 10 mg. Boxes represent the median and IQR, and whiskers extend to the minimum and maximum values.

\( \text{AUC}_{\text{ss}} \), steady-state area under the concentration time curve from 0 to 8 hours; IQR, interquartile range
**Figure S3.18.** Methadone simulation (n = 1,000 virtual children for each age group) of current recommended oral dosing (0.2 mg/kg every 8 hours) for CYP2B6 *1/*1 and *6/*6 (a, c, e) as well as dosing adjustments of 0.1 mg/kg for CYP2B6 *6/*6 (b, d, f) to match wildtype *1/*1 exposure. Shaded regions represent the 90% PBPK model prediction interval. The dashed line represents the target \( C_{ss,\text{min}} \) of 30 ng/mL, the MEC for adult perioperative and cancer pain patients.\(^{59,60}\)

\( C_{ss,\text{min}} \), steady-state trough concentration; CYP, cytochrome P450; MEC, minimum effective concentration; PBPK, physiologically-based pharmacokinetic
### 3.8. Methadone Supplementary Tables

**Table S3.1.** Population Demographics and PBPK Model Simulation Results for Digitized Methadone Studies

| Demographics | Value |
|--------------|-------|
| **Dale *et al* (2002)***
  | Patient population | Adults, healthy volunteers |
| N | 8 |
| Age, y\(^a\) | (19-33) |
| Weight, kg\(^b\) | 74 (59, 99), males |
| | 59 (57-61), females |
| Male\(^c\) | 6 (75%) |
| Dose, mg | 10, IV and PO |
| AFE | |
| IV, Methadone | 0.70 |
| IV, EDDP | 0.64 |
| PO, Methadone | 0.55 |
| PO, EDDP | 0.24 |

**Horst *et al* (2016)***

| Patient population | Adults, sickle cell disease |
| N | 12 |
| Age, y\(^d\) | 24.5 ± 6.3 |
| Weight, kg | NR |
| Male\(^c\) | 8 (67%) |
| Dose, mg/kg | 0.1 (IV) |
| AFE | |
| IV, R-Methadone | 0.79 |
| IV, S-Methadone | 0.63 |
| IV, EDDP | 1.66 |

**Inturrisi *et al* (1987)***

| Health status | Adults, chronic pain |
|                          |       |
|--------------------------|-------|
| N                        | 8     |
| Age, y<sup>d</sup>       | 50 ± 12 |
| Weight, kg<sup>d</sup>   | 66 ± 28 |
| Male<sup>c</sup>         | 3 (38%) |
| Dose, mg                 | 10-30 (IV) |
| AFE                      |       |
| IV, Methadone            | 0.64  |

Inturrisi <i>et al</i> (1990)<sup>47</sup>

|                          |       |
|--------------------------|-------|
| Patient population       | Adults, cancer pain |
| N                        | 15    |
| Age, y<sup>d</sup>       | 48 ± 18 |
| Weight, kg<sup>d</sup>   | 63 ± 14 |
| Male<sup>c</sup>         | 11 (73%) |
| Dose, mg                 | 2-25 (IV inf, 3-4.5 h) |
| AFE                      |       |
| IV, Methadone            | 0.71  |

Kharasch <i>et al</i> (2009)<sup>45</sup>

|                          |       |
|--------------------------|-------|
| Patient population       | Adults, healthy volunteers |
| N                        | 12    |
| Age, y<sup>e</sup>       | 23 (18, 34) |
| Weight, kg<sup>e</sup>   | 68 (50, 95) |
| Male<sup>c</sup>         | 6 (50%) |
| Dose, mg                 | 9.86 (IV), 5.3 (PO) |
| AFE                      |       |
| IV, R-Methadone          | 0.75  |
| IV, S-Methadone          | 0.72  |
| IV, EDDP                 | 1.71  |
| PO, R-Methadone          | 0.98  |
| PO, S-Methadone          | 0.69  |
| PO, EDDP                 | 0.45  |
| Meresaar et al (1981)\textsuperscript{62} |          |
|---------------------------------|----------|
| Patient population              | Adults, methadone maintenance treatment |
| N                               | 8        |
| Age, y\textsuperscript{c}       | 25 (21, 29) |
| Weight, kg\textsuperscript{c}   | 69 (56, 79) |
| Male\textsuperscript{c}         | 7 (88%)  |
| Dose, mg                        | 20, IV and PO (simultaneous) |
| AFE                             |          |
| IV, Methadone                   | 0.72     |
| PO, Methadone                   | 1.06     |

