

MESTRADO
EVIDÊNCIA E DECISÃO EM SAÚDE

PBPK Modeling to Predict Pharmacokinetics of Indomethacin and Phenytoin: A Focus on Pregnancy and Enzyme-Mediated Drug-Drug Interactions

Mariana Miranda Godinho

M

2025





Agradecimentos

Ao longo da realização desta dissertação, tive o privilégio de contar com o apoio, orientação e incentivo de pessoas fundamentais, sem as quais este trabalho não teria sido possível.

Em primeiro lugar, gostaria de expressar a minha mais profunda gratidão ao meu orientador, Professor Doutor Nuno Vale, pela confiança depositada em mim, pela orientação científica e pela constante disponibilidade. A sua dedicação e incentivo foram determinantes para ultrapassar cada desafio deste percurso.

Um agradecimento muito especial à Lara Marques, pela sua constante disponibilidade, dedicação e pela forma generosa com que partilhou os seus conhecimentos ao longo deste percurso. O seu apoio incansável, aliado à prontidão em ajudar sempre que necessário, representou um contributo inestimável e determinante para a concretização desta dissertação.

Aos meus pais, o mais sincero e profundo agradecimento. Por todo o apoio incondicional e por todos os valores que me transmitiram e que me guiaram até aqui. Todo o esforço, dedicação e amor que me deram são a base do meu percurso académico e pessoal.

À minha irmã, agradeço de forma especial pelo apoio, força e compreensão que sempre demonstrou ao longo do meu percurso.

Às minhas amigas, Ana e Diana, agradeço de forma especial pela amizade genuína, pela compreensão nos momentos difíceis e por todos os momentos de alegria que partilhamos.

Ao meu namorado Luís Monteiro, agradeço profundamente todo o apoio, carinho, compreensão e motivação ao longo deste caminho. A presença constante, as palavras de encorajamento e a capacidade de me transmitir força mesmo nos momentos mais desafiantes foram determinantes para que nunca perdesse o foco e acreditasse sempre que seria capaz de alcançar esta meta.

Por fim, a todos os que, de forma direta ou indireta, contribuíram para a realização desta etapa, deixo o meu sincero agradecimento

Abstract

The use of medications during pregnancy is very common, however, for most drugs available data on pharmacokinetics (PK) and safety in this population remain limited. Pregnant women are rarely included in clinical trials, and the physiological changes during pregnancy significantly affect drug absorption, distribution, metabolism and excretion (ADME). This limitation can result in unpredictable drug levels, with risks of underexposure or toxicity. Physiologically based pharmacokinetic (PBPK) modelling emerges as a promising tool to overcome this limitation, enabling the prediction of drug disposition in specific contexts.

The purpose of this dissertation was to develop and validate PBPK models for indomethacin and phenytoin, assessing the impact of age, different dosing regimens, pregnancy, and potential drug-drug interactions (DDIs). Models were built from scratch in GastroPlus™ v9.8.3 and validated with clinical data.

Indomethacin exhibited linear pharmacokinetics, with systemic exposure proportional to dosing frequency, whereas phenytoin exhibited nonlinear behavior, with fractionated regimens of 100 mg every 8 hours resulting in greater exposure compared to a single daily dose of 300 mg every 24 hours. During pregnancy, indomethacin exposure decreased, while phenytoin exhibited variable exposure.

The DDI study, in which phenytoin acted as a perpetrator and indomethacin as a victim, revealed no clinically relevant alterations in non-pregnant women, despite both drugs being metabolized by the CYP2C9 enzyme.

PBPK modelling proved to be effective in predicting pharmacokinetic changes in underrepresented populations, emphasizing the need for personalized treatment strategies and demonstrating the value of *in silico* models to promote safer pharmacotherapy during pregnancy.

Resumo

O uso de medicamentos durante a gravidez é muito frequente, contudo, para a maioria, os dados disponíveis de farmacocinética e segurança nesta população permanecem limitados. As mulheres grávidas raramente são incluídas em ensaios clínicos, e as adaptações fisiológicas da gestação afetam profundamente a absorção, distribuição, metabolismo e excreção dos fármacos. Esta lacuna reflete um risco de exposições subterapêuticas ou tóxicas. A modelação farmacocinética baseada na fisiologia (PBPK) surge como uma ferramenta promissora para suprir esta limitação, permitindo prever a disposição de fármacos em contextos específicos.

O objetivo desta dissertação foi desenvolver e validar modelos PBPK para a indometacina e fenetoína, avaliando o impacto da idade, de diferentes regimes de dosagem, da gravidez e de potenciais interações medicamentosas (DDIs). Os modelos foram construídos de raiz no GastroPlus™ v9.8.3 e validados com dados clínicos.

A indometacina apresentou uma farmacocinética linear, com exposição proporcional à frequência de administração, enquanto a fenetoína apresentou um comportamento não linear, em que regimes fracionados de 100 mg de 8 em 8 horas resultaram numa maior exposição do que a administração única diária de 300 mg a cada 24 horas. Durante a gravidez, a exposição da indometacina diminuiu, enquanto a apresentou variabilidade.

O estudo da interação medicamentosa entre a fenetoína como perpetradora e da indometacina como vítima, não revelou alterações relevantes em mulheres não grávidas, apesar de ambos os fármacos serem metabolizados pela enzima CYP2C9.

A modelação PBPK mostrou-se eficaz na previsão de alterações farmacocinéticas em populações sub-representadas, mostrando a importância de abordagens individualizadas e evidenciando o contributo dos modelos *in silico* para uma utilização mais segura de fármacos durante a gravidez.

Table of Contents

Agradecimientos	iv
Abstract	vi
Resumo.....	vii
Table of Contents	viii
List of Figures	x
List of Tables	xii
List of Abbreviations	xiv
1. Introduction.....	1
1.1 The Need for PBPK Modeling in Pregnant Populations	1
1.2 Physiological and Pharmacokinetic Changes During Pregnancy	3
1.3 Enzyme-Mediated Drug-Drug Interactions in Pregnancy	8
1.4 Indomethacin in the context of Pregnancy	9
1.4.1 Chemistry	10
1.4.2 Pharmacokinetics and Metabolism	11
1.4.3 Pharmacodynamic and Mechanism of Action.....	12
1.4.4 Adverse Effects	15
1.5 Phenytoin in Pregnancy.....	16
1.5.1 Chemistry	17
1.5.2 Pharmacokinetics and Metabolism	18
1.5.3 Pharmacodynamics and Mechanism of Action	20
1.5.4 Adverse Effects	22
1.6 Objectives	23
2. Materials and Methods	24
2.1 Data sources and information selection	24
2.2 PBPK model development.....	26
2.3 Model validation	27
2.4 Simulated dosing regimens	27
2.5 Drug–drug interaction (DDI) study.....	27
3. Results and Discussion	28
3.1 Influence of age on pharmacokinetic parameters	31
3.1.1 Indomethacin: impact of age (20–45 years) on pharmacokinetics.....	31

3.1.2 Phenytoin: impact of age (20–45 years) on pharmacokinetics.....	33
3.2 Influence of dosing regimens on pharmacokinetics.....	36
3.2.1 Indomethacin: comparison between dosing regimens (AUC, C _{max} , F _a)	36
3.2.2 Phenytoin: comparison between dosing regimens (AUC, C _{max} , F _a)	38
3.3 Influence of pregnancy on pharmacokinetics.....	40
3.3.1 Effect of pregnancy on indomethacin pharmacokinetics	40
3.3.2. Effect of pregnancy on phenytoin pharmacokinetics	45
3.4 Drug–drug interaction (DDI) evaluation	50
3.4.1 Impact of co-administration on indomethacin pharmacokinetics in non- pregnant women.....	50
3.5 Integrative Discussion.....	54
3.6 Study limitations.....	58
3.7 Future perspectives	60
4.Conclusion	61
References	63
List of Appendices	71
Appendix A – Indomethacin simulations.....	71
Appendix B- Phenytoin simulations	83

List of Figures

Figure 1. Chemical structure of indomethacin.

Figure 2. Metabolism of Indomethacin. Adapted from Gliszczynska & Nowaczyk, 2021 (Molecules, 26, 1576), under CC BY 4.0 license.

Figure 3. Mechanism of Action of Indomethacin. Adapted from Gliszczynska & Nowaczyk, 2021 (Molecules, 26, 1576), under CC BY 4.0 license.

Figure 4. Chemical structure of phenytoin.