| Horst et al (2016)\textsuperscript{40} |
|---------------------------------|----------|
| Patient population              | Pediatrics, sickle cell disease |
| N                               | 12       |
| Age, y\textsuperscript{d}       | 14.5 ± 2.3 |
| Weight, kg\textsuperscript{d}   | NR       |
| Male\textsuperscript{f}         | 9 (75%)  |
| Dose, mg/kg                     | 0.1 (10min IVinf) |
| AFE                             |          |
| IV, R-Methadone                 | 0.77     |
| IV, S-Methadone                 | 0.72     |
| IV, EDDP                        | 1.19     |

\textsuperscript{a}(range); \textsuperscript{b}median ± standard deviation; \textsuperscript{c}n (%); \textsuperscript{d}mean ± standard deviation; \textsuperscript{e}mean (range) \textsuperscript{f}median (interquartile range)

AFE, average fold error; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; IV, intravenous; IV inf, intravenous infusion; NR, not reported; PBPK, physiologically-based pharmacokinetic; PO, oral
Table S3.2. Population Demographics for Pediatric Subjects Receiving Methadone in the POP01 and MTH01 Studies and Combined

| Demographics | Children without Obesity (N = 33) | Children with Obesity (N = 22) | Combined (N = 55) |
|--------------|-----------------------------------|-------------------------------|------------------|
| n, samples   | 220                               | 130                           | 350              |
| Age, years   | 7.5 (2.1, 19.01)                  | 12.8 (2.1, 17.3)              | 10.2 (2.1, 19.01) |
| Age groups   |                                   |                               |                  |
| 2-<6 years   | 13 (39.4%)                        | 4 (18.2%)                     | 17 (30.9%)       |
| 6-<12 years  | 11 (33.3%)                        | 6 (27.3%)                     | 17 (30.9%)       |
| 12-21 years  | 9 (27.3%)                         | 12 (54.5%)                    | 21 (38.2%)       |
| Male         | 15 (45.5%)                        | 11 (50.0%)                    | 26 (47.3%)       |
| Weight, kg   | 22.6 (8.1, 82.0)                  | 84.8 (14.3, 159.0)            | 31.6 (8.1, 159.0) |
| Height, cm   | 122.0 (75.5, 180)                 | 157.2 (81.0, 189.0)           | 133.0 (75.5, 189.0) |
| BMI, kg/m²   | 15.7 (12.7, 30.1)                 | 27.2 (19.2, 62.1)             | 19.2 (12.7, 62.1) |
| BMI percentile, % | 28.1 (0, 94.0) | 98.8 (95.0, 100.0) | 86.0 (0, 100.0) |
| Extended BMI Percentile, % | 76.1 (50.2, 97.1) | 116.5 (99.9, 257.0) | 89.4 (50.2-257.0) |
| Weight classification |                                 |                               |                  |
| Without obesity | 33 (100%)                        | 0 (0%)                        | 33 (60.0%)       |
| With obesity, Class I | 0 (0%)                     | 12 (54.5%)                    | 12 (21.8%)       |
| With obesity, Class II | 0 (0%)                      | 5 (22.7%)                     | 5 (9.1%)         |
| With obesity, Class III | 0 (0%)                      | 5 (22.7%)                     | 5 (9.1%)         |
| Race          |                                   |                               |                  |
| White         | 24 (72.7%)                        | 17 (77.3%)                    | 41 (74.5%)       |
| Black or African American | 6 (18.2%)                 | 4 (18.2%)                     | 10 (18.2%)       |
| Asian         | 0 (0%)                            | 0 (0%)                        | 0 (0%)           |
| American Indian/Alaskan Native | 0 (0%)                  | 0 (0%)                        | 0 (0%)           |
| Native Hawaiian/Pacific Islander | 0 (0%)              | 0 (0%)                        | 0 (0%)           |
| Other         | 0 (0%)                            | 0 (0%)                        | 0 (0%)           |
| Multiple races | 3 (9.1%)                        | 1 (4.5%)                      | 4 (7.3%)         |
| Unknown/Not reported | 0 (0%) | 0 (0%) | 0 (0%) |
|----------------------|--------|--------|--------|
| **Ethnicity**        |        |        |        |
| Hispanic/Latino      | 4 (12.1%) | 0 (0%) | 4 (7.3%) |
| Not Hispanic/Latino  | 29 (87.9%) | 21 (95.5%) | 50 (90.9%) |
| Unknown/Not Reported | 0 (0%) | 1 (4.5%) | 1 (1.8%) |
| **CYP2B6 Genotype**  |        |        |        |
| *1/*1                | 15 (45.5%) | 1 (4.5%) | 16 (29.1%) |
| *1/*6                | 9 (27.3%) | 5 (22.7%) | 14 (25.5%) |
| *6/*6                | 2 (6.1%) | 2 (9.1%) | 4 (7.3%) |
| *4/X                 | 1 (3.0%) | 1 (4.5%) | 2 (3.6%) |
| *1/*9                | 0 (0%) | 1 (4.5%) | 1 (1.8%) |
| **ND**               | 6 (18.2%) | 12 (54.5%) | 18 (32.7%) |
| **N, subjects per study** |        |        |        |
| POP01                | 22 (66.7%) | 16 (72.7%) | 38 (69.1%) |
| MTH01                | 11 (33.3%) | 6 (27.3%) | 17 (30.9%) |
| **AAG, mg/mL**       | 2.23 (1.02-5.76) [66.7%] | 2.43 (1.68-5.74) [72.7%] | 2.30 (1.02-5.76) [69%] |

Values are medians (range) [percent missing] for continuous variables and counts (%) for categorical variables. Demographics recorded at the time of the first study dose were used to calculate descriptive statistics.

*_Weight classifications are assigned using conventional definitions: Extended BMI Percentile < 100% is without obesity, 100% - < 120% is Class I obesity, 120% - < 140% is Class II obesity, and ≥ 140% is Class III obesity._

AAG, α1-acid glycoprotein; BMI, body mass index; CYP, cytochrome P450; MTH01, Pharmacokinetics of Multiple Dose Methadone in Children Treated for Opiate Withdrawal study; ND, not determined; POP01, Pharmacokinetics of Understudied Drugs Administered to Children per Standard of Care study.
Table S3.3. Parameters used in Methadone PBPK Model Development

| Parameter                          | R-Methadone | S-Methadone | EDDP   | Source          |
|------------------------------------|-------------|-------------|--------|----------------|
| **PHYSICOCHEMICAL PROPERTIES**     |             |             |        |                |
| Molecular weight, g/mol            | 309.40      | 309.40      | 277.41 | Wishart *et al* |
| Effective molecular weight, g/mol  | 309.40      | 309.40      | 277.41 | Wishart *et al* |
| pKa value                          | 9.20        | 9.20        | ---    | Wishart *et al* |
| Compound type                      | weak base   | weak base   | ---    | Wishart *et al* |
| Lipophilicity                      | 2.89        | 2.89        | 2.30   | Optimized      |
| Protein binding partner            | AAG         | AAG         | AAG    | Yang *et al*   |
| Fraction unbound                   | 0.14        | 0.10        | 0.14   | Yang *et al*   |
| Fraction unbound CV, %             | 50          | 50          | 50     | Optimized      |
| Solubility, mg/mL                  | 120         | 120         | 120    | Wishart *et al* |
| Solubility reference pH            | 7.0         | 7.0         | 7.0    | Wishart *et al* |
| Solubility gain per charge         | 1000        | 1000        | 1000   | Wishart *et al* |
| Blood to plasma ratio              | 1.19        | 1.01        | 0.74   | Calculated value^a |
| **ABSORPTION**                     |             |             |        |                |
| Dissolution function               | Weibull     | Weibull     | ---    | Optimized      |
| Dissolution time, min              | 85          | 85          | ---    | Optimized      |
| Dissolution shape                  | 0.92        | 0.92        | ---    | Optimized      |
| Lag time, h                        | 0.30        | 0.30        | ---    | Foster *et al* |