Figure 5. Metabolism of Phenytoin. Adapted from Patocka, J., Wu, Q., Nepovimova, E., & Kuca, K. (2020). *Phenytoin – An anti-seizure drug: Overview of its chemistry, pharmacology and toxicology. Food and Chemical Toxicology*, 142, 111393.

Figure 6. Mechanism of Action of Phenytoin. Adapted from Ochoa, J. G. (2024). *Antiepileptic Drugs*. In: Benbadis, S. R. (Ed.), Medscape. Updated June 7, 2024.

Figure 7. Simulated plasma concentration-time curve for indomethacin in the standard PBPK model representing a healthy, fasting, 30-year-old American woman (60 kg) after a single oral dose of 50 mg.

Figure 8. Simulated plasma concentration-time curve for phenytoin in the standard PBPK model representing a healthy, fasting, 30-year-old American woman (60 kg) after a single oral dose of 300 mg.

Figure 9. Simulated PK parameters of indomethacin in non-pregnant women aged 20 to 45 years. The panels illustrate differences between different ages for: (a) T_{max} (h), (b) C_{max} ($\mu\text{g/mL}$), (c) $AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$), (d) AUC_{0-t} ($\mu\text{g}\cdot\text{h/mL}$), (e) liver C_{max} ($\mu\text{g/mL}$), (f) fraction absorbed F_a (%), (g) oral bioavailability F (%), and (h) fraction dissolved in the intestine FDp (%).

Figure 10. Simulated PK parameters of phenytoin in non-pregnant women aged 20 to 45 years. The panels illustrate differences between different ages for: (a) T_{max} (h), (b) C_{max} ($\mu\text{g/mL}$), (c) $AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$), (d) AUC_{0-t} ($\mu\text{g}\cdot\text{h/mL}$), (e) liver C_{max} ($\mu\text{g/mL}$), (f) fraction absorbed F_a (%), (g) oral bioavailability F (%), and (h) fraction dissolved in the intestine FDp (%).

Figure 11. Simulated plasma concentration-time profiles of indomethacin in the standard PBPK model (healthy, fasting, 30-year-old woman, 60 kg) under different dosing regimens: 50 mg q24h, 50 mg q12h, and 50 mg q8h over 240 hours.

Figure 12. Simulated plasma concentration–time profiles of phenytoin in the standard PBPK model (healthy, fasting, 30-year-old woman, 60 kg) under different dosing regimens: 300 mg q24h for 168 h, 100 mg q8h for 168 h, 300 mg q24h for 240 h, and 100 mg q8h for 240 h

List of Tables

Table 1. Predicted physicochemical properties of indomethacin.

Table 2. Predicted physicochemical properties of phenytoin.

Table 3. Validation of the PBPK model for indomethacin through comparison of observed PK parameters with simulated values.

Table 4. Validation of the PBPK model for phenytoin through comparison of observed PK parameters with simulated values.

Table 5. Simulated pharmacokinetic parameters ($C_{\max}$, $AUC_{0-\infty}$, Fa%) of indomethacin under different dosing regimens in the standard PBPK model.

Table 6. Simulated pharmacokinetic parameters ($C_{\max}$, $AUC_{0-\infty}$, Fa%) of phenytoin under different dosing regimens in the standard PBPK model.

Table 7. Simulated pharmacokinetic parameters of indomethacin in 20-year-old pregnant and non-pregnant women.

Table 8. Simulated pharmacokinetic parameters of indomethacin in 25-year-old pregnant and non-pregnant women.

Table 9. Simulated pharmacokinetic parameters of indomethacin in 30-year-old pregnant and non-pregnant women.

Table 10. Simulated pharmacokinetic parameters of indomethacin in 35-year-old pregnant and non-pregnant women.

Table 11. Simulated pharmacokinetic parameters of indomethacin in 40-year-old pregnant and non-pregnant women.

Table 12. Simulated pharmacokinetic parameters of indomethacin in 45-year-old pregnant and non-pregnant women.

Table 13. Simulated pharmacokinetic parameters of phenytoin in 20-year-old pregnant and non-pregnant women.

Table 14. Simulated pharmacokinetic parameters of phenytoin in 25-year-old pregnant and non-pregnant women.

Table 15. Simulated pharmacokinetic parameters of phenytoin in 30-year-old pregnant and non-pregnant women.

Table 16. Simulated pharmacokinetic parameters of phenytoin in 35-year-old

pregnant and non-pregnant women.

Table 17. Simulated pharmacokinetic parameters of phenytoin in 40-year-old pregnant and non-pregnant women.

Table 18. Simulated pharmacokinetic parameters of phenytoin in 45-year-old pregnant and non-pregnant women.

Table 19. Pharmacokinetic parameters of indomethacin in a 20-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

Table 20. Pharmacokinetic parameters of indomethacin in a 25-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

Table 21. Pharmacokinetic parameters of indomethacin in a 30-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

Table 22. Pharmacokinetic parameters of indomethacin in a 35-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

Table 23. Pharmacokinetic parameters of indomethacin in a 40-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

Table 24. Pharmacokinetic parameters of indomethacin in a 45-year-old female simulation under drug–drug interaction (DDI) with phenytoin

List of Abbreviations

AED - Antiepileptic Drug

ADME - Absorption, Distribution, Metabolism and Excretion

ADMET - Absorption, Distribution, Metabolism, Excretion, Toxicity

AUC - Area Under the Curve

AUC_{0-∞} - Area Under the Curve from time zero to infinity

AUC_{0-t} - Area Under the Curve from time zero to last measurable concentration

BCS - Biopharmaceutics Classification System

BCRP - Breast Cancer Resistance Protein

C_{max} - Maximum Plasma Concentration

COX - Cyclooxygenase

COX-1 - Constitutive isoform of Cyclooxygenase

COX-2 - Inducible isoform of Cyclooxygenase

COMT - Catechol-O-Methyltransferase

CYP - Cytochrome P450

CYP1A2 / CYP2A6 / CYP2B6 / CYP2C8 / CYP2C9 / CYP2C18 /

CYP2C19 / CYP2D6 / CYP2E1 / CYP3A4 / CYP3A5 - Specific isoenzymes of Cytochrome P450

DBI-O-deschlorobenzoyl- indomethacin (metabolite)

DMBI-O-desmethyl-N-deschlorobenzoyl- indomethacin (metabolite)

DMI-O-desmethyl-indomethacin (metabolite)

DDI- Drug-Drug Interaction

EPHX1 - Epoxide Hydrolase 1

F_a / F_a% - Fraction Absorbed

FD_p / FD_p% - Fraction Dissolved / Fraction of Dose in Portal Vein

F% - Fraction Bioavailable / Oral Bioavailability

FDA - Food and Drug Administration

MATE1 / MATE2-K - Multidrug and Toxin Extrusion Protein 1 / 2-K

Na⁺ - Sodium Ion

NCBI - National Center for Biotechnology Information

NQO1 - NAD(P)H Quinone Dehydrogenase 1

NSAID - Nonsteroidal Anti-Inflammatory Drug

OATP - Organic Anion Transporting Polypeptides

OCT2 - Organic Cation Transporter 2

PBPK - Physiologically Based Pharmacokinetic

PGG₂ - Prostaglandin G₂

PGH₂ - Prostaglandin H₂

PGE₁ / PGE₂ - Prostaglandin E₁ / E₂

PGI₂ - Prostacyclin (Prostaglandin I₂)

PDA - Patent Ductus Arteriosus

PK – Pharmacokinetics

p-HPPH – 5-(4'-hydroxyphenyl)-5-phenylhydantoin (phenytoin metabolite)

pKa – Acid Dissociation Constant

P-gp – P-glycoprotein

PTL – Preterm Labor

PTB – Preterm Birth

q8h / q12h / q24h – Every 8 / 12 / 24 hours (dosing interval)

S1–S6 – Transmembrane α-helical segments of sodium channels

SJS / TEN / SJS-TEN – Stevens–Johnson Syndrome / Toxic Epidermal Necrolysis

SUDEP – Sudden Unexpected Death in Epilepsy

T_{max} – Time to Maximum Plasma Concentration

UGT / UGT1A1 / UGT1A4 / UGT1A6 / UGT1A9 / UGT2B15 – UDP-Glucuronosyltransferases

V_d – Volume of Distribution

α -subunit – Alpha subunit of sodium channel (pore-forming)

β -subunit – Beta subunit of sodium channel (auxiliary, modulates gating)

P-loop – Pore loop of sodium channel

1. Introduction

1.1 The Need for PBPK Modeling in Pregnant Populations

The use of medication is very common during pregnancy however, most drug labels lack specific information for use during pregnancy (Ke, Greupink, & Abduljalil, 2018). It is estimated that around 90% of pregnant women take at least one medication during pregnancy, and this trend continues to rise (Smolina, Hanley, Mintzes, Oberlander, & Morgan, 2015; Engeland, Bjørge, Klungsøyr, Hjellvik, Skurtveit, & Furu, 2018). Nevertheless, fewer than 10% of these drugs have been adequately studied and labelled for use in pregnancy, which represents a serious gap in the available evidence (Van Calsteren et al., 2016). This lack of data compromises the safety and efficacy of pharmacotherapy in this population and poses a significant public health challenge. The COVID-19 pandemic further highlighted this concern by exposing the scarcity of robust data to guide safe treatment of pregnant women in critical settings (Davis-Floyd, Gutschow, & Schwartz, 2020; Male, 2021; Jaffe, Lyerly, & Goldfarb, 2021).

Pregnant women are rarely included in clinical trials for numerous practical, legal, and ethical reasons. The profound physiological complexity of pregnancy introduces additional variability that complicates trial design and result interpretation (van der Graaf et al., 2018). Legally, historical tragedies such as thalidomide and diethylstilbestrol led to stringent restrictions that discouraged the inclusion of women of reproductive age, and these concerns about liability continue to shape regulatory frameworks (Blehar et al., 2013; Lyerly et al., 2008). Ethically, the principle of fetal protection has often outweighed maternal autonomy, resulting in the systemic exclusion of pregnant women from research despite the injustice of denying them evidence-based treatment (Zur, 2023). As a result, less than 0.5% of clinical trials in the European Union currently include pregnant women (EMA, 2025), although recent draft guidance such as the ICH E21 guideline encourages more inclusive approaches by balancing risks and benefits (FDA, 2025).

Therefore, most medications are prescribed off-label during pregnancy, with dosing typically based on men or non-pregnant women, combined with the clinician's

judgment, often without reliable clinical data on maternal and fetal effects (Ke, Greupink, & Abduljalil, 2018). This reliance on extrapolation highlights a significant gap between clinical practice and scientific evidence, raising concerns about both maternal safety and neonatal outcomes. In many cases, therapeutic decisions are guided by the urgency of the clinical situation than by robust data, reinforcing the vulnerability of this population.

Pregnancy is one of the most complex physiological periods for both the mother and the fetus. It is associated with constant physiological and metabolic changes that significantly affect drug absorption, distribution, metabolism, and excretion (ADME) (Alkema et al., 2016; Say et al., 2014). These alterations are not static, instead, they evolve across trimesters, meaning that the PK behavior of a drug at early gestation may differ substantially from that observed closer to term. This temporal variability further complicates clinical management, as dose adjustments may need to be individualized throughout pregnancy.

Physiologically based pharmacokinetic (PBPK) modelling has gained relevance in the study of chemical compounds and their resulting effects. The increasing development of biomedical information systems, along with support from regulatory agencies, has contributed to the gradual rejection of invasive experimental methods and the preference for *in silico* trials (Ferreira, 2015). Given the profound physiological changes during pregnancy that influence drug pharmacokinetics (PK), more specific and cautious approaches are needed. PBPK models represent a promising tool to predict how pregnant individuals respond to drug administration and has emerged as an especially effective approach for anticipating drug disposition during this time (Miller, Reddy, Heikkinen, Lukacova, & Parrott, 2019). PBPK modelling integrates physiological parameters with drug-specific characteristics and is applied in multiple clinical settings, including dosage optimization, (DDI) assessment, generation of complementary evidence on therapeutic efficacy, clinical trial planning, and regulatory policy development (Ke, Greupink, & Abduljalil, 2018). Despite its growing application, PBPK modelling specifically focused on pregnancy remains relatively limited.

Taken together, these challenges illustrate why pregnancy cannot be treated as a simple extension of non-pregnant physiology. Instead, it requires a deeper

understanding of the unique transformations that occur during gestation.. PBPK modelling, by offering mechanistic insight and predictive capacity, emerges as one of the few methodologies capable of integrating this complexity into clinically useful evidence.

For this reason, the following section explores in detail the physiological and PK changes that characterize pregnancy and their implications for drug disposition

.

1.2 Physiological and Pharmacokinetic Changes During Pregnancy

Pregnancy is a period of profound change in a woman's life, among the most significant are physiologic adaptations that also alter drug PK, from absorption through distribution, metabolism, and excretion. Using standard dosing regimens can lead to reduced efficacy or increased risk of adverse events. This is particularly concerning for drugs with a narrow therapeutic index, where dosing must be precise. Changes in drug handling during pregnancy, combined with the limited number of PK trials in pregnant populations, leave clinicians with an incomplete understanding of the medications they prescribe to pregnant patients and consequently increase the risk of subtherapeutic or toxic concentrations and risk of adverse events (Pinheiro & Stika, 2020).

Pregnancy triggers a broad set of physiological changes that substantially alter hormone levels, fluid and fat volumes, cardiac output, glomerular filtration rate, plasma protein concentrations, and drug-metabolizing enzymes (Pinheiro & Stika, 2020). During pregnancy, progesterone and estrogen concentrations rise substantially. In a non-pregnant reproductive age woman after ovulation, progesterone is about 10 to 35 ng/mL in the luteal phase but during pregnancy it can rise to 100-300 ng/mL (Tal & Taylor, 2024). Estradiol increases even more across pregnancy, early follicular phase concentrations are about 50 pg/mL, whereas during pregnancy they can reach up to about 25,000 pg/mL (Tal & Taylor, 2024). These hormonal changes impact drug pharmacokinetics. Progesterone slows gastric emptying, estrogens increase plasma binding proteins, and together they alter drug absorption and distribution. Both hormones regulate metabolic enzymes, inducing CYP3A4, CYP2D6, and UGT1A4 while inhibiting CYP1A2, and enhance

renal clearance, which frequently reduces systemic drug exposure during pregnancy (Anderson, 2025; Isoherranen & Thummel, 2013; Ke et al., 2018; Pariente et al. 2016).

The volume of distribution (V_d) is influenced by lipophilicity, fluid volume, and protein binding (Wade, 2019). During pregnancy, total body fluid increases in part through estrogen-mediated activation of the renin angiotensin aldosterone system, plasma volume increases by about 1,200 to 1,600 mL, roughly 40% above baseline, beginning at about 6 to 8 weeks and becoming more pronounced with multiple gestations (Halpern et al., 2019; Hytten, 1985).

Concerning cardiac output, there is an increase at about 30 to 50% to compensate for the diversion of 12 to 20% of flow to the placenta. This rise begins around 5 weeks of gestation and peaks at approximately 32 weeks. In twin pregnancies, there may be a further increase of up to about 15% (Meah, Cockcroft, Backx, Shave, & Stohr, 2016; Cunningham et al., 2018; Halpern et al., 2019).

During pregnancy, increased renal blood flow and glomerular filtration rate result in a 30-50% increase in renal clearance (Odutayo & Hladunewich, 2012). Renal plasma flow increases early and substantially, with an increase of about 80% by the end of the first trimester (Cunningham et al., 2018). This higher clearance can reduce plasma concentrations of renally eliminated drugs and or their metabolites (Anderson, 2020).

Maternal renal transporters also change during pregnancy. Upregulation of P-glycoprotein (P-gp) and organic anion transporting polypeptides (OATP) can increase tubular secretion and produce clearance that exceeds what would be predicted from filtration alone (Hebert et al., 2008). There is also greater secretion of metformin, a substrate for OCT2 and the transporters (MATE1 and MATE2 K) (Eyal et al., 2010; Bergagnini Kolev et al., 2017). These changes can lead to a clearance higher than expected from glomerular filtration rate alone.

Both PK and pharmacodynamics (PD) are influenced by plasma protein binding. Drugs bound to proteins are not pharmacologically active, so protein binding has a major impact on drug effect (Buxton, 2017). Only the free fraction is available for

distribution, metabolism, and renal excretion. When binding decreases, the free fraction increases, which can yield higher tissue concentrations and lower total plasma concentrations at the same dose. During pregnancy, albumin and α 1-acid glycoprotein concentrations are lower, which tends to increase the free fraction of highly protein-binding drugs (Bardy et al., 1990). The impact is drug-specific, and not all drugs show relevant changes (Hebert et al., 2009). Even small shifts in binding can substantially change exposure, so monitoring of the free fraction is recommended for highly protein-binding drugs with a narrow therapeutic index (Buxton, 2017).