| Specific intestinal permeability, cm/min | 7.69e⁻⁵   | 7.69e⁻⁵   | 3.23e⁻⁵ | Calculated value^b |
| Specific organ permeability, cm/min | 0.03        | 0.03        | 0.01   | Calculated value^c |
| DISTRIBUTION | Rodgers & Rowland | Rodgers & Rowland | Rodgers & Rowland | Rodgers et al<sup>18</sup> |
|--------------|-------------------|-------------------|-------------------|--------------------------|
| Cellular permeabilities | PK-Sim® Standard | PK-Sim® Standard | PK-Sim® Standard | Willmann et al<sup>19,20</sup> |
| Renal transporter | | | | |
| Concentration, μmol/L | --- | --- | 3.0 | Campbell et al<sup>50</sup> |
| V<sub>max</sub>, μmol/min/L | --- | --- | 128.9 | Campbell et al<sup>50</sup> |
| K<sub>m</sub>, μM | --- | --- | 6.3 | Campbell et al<sup>50</sup> |
| k<sub>cat</sub>, 1/min | --- | --- | 43.0 | Optimized<sup>d</sup> |
| METABOLISM | | | | |
| CYP2B6 | | | | |
| Concentration, μmol/L | 1.56 | 1.56 | --- | Rodrigues et al<sup>29</sup> |
| Fraction metabolized | 0.44 | 0.59 | --- | Ke et al<sup>49</sup> |
| Specific clearance, 1/min | 0.15 | 0.24 | --- | Optimized |
| Clearance, L/μmol/min | 0.09 | 0.16 | --- | Optimized |
| CYP2B6 *1/*6 | | | | |
| Concentration, μmol/L | 1.56 | 1.56 | --- | Rodrigues et al<sup>29</sup> |
| Specific clearance, 1/min | 0.15 | 0.24 | --- | Optimized |
| Clearance, L/μmol/min | 0.09 | 0.12 | --- | Optimized |
| CYP2B6 *6/*6 | | | | |
| Concentration, μmol/L | 1.56 | 1.56 | --- | Rodrigues et al<sup>29</sup> |
| Specific clearance, 1/min | 0.15 | 0.24 | --- | Optimized |
| Clearance, L/μmol/min | 0.05 | 0.04 | --- | Optimized |
| **CYP2B6 *4/X** | | | | | |
|---|---|---|---|---|
| Concentration, μmol/L | 1.56 | 1.56 | --- | Rodrigues et al 29 |
| Specific clearance, 1/min | 0.15 | 0.24 | --- | Optimized |
| Clearance, L/μmol/min | 0.37 | 0.51 | --- | Optimized |

| **CYP2C19** | | | | | |
|---|---|---|---|---|
| Concentration, μmol/L | 0.76 | 0.76 | --- | Rodrigues et al 29 |
| Fraction metabolized | 0.09 | 0.09 | --- | Ke et al 49 |
| Specific clearance, 1/min | 0.03 | 0.04 | --- | Optimized |
| Clearance, L/μmol/min | 0.04 | 0.05 | --- | Optimized |

| **CYP3A4** | | | | | |
|---|---|---|---|---|
| Concentration, μmol/L | 4.32 | 4.32 | --- | Rodrigues et al 29 |
| Fraction metabolized | 0.46 | 0.32 | --- | Ke et al 49 |
| Specific clearance, 1/min | 0.15 | 0.13 | --- | Optimized |
| Clearance, L/μmol/min | 0.04 | 0.03 | --- | Optimized |

| **CYP2B6 autoinduction** | | | | | |
|---|---|---|---|---|
| E<sub>max</sub> | 2.1 | 2.1 | --- | Campbell et al 50 |
| EC<sub>50</sub>, μmol/L | 0.01 | 0.01 | --- | Optimized |