Regional blood flow changes significantly in pregnancy, with increases to the uterus, kidneys, skin, and breasts, and decreases to skeletal muscle and the splanchnic circulation (Cunningham et al., 2018; Thaler et al., 1990). Data on hepatic blood flow is contradictory. Some studies suggest stability, while others indicate an increase in the third trimester (Robson et al., 1990; Nakai et al., 2002). Higher hepatic flow can accelerate the metabolism and elimination of high-extraction compounds (Halpern et al., 2019; Buxton, 2017).

During pregnancy, reduced splanchnic perfusion combined with progesterone-mediated reductions in gastrointestinal motility leads to slower gastric emptying and prolonged intestinal transit time, which can alter drug absorption (Halpern et al., 2019; Cunningham et al., 2018). Additionally, an elevation in gastric pH during pregnancy may theoretically influence the absorption profiles of weak acids and bases (Philipson, 1977).

The placenta functions as an anatomic and functional barrier that actively regulates transport between maternal and fetal circulations. Small nonpolar compounds can cross by passive diffusion (Syme et al., 2004; Griffiths & Campbell, 2015). In addition, the placenta expresses multiple influx and efflux transporters on the apical and basal membranes that control the movement of drugs toward the fetus or back to the mother (Syme, Paxton, & Keelan, 2004; Iqbal et al., 2012; Tetro et al., 2018; Lee et al., 2018; Joshi et al., 2016). Transporter expression varies with gestational age, for example P-gp is more highly expressed early in pregnancy, whereas BCRP activity appears relatively constant (Syme, Paxton, & Keelan, 2004;

Iqbal et al., 2012; Tetro et al., 2018; Lee et al., 2018; Joshi et al., 2016). Transporter activity can produce unequal distribution of drugs between maternal and fetal compartments, influencing both efficacy and toxicity during pregnancy (Syme, Paxton, & Keelan, 2004; King et al., 1984; Sit et al., 2011; Paulzen et al., 2017a; Paulzen et al., 2017b). Fetal pharmacogenomics may also influence placental transport.

Furthermore, cytochrome P450 (CYPs) enzymes participate in Phase I metabolism for many drugs. They are hemoproteins grouped into 18 families based on amino acid homology, with families CYP1, CYP2, and CYP3 most involved in drug metabolism (Tornio & Backman, 2018). These families are further divided into subfamilies, each with multiple related isoenzymes (Tornio & Backman, 2018). There is redundancy in Phase I metabolism because many drugs are metabolized by more than one CYP, and the activity of several CYPs can change during pregnancy (Tornio & Backman, 2018).

Regarding the CYP3A family, which metabolizes more than 50% of all medications and is upregulated in pregnancy. Its activity increases at about 14 to 18 weeks of gestation and remains elevated through the remainder of pregnancy (Tornio & Backman, 2018; Tracy et al., 2005).

CYP2D6 is the second most active CYP enzyme and is responsible for the metabolism of approximately 25% of medications, including many psychotropic agents, and it shows high phenotypic variability due to genetic polymorphisms (Tornio & Backman, 2018; Borges et al., 2010). Metabolic phenotypes range from poor to intermediate, extensive, and ultrarapid, with distributions that vary across ethnic groups (Bernard et al., 2006). Although CYP2D6 is not traditionally considered an inducible enzyme, pregnancy alters its activity. Extensive metabolizers show increased activity over gestation, whereas poor metabolizers show further reductions (Wadelius et al., 1997).

As for the CYP2B6 subfamily which contributes to the clearance of about 3 to 12% of drugs. Its activity is difficult to characterize because it often acts together with other cytochrome P450 enzymes (Wang & Tompkins, 2008). During pregnancy,

CYP2B6-mediated clearance is higher than in the postpartum period (Pond et al., 1985; Bogen et al., 2013). *In vitro* studies show that estradiol induces CYP2B6 transcription and increases its metabolic activity (Dickmann & Isoherranen, 2013; Wade, 2019).

CYP1A2 is responsible for the metabolism of several commonly used drugs (Tornio & Backman, 2018; Dixon et al., 1995). Its activity decreases during pregnancy, with evidence of a progressive reduction in clearance across gestation (Aldridge et al., 1981).

CYP2C19 is responsible for the metabolism of several drugs and, like CYP2D6, is highly polymorphic with pharmacogenomic variants of clinical relevance (Tornio & Backman, 2018; Hicks et al., 2017; Hicks et al., 2015). Evidence suggests that its activity may be reduced during pregnancy, possibly due to estrogen effects. However, despite its importance, the pharmacogenomics of CYP2C19 in pregnancy remains largely unexplored (Wattanagoon et al., 1987; McGready et al., 2003).

Many medications are metabolized by multiple CYP enzymes (Obach et al., 2005). During pregnancy, the activity of CYP2B6, CYP2C9, and CYP3A4/5 increases, CYP2C19 activity decreases, and CYP2D6 activity varies with genotype (Wadelius et al., 1997; Anderson, 2005). In such cases, where multiple enzymes contribute to the metabolism of the same drug, it becomes difficult to predict the overall impact on dosing requirements needed to maintain efficacy across pregnancy (Pinheiro & Stika, 2020).

Because medication use in pregnancy is widespread, and because the risk-benefit profile and PK changes are distinctive in this period, there is a clear need for targeted research in pregnancy pharmacology to support better clinical practice. It is essential to deepen the understanding of the physiological changes that occur and their consequences for therapeutic effectiveness and adverse event risk (Pinheiro & Stika, 2020).

These physiological adaptations not only alter the PKs of individual drugs but also create a background in which DDIs may reflect an even greater influence. The

combined effect of endogenous adaptations and exogenous enzyme modulation emphasizes the importance of assessing enzyme-mediated DDIs.

1.3 Enzyme-Mediated Drug-Drug Interactions in Pregnancy

Drug-drug interactions represent a major concern in clinical pharmacology because they can significantly alter therapeutic outcomes and patient safety. Among the different mechanisms of DDIs, enzyme-mediated interactions are particularly important, as they occur when a perpetrator drug modifies the metabolic activity of enzymes responsible for the biotransformation of a victim drug. This modulation can lead to altered systemic exposure, resulting either in reduced efficacy or in increased toxicity (Bjornsson et al., 2003; Wienkers & Heath, 2005).

These alterations are not always predictable, since they depend on the strength of the inducer or inhibitor, the specific enzyme isoform affected, and the PK characteristics of the victim drug.

The cytochrome P450 (CYP) superfamily is the most common enzymatic system involved in enzyme-mediated DDIs. Isoforms such as CYP3A4, CYP2C9, CYP2C19, and CYP2D6 are responsible for the metabolism of a large proportion of clinically used drugs, while phase II enzymes, particularly UDP-glucuronosyltransferases (UGTs), also play an essential role (Zhang et al., 2009; Zanger & Schwab, 2013). Enzyme inhibition, which can occur rapidly, often results in elevated drug concentrations and a higher risk of adverse effects, whereas enzyme induction tends to develop more gradually but can reduce plasma concentrations below the therapeutic range (Lin & Lu, 2001; Shitara et al., 2013).

The clinical impact of these interactions is especially critical for drugs with a narrow therapeutic index, where small changes in plasma concentrations can have major consequences for efficacy and safety (Holford & Buclin, 2012). This risk is further amplified in populations with altered physiology, such as pregnant women. In this group, profound endogenous changes already influence drug disposition, and the superimposition of enzyme-mediated DDIs can create unpredictable patterns of maternal and fetal exposure (Anderson, 2005; Pariente et al., 2016).

For example, an inducer that accelerates clearance may further drug reduce exposure already lowered by pregnancy related physiological changes, whereas an inhibitor may cause higher concentrations.

Given these challenges, PBPK modeling has emerged as a valuable tool to investigate enzyme-mediated DDIs. PBPK frameworks enable the integration of drug-specific parameters with enzyme kinetics and physiological variables, allowing for the simulation of both inhibitory and inductive interactions under pregnancy-specific conditions. Regulatory agencies increasingly recommend such approaches to guide dose optimization, anticipate clinical risks, and reduce the reliance on empirical trial-and-error dosing in vulnerable populations (Jones et al., 2015; Wagner et al., 2021).

Altogether, the evidence highlights how pregnancy amplifies the complexity of both intrinsic PK variability and extrinsic DDIs. This context justifies the application of PBPK modeling to drugs of clinical relevance in women of reproductive age. The following sections introduce indomethacin and phenytoin, two representatives compounds selected for this thesis, outlining their pharmacological properties and clinical significance in pregnancy.