| **CYP3A4 autoinduction** | | | | | |
|---|---|---|---|---|
| E<sub>max</sub> | 2.5 | 2.5 | --- | Campbell et al 50 |
| EC<sub>50</sub>, μmol/L | 0.01 | 0.01 | --- | Optimized |

| **EXCRETION** | | | | | |
|---|---|---|---|---|
| Renal clearance, mL/min | 27.4 | 15.1 | Sink<sup>e</sup> | Foster et al 38 |
\[ a\left( f_{\text{water}_{\text{rbc}}} + f_{\text{lipids}_{\text{rbc}}} \times 10^{\log P} + f_{\text{proteins}_{\text{rbc}}} \times K_{\text{Prot}} \right) \times f_u \times HCT \] − HCT + 1; where \( f_{\text{water}_{\text{rbc}}} \) is the fractional volume content of water in blood cells, \( f_{\text{lipids}_{\text{rbc}}} \) is the fractional volume content of lipid in blood cells, \( \log P \) is the lipophilicity measure, \( f_{\text{proteins}_{\text{rbc}}} \) is the fractional volume content of protein in blood cells, \( K_{\text{Prot}} \) is partition coefficient of water to protein, \( f_u \) is the fraction unbound, and \( HCT \) is the hematocrit.

\[ b\ 266 \times \left( M_{\text{W}_{\text{eff}}} \times 10^9 \right)^{-4.5} \times 10^{\log P} \times 60 \times 10^{-1}; \] where \( M_{\text{W}_{\text{eff}}} \) is the effective molecular weight and \( \log P \) is the lipophilicity measure.

\[ c\left( \frac{M_{\text{W}_{\text{eff}}} \times 10^9}{336} \right)^{-6} \times \frac{10^{\log P}}{5} \times 10^{-5}; \] where \( M_{\text{W}_{\text{eff}}} \) is the effective molecular weight and \( \log P \) is the lipophilicity measure.

\[ d\ V_{\text{max}} / \text{Transporter concentration} \]

\[ e\ EDDP \text{ renal clearance set arbitrarily high (sink) to reflect it’s known formation rate limited clearance.} \]

AAG, \( \alpha_1 \)-acid glycoprotein; CYP, cytochrome P450; EC_{50}, concentration at half maximal autoinduction effect; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; \( E_{\text{max}} \), maximum autoinduction effect; \( k_{\text{cat}} \), catalytic activity; \( K_m \), concentration of half-maximal metabolism or transport; PBPK, physiologically-based pharmacokinetic; pKa, negative log of the acid dissociation constant; \( V_{\text{max}} \), maximal rate of metabolism or transport
### Table S3.4. Methadone Target Achievement in Virtual Children with and without Obesity

| Target\(^a\) | **Children without Obesity** | **Children with Obesity** |
|--------------|-----------------------------|--------------------------|
|              | 2–<6 years | 6–<12 years | 12–18 years | 2–<6 years | 6–<12 years | 12–18 years |
| **NO DOSE CAPPING\(^b\)** | | | | | | |
| Efficacy    | \(C_{\text{ss,min}} \geq 30 \text{ ng/mL}\) | 95.2\% | 98.4\% | 99.6\% | 97.0\% | 99.4\% | 99.4\% |
| **DOSE CAPPING\(^b\)** | | | | | | |
| Efficacy    | \(C_{\text{ss,min}} \geq 30 \text{ ng/mL}\) | 95.2\% | 98.6\% | 99.4\% | 97.0\% | 99.2\% | 98.2\% |

\(^a\)The efficacy target for methadone is a \(C_{\text{ss,min}} \geq 30 \text{ ng/mL}\), the MEC reported for adult perioperative and cancer pain patients.\(^{59,60}\)

\(^b\)All virtual children received oral dosing of 0.2 mg/kg every 8 hours, with or without a dose cap of 10 mg.\(^{58}\)

\(C_{\text{ss,min}}\), steady-state minimum concentration; MEC, minimum effective concentration
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