1.4 Indomethacin in the context of Pregnancy

During pregnancy, preterm labor (PTL) and preterm birth (PTB) remain major causes of morbidity and mortality worldwide (Rytting et al., 2014). The onset of labor is thought to be closely linked to the local synthesis of prostaglandins, which play a key role in regulating cervical ripening, promoting the formation of gap junctions in the myometrium, and stimulating uterine contractions (Gamissans & Balasch, 1993; Zuckerman et al., 1984). Indomethacin, a prostaglandin synthetase inhibitor, has been used as a treatment for PTL since the early 1970s (Zuckerman et al., 1974; Wqvist, 1975; Rane et al., 1978). Its mechanism of action, by targeting prostaglandin pathways, makes it one of the most effective pharmacological options for temporarily delaying preterm labor and buying critical time for fetal maturation or maternal transfer to specialized care units. Despite this clinical utility,

the use of indomethacin is not without controversy, particularly due to safety concerns for both mother and fetus.

The use of indomethacin during pregnancy is primarily supported by clinical experience gained from trials in which the drug was administered to prevent PTL (Abramovici et al., 2012). However, the literature lacks PK data comparing exposure and response to indomethacin in pregnant women, as most available data have been derived from studies in men and non-pregnant women (Rytting et al., 2014).

Therefore, it is important to investigate the relationship between dose, exposure, and clinical response when indomethacin is prescribed during pregnancy.

1.4.1 Chemistry

Indomethacin, Figure 1 is a nonsteroidal anti-inflammatory drug (NSAID) belonging to the class of indole-3-acetic acid derivatives (National Center for Biotechnology Information (NCBI), PubChem, 2025). It appears as a fine white powder, practically odorless, with a slightly perceptible or almost imperceptible taste (NTP, 1992) and has a molecular weight of 357.8 g/mol (PubChem, 2025)

Structurally, it consists of an indole-3-acetic acid moiety substituted at positions 1, 2, and 5 of the indole ring with *p*-chlorobenzoyl, methyl, and methoxy groups, respectively. Chemically, it is classified as an N-acylindole, an aromatic ether, a member of monochlorobenzenes, and a derivative of indole-3-acetic acids (NCBI, PubChem, 2025).

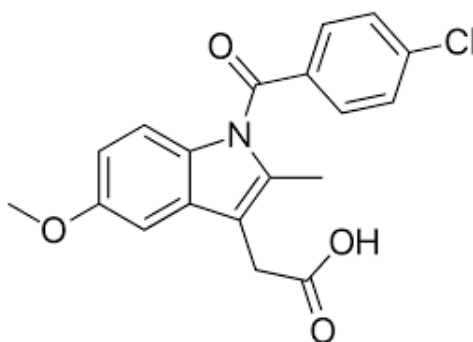


Figure 1: Chemical structure of Indomethacin.

1.4.2 Pharmacokinetics and Metabolism

Indomethacin exhibits a linear PK profile, with plasma concentrations and area under the curve (AUC) directly proportional to the administered dose. Nevertheless, its elimination half-life and both plasma and renal clearance vary as a function of dose (Dodick, 2004). Following oral administration, indomethacin is rapidly absorbed from the gastrointestinal tract, achieving a bioavailability close to 100% (Dodick, 2004), with approximately 90% of the dose absorbed within the first four hours (U.S. Food and Drug Administration, 2025).

Oral absorption demonstrates notable inter- and intraindividual variability and is reduced when the drug is not administered under fasting conditions (Helleberg, 1981). In the fasted state, peak plasma concentrations are typically attained within 30 minutes to 2 hours post-administration, with values ranging from 2 to 3 µg/mL (Helleberg, 1981). Under a dosing regimen of 25 to 50 mg three times daily, steady-state plasma concentrations are approximately 1.4-fold higher than the maximum concentrations observed after the initial dose (U.S. Food and Drug Administration, 2025).

Indomethacin displays extensive plasma protein binding (90-99%), primarily to albumin (Weber et al., 2013; Nalamachu & Wortmann, 2014). Elimination occurs predominantly through hepatic metabolism and biliary excretion, followed by hydrolysis and subsequent reabsorption (Weber et al., 2013). After oral administration, approximately 60% of the dose is recovered in urine in non-metabolized form and metabolites in urine, whereas about 33% is excreted in feces, of which 1.5% corresponds to unchanged indomethacin (U.S. Food and Drug Administration, 2025).

The primary hepatic metabolites include O-desmethyl-indomethacin (DMI), O-deschlorobenzoyl-indomethacin (DBI), O-desmethyl-N-deschlorobenzoyl-indomethacin (DMBI), and their respective glucuronide conjugates. These metabolites are largely pharmacologically inactive (Duggan et al., 1972). The high

oral bioavailability suggests that indomethacin is unlikely to undergo significant first-pass metabolism (Weber et al., 2013).

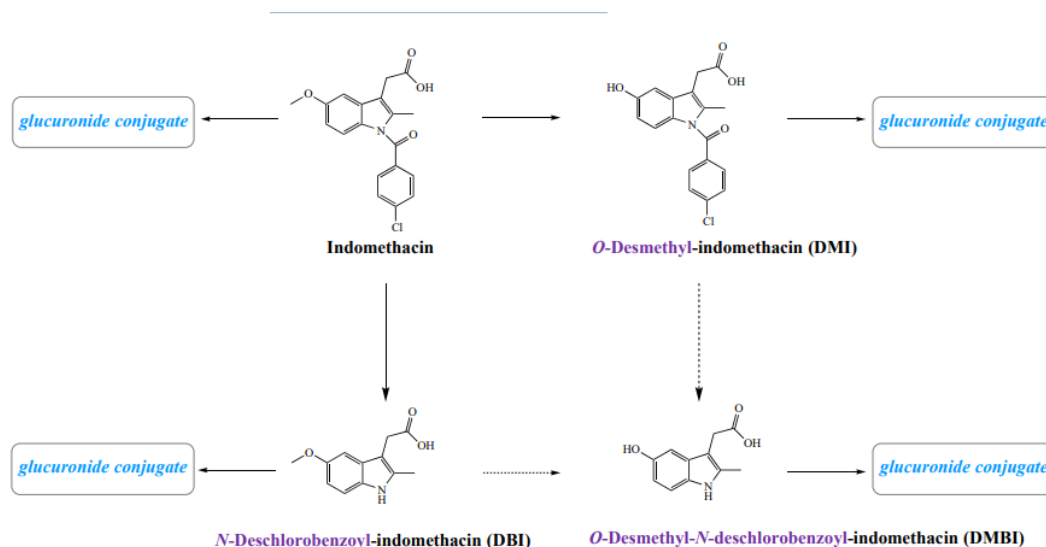


Figure 2. Metabolism of Indomethacin. Adapted from Gliszczynska & Nowaczyk, 2021 (Molecules, 26, 1576), under CC BY 4.0 license.

1.4.3 Pharmacodynamic and Mechanism of Action

Indomethacin is a NSAID with analgesic and antipyretic properties, exerting its pharmacological effects primarily through the inhibition of the synthesis of mediators involved in pain, fever, and inflammation (U.S. Food and Drug Administration, 2025). Indomethacin acts via non-selective and reversible inhibition of the cyclooxygenase (COX) enzyme, which exists in two isoforms COX-1 present in most tissues, and COX-2 expressed predominantly in response to inflammatory stimuli (Lucas, 2016).

COX-1 is constitutively expressed and plays an essential role in physiological processes such as gastric mucosal protection, platelet aggregation, and renal function. It catalyzes the conversion of arachidonic acid to prostaglandin G_2 (PGG_2) and subsequently to prostaglandin H_2 (PGH_2) (Lucas, 2016). Although COX-2 may also be constitutively expressed in certain tissues, it is highly inducible by inflammatory stimuli and is found in the central nervous system, kidneys, uterus, and other organs. Like COX-1, it converts arachidonic acid to PGG_2 and subsequently to PGH_2 however, in the COX-2-mediated pathway, PGH_2 is further converted into prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2). PGE_2 plays a central role in mediating inflammation, pain, and fever, and its inhibition attenuates these responses (Lucas, 2016).

Because indomethacin inhibits both COX isoforms, with a greater selectivity for COX-1, its use is associated with an increased risk of gastrointestinal adverse effects. It binds to the enzyme's active site, preventing the interaction with its substrate, arachidonic acid, and inhibits phospholipase A_2 , the enzyme responsible for releasing arachidonic acid from membrane phospholipids.

Although the precise mechanism by which indomethacin promotes the closure of a patent ductus arteriosus (PDA) remains incompletely understood, it is believed to involve inhibition of prostaglandin synthesis (U.S. Food and Drug Administration, 2025). After birth, the rise in oxygen tension triggers the physiological closure of the ductus arteriosus (Pacifici, 2013). In preterm neonates, PGE_1 counteracts this process by inducing smooth muscle relaxation, thereby maintaining ductal patency (Pacifici, 2013). By lowering prostaglandin levels, indomethacin facilitates ductal closure (U.S. Food and Drug Administration, 2025).

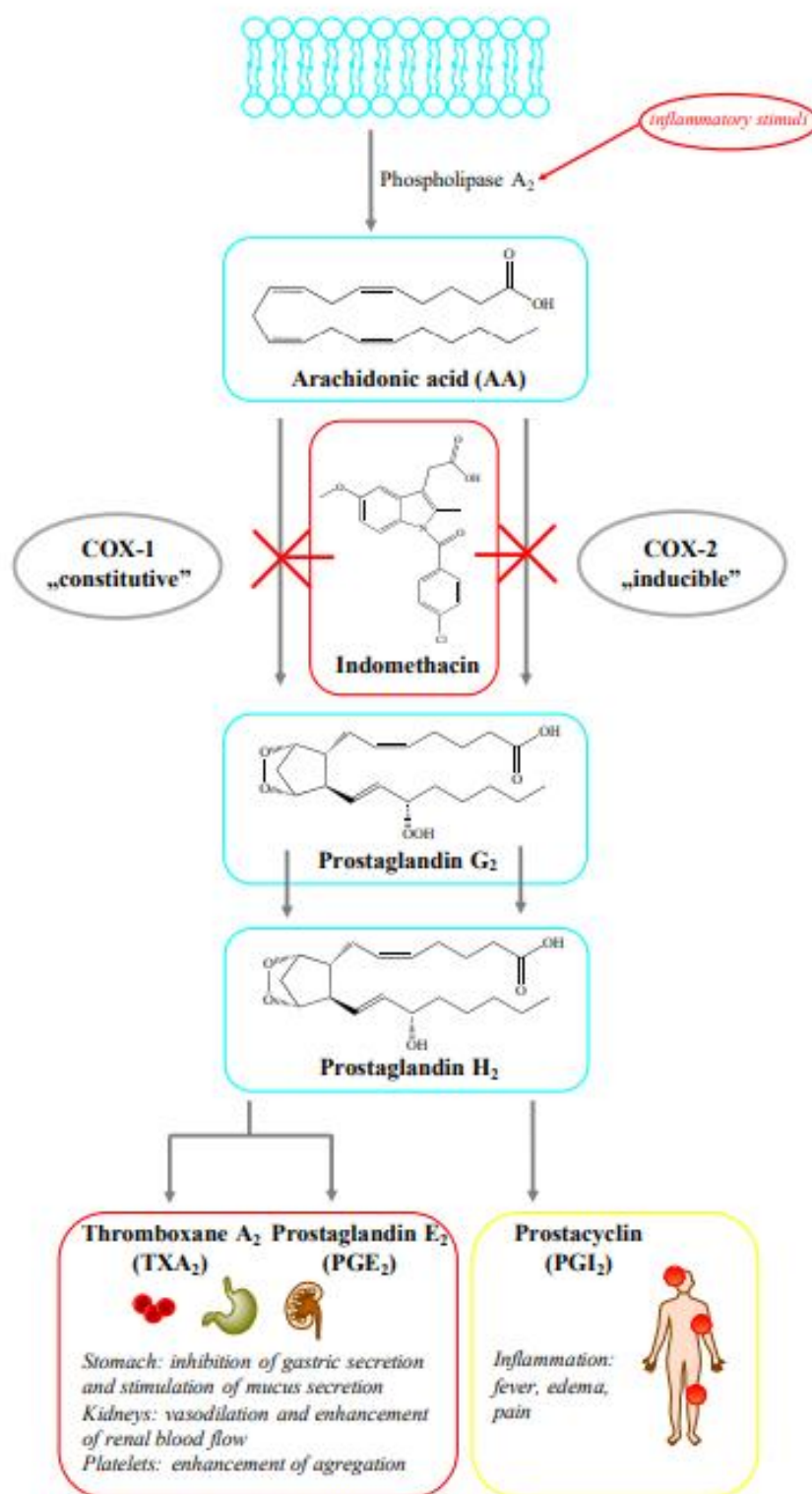


Figure 3. Mechanism of Action of Indomethacin. Adapted from Gliszczyńska & Nowaczyk, 2021 (Molecules, 26, 1576), under CC BY 4.0 license. Adapted from Gliszczyńska & Nowaczyk, 2021 (Molecules, 26, 1576), under CC BY 4.0 license.

1.4.4 Adverse Effects

The analgesic, antipyretic, and anti-inflammatory effects of indomethacin, as well as its associated adverse effects, result from the reduced synthesis of prostaglandins. Indomethacin inhibits both COX isoforms, although with greater selectivity for COX-1, which explains its higher incidence of gastrointestinal adverse effects, since prostaglandins produced by COX-1 play a crucial role in maintaining gastric mucosal integrity (DrugBank, 2025).

Although indomethacin is widely used for the treatment of various headache disorders, it can also induce headache, dizziness, dyspepsia, and nausea (Dougherty, 2021). It may also trigger hypersensitivity reactions, such as anaphylaxis, urticaria, and angioedema (Kowalski & Makowska, 2006). Within the nervous system, reported symptoms include tinnitus, vertigo, depression, and dizziness in more severe cases, aseptic meningitis, psychosis, and cognitive deficits have been documented (Hoppmann, Peden, & Ober, 1991).

Adverse effects may also extend to the cardiopulmonary system, resulting in acute respiratory distress syndrome, pulmonary edema, and congestive heart failure (Wan et al., 2023; Arfè et al., 2016).

Arachidonic acid is the key substrate for prostaglandin synthesis via COX enzymes. When these enzymes are inhibited, arachidonic acid may be diverted towards the leukotriene synthesis pathway, which may contribute to the development of nasal polyps and respiratory difficulties. Other possible effects include generalized fatigue and somnolence (Kowalski & Makowska, 2006)

1.5 Phenytoin in Pregnancy

Pregnant women who suffer from epilepsy often experience a higher frequency of seizures during gestation, frequently accompanied by pregnancy-related complications. For seizure control, antiepileptic drugs (AEDs) are commonly prescribed (Veroniki et al., 2017).

Epilepsy is one of the most common chronic neurological disorders, affecting approximately 0.6-1% of the population (Hauser & Hesdorffer, 1990; Wiebe et al., 1999). During pregnancy, seizure frequency may increase, thereby elevating the risk of obstetric complications (Morrell, 2002; Zahn et al., 1998).

The potential consequences of uncontrolled seizures extend beyond maternal morbidity, as they can also compromise fetal oxygenation and development. This dual vulnerability makes seizure management during pregnancy uniquely challenging, requiring careful balancing between efficacy and safety.

AEDs are prescribed not only to reduce the severity of epilepsy but also to manage other conditions, including pain, psychiatric disorders, and migraine (Spina & Perugi, 2004).

Pregnant women receiving AED therapy have an increased risk of spontaneous abortion and teratogenicity, with a 4-8% probability of delivering a child with a major congenital malformation (CM), as these drugs can cross the placental barrier (Morrell, 2002; Zahn et al., 1998; Harden et al., 2009; Meador et al., 2008).

Physiological changes during pregnancy, such as increased plasma volume and accelerated hepatic metabolism mediated by microsomal enzymes (CYP2C9 and CYP2C19), may contribute to reduced total plasma concentrations of phenytoin (Tomson & Battino, 2012; Harden et al., 2009). However, reductions in total plasma levels do not necessarily correlate with reduced clinical effect, since protein binding alterations can increase the free fraction of the drug. This discrepancy complicates therapeutic monitoring and reinforces the need for individualized dose adjustments.

Phenytoin use during pregnancy can also interfere with vitamin K metabolism, thereby increasing the risk of hemorrhagic disease of the newborn (Shearer, 2009). For this reason, vitamin K supplementation is recommended during the last 4-6 weeks of pregnancy (American Academy of Pediatrics Committee on Fetus and Newborn, 2003). This preventive approach exemplifies how pharmacological interventions in pregnancy often require additional supportive strategies to mitigate drug-related risks for the neonate.

The decision to maintain AED therapy should be based on an individualized risk-benefit assessment. Discontinuation of treatment is generally not advised, as uncontrolled seizures pose significant risks to both mother and fetus, including hypoxia, trauma, and sudden unexpected death in epilepsy (SUDEP) (Tomson et al., 2011). In such cases, therapeutic drug monitoring, individual dose adjustment, and multidisciplinary follow-up are essential to optimize clinical outcomes and minimize risks (Pennell, 2003).

1.5.1 Chemistry

Phenytoin (5,5-diphenylimidazolidine-2,4-dione) is a hydantoin derivative (imidazolidine-2,4-dione) structurally characterized by the presence of two phenyl groups at the 5-position. It has the molecular formula $C_{15}H_{12}N_2O_2$ and a molecular weight of 252.27 g/mol (National Center for Biotechnology Information, 2025). Phenytoin appears as a white or almost white crystalline powder (National Center for Biotechnology Information, 2025), practically insoluble in water, moderately soluble in ethanol, and sparingly soluble in methylene chloride. According to the Biopharmaceutics Classification System (BCS), it is classified as a Class II drug, characterized by low aqueous solubility and high permeability (Rodriguez-Vera et al., 2023). The pKa value is 8.3 (Patil et al., 2012), and it is functionally related to other hydantoin derivatives.

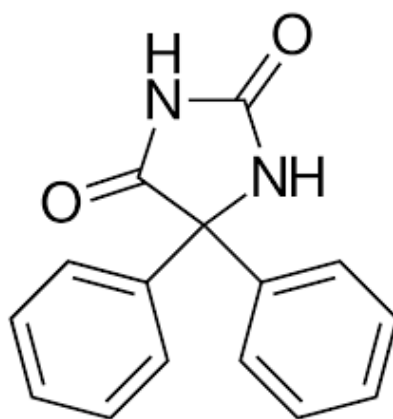


Figure 4. Chemical structure of Phenytoin.

1.5.2 Pharmacokinetics and Metabolism

Phenytoin has a narrow therapeutic index, making therapeutic drug monitoring essential for individualizing dosing and minimizing the risk of toxicity (Gupta & Tripp, 2023; Abdelsayed & Sokolov, 2013). Following oral administration, the drug is well absorbed, with peak plasma concentrations generally occurring 1.5-3 hours after intake of the immediate-release formulation and 4-12 hours after the extended-release formulation (Iorga & Horowitz, 2023; Gupta & Tripp, 2023). In cases of acute ingestion, absorption can be substantially delayed (Gupta & Tripp, 2023).

The apparent volume of distribution is relatively small, ranging from 0.6 to 0.8 L/kg (Bergen DC, 2009), reflecting the drug's extensive plasma protein binding (90-95%), primarily to albumin (Richens, 1979). Conditions that reduce serum albumin such as pregnancy, hepatic impairment, severe burns, or malnutrition may increase the unbound fraction of phenytoin, thereby amplifying both therapeutic effects and the potential for toxicity (Thorn et al., 2012).

Phenytoin is primarily metabolized to the inactive hydroxyphenytoin, designated 5-(4'-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) (Leeder, 1998). Approximately 90% of the administered dose is converted to p-HPPH, which subsequently undergoes glucuronidation and is eliminated in the urine (Nakajima et al., 2007). Two stereoisomers, (R)-p-HPPH and (S)-p-HPPH, are formed in variable proportions

among individuals (Argikar et al., 2006; Fritz et al., 1987). When catalyzed by CYP2C19, the isomeric ratio is approximately 1:1 (Bajpai et al., 1996), whereas CYP2C9 preferentially produces the S-isomer, with up to a 40-fold greater yield compared to the R-isomer (Argikar et al., 2006; Bajpai et al., 1996). These stereoisomeric patterns can serve as biomarkers for genetic variants of CYP2C9 and CYP2C19.

The formation of p-HPPH proceeds through a reactive arene oxide intermediate (Claesen et al., 1982), which has been implicated in hypersensitivity reactions, Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), hepatotoxicity, and other idiosyncratic toxicities. This intermediate can be converted to a dihydrodiol by epoxide hydrolase (EPHX1) or by several cytochrome P450 enzymes, including CYP1A2, CYP2C19, CYP2E1, CYP2A6, CYP2D6, CYP2C8, CYP2C9, and CYP3A4 (Komatsu et al., 2000), and subsequently transformed into a catechol (Leeder, 1998).

Hydroxyphenytoin can be further metabolized to generate the metabolite catechol 3',4'-diHPPH, primarily via CYP2C19, although CYP2C9 and CYP3A4 are responsible for most of this conversion due to their high hepatic expression (Komatsu et al., 2000; Cuttle et al., 2000). CYP2C18, expressed in the skin, also contributes to hydroxylation steps and is relevant to cutaneous adverse reactions (Kinobe et al., 2005). The catechol can be oxidized to a reactive quinone, which may be reduced back to catechol by NQO1 or methylated by COMT to methylcatechol, subsequently excreted in the urine (Leeder, 1998).

Glucuronidation of hydroxyphenytoin, catalyzed by UGT1A1, UGT1A4, UGT1A6, and UGT1A9, prevents the formation of toxic metabolites via peroxidase-mediated pathways (Yamanaka et al., 2005). This process is stereoselective, UGT1A1 preferentially acts on the S-isomer, while UGT1A9 and UGT2B15 preferentially conjugate the R-isomer (Nakajima et al., 2007).

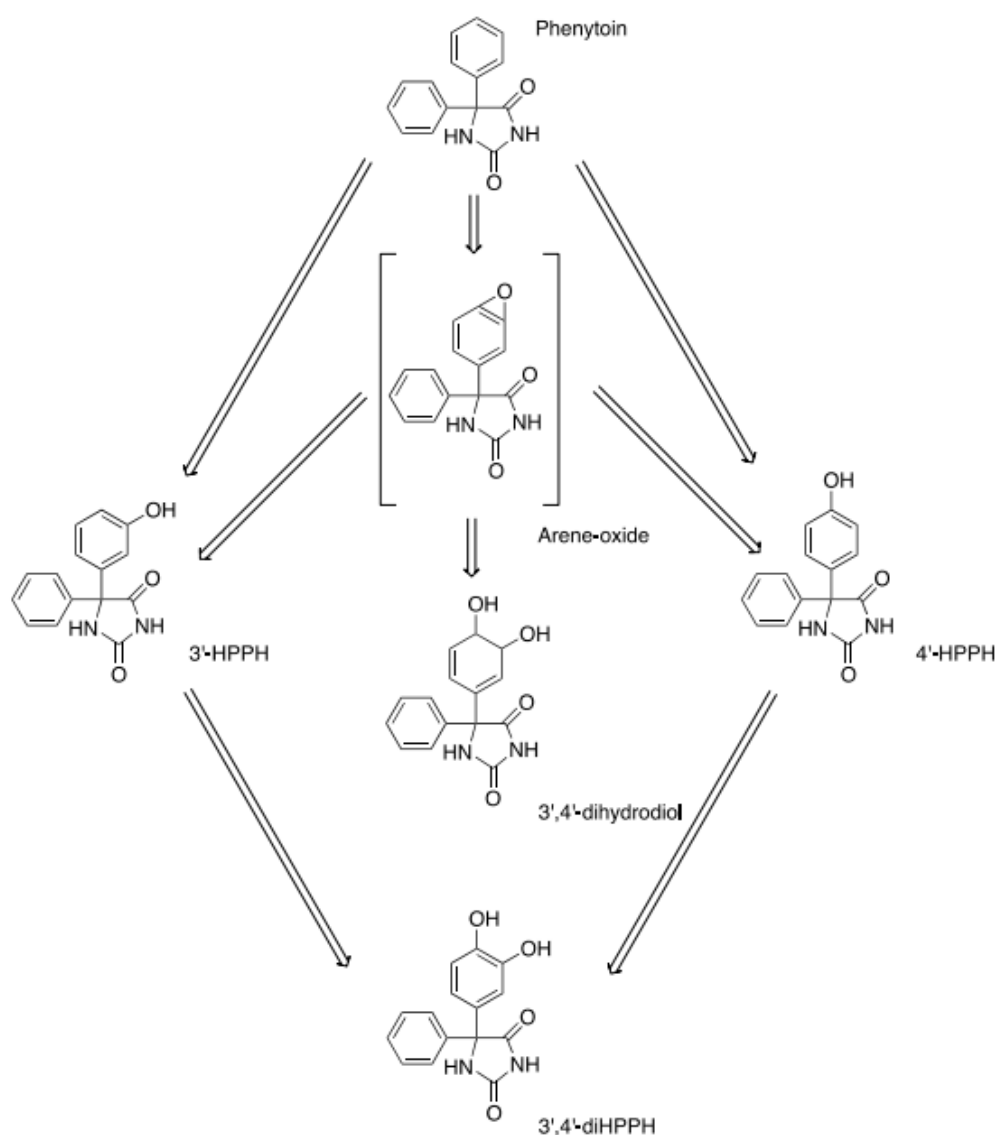


Figure 5. Metabolism of Phenytoin. Adapted from Patocka, J., Wu, Q., Nepovimova, E., & Kuca, K. (2020). *Phenytoin – An anti-seizure drug: Overview of its chemistry, pharmacology and toxicology. Food and Chemical Toxicology*, 142, 111393.

1.5.3 Pharmacodynamics and Mechanism of Action

The antiepileptic effect of phenytoin results from its preferential binding to voltage-gated sodium (Na^+) channels in their inactivated state. This interaction prevents the repetitive conduction of action potentials in hyperexcitable neurons, typical of ictal activity, while preserving normal electrical transmission in healthy neurons (Rogawski, 2002; Rogawski et al., 2004).

Voltage-gated Na⁺ channels are composed of a central α -subunit and auxiliary β -subunits ($\beta 1$ – $\beta 4$). The α -subunit forms the pore through which ions pass and is responsible for detecting and responding to changes in membrane potential. It consists of four homologous domains (I–IV), each containing six transmembrane α -helical segments (S1–S6) and a re-entrant loop (P-loop) between S5 and S6 that contributes to ion selectivity. The S4 segment, enriched in positively charged amino acid residues, functions as a voltage sensor, coupling membrane depolarization to channel opening and closure. The β -subunits do not directly mediate ion conduction but modulate channel expression and influence gating properties (Rogawski, 2002; Mantegazza et al., 2012).

During the initiation of an action potential, membrane depolarization reaches the threshold required to activate the voltage sensors of Na⁺ channels. Within a few hundred microseconds, this activation triggers the transition from the closed (non-conducting) state to the open state, enabling a rapid influx of Na⁺ ions. After several milliseconds, the channel undergoes fast inactivation a transient block mediated by a loop of amino acid residues located between domains III and IV of the α -subunit, which acts as an intracellular “gate.” Under conditions of prolonged depolarization such as during repetitive neuronal firing, a second process known as slow inactivation can occur. This state is characterized by slower recovery kinetics and involves additional regions of the channel, including the outer pore. Phenytoin, like other sodium channel-blocking antiepileptic drugs, preferentially stabilizes the inactivated state, thereby reducing the spread of ictal activity (Rogawski, 2002; Rogawski et al., 2004; Mantegazza et al., 2012).

Phenytoin promotes channel occupancy in the inactivated state and delays recovery to the functional state, making it more effective in neurons firing at high frequency. This confers selectivity for hyperactive neuronal tissue. Binding to the channel occurs mainly through hydrophobic and dipole-induced interactions, with the two phenyl rings of the molecule playing a critical role (Rogawski, 2002). The potency of block increases markedly at less negative membrane potentials, and phenytoin’s affinity for channels in the inactivated state 7 $\mu\text{mol/L}$ is substantially

higher than for channels in the resting state $>600\text{ }\mu\text{mol/L}$ (Rogawski, 2002; Rogawski et al., 2004).

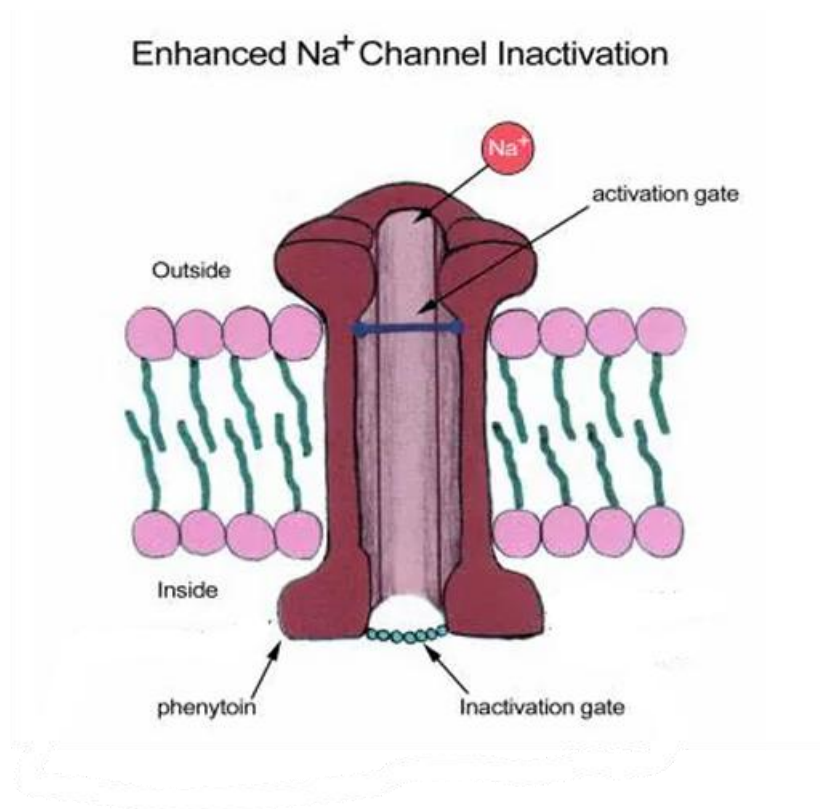


Figure 6. Mechanism of Action of Phenytoin. Adapted from Ochoa, J. G. (2024). *Antiepileptic Drugs*. In: Benbadis, S. R. (Ed.), Medscape. Updated June 7, 2024.

1.5.4 Adverse Effects

The adverse effects of phenytoin can be classified as either dependent or independent of its serum concentration. Concentration-dependent effects are generally predictable, explained by the pharmacodynamic properties of the drug, and primarily include neurological manifestations such as visual and motor disturbances, whose severity increases with concentration, and which tend to be reversible upon dose reduction or drug discontinuation (Craig, 2005; Crepeau et al., 2012). Intravenous administration may also lead to immediate effects, including local complications such as venous irritation, pain, and thrombophlebitis, as well as cardiac complications such as hypotension, bradycardia, arrhythmias, and

cardiovascular collapse, the latter being more frequent with rapid infusions and generally reversible with infusion rate reduction or treatment discontinuation (Fuller et al., 2013).

Concentration-independent effects are idiosyncratic, unpredictable, and vary in severity. These may present as frequent but less severe reactions, or as rare and serious events requiring life support. They include antiepileptic hypersensitivity syndrome, dermatological manifestations such as rash, Stevens-Johnson syndrome, Lyell's syndrome, hirsutism, and exfoliative dermatitis. Other adverse events may involve gastrointestinal, endocrine, and metabolic effects such as folate depletion and hyperglycemia; hematological effects such as thrombocytopenia, agranulocytosis, and megaloblastic anemia, among others; as well as neuromuscular manifestations including peripheral neuropathy and osteomalacia, along with hepatitis and gingival hyperplasia (Crepeau et al., 2012; Scheinfeld, 2004).

1.6 Objectives

The purpose of this thesis is to develop and validate (PBPK) models for indomethacin and phenytoin, ensuring consistency with available *in vitro* and *in vivo* data.

These models aim to characterize the PK profiles of both drugs across different age groups (20-45 years) and evaluate the impact of pregnancy on drug disposition.

This thesis also seeks to investigate different dosing regimens of indomethacin and phenytoin in order to assess linearity and possible alterations in systemic exposure.

Finally, it aims to assess the potential DDI between phenytoin, as a perpetrator, and indomethacin, as a victim, in non-pregnant women, and to determine the clinical relevance of this interaction.

2. Materials and Methods

This study was conducted using *in silico* methodologies based on PBPK modeling, with the aim of characterizing the PK profiles of indomethacin and phenytoin under different clinical scenarios and assessing their potential DDI, simulations were performed using GastroPlus™ v9.8.3 (Simulations Plus, Lancaster, CA, USA), supported by MedChem Designer™ v10.0 and ADMET Predictor™ v10.3 for the prediction and optimization of physicochemical and pharmacokinetic properties.

2.1 Data sources and information selection

Physicochemical and PK properties of indomethacin and phenytoin were collected from published scientific literature, predictions generated with ADMET Predictor™ v10.3, and experimental data reported in regulatory drug databases, including PubChem, DrugBank, and the Food and Drug Administration. Structural characterization of each compound was performed using MedChem Designer™ v10.0. To systematize the information, the key physicochemical parameters of each drug, along with their respective sources, are summarized in dedicated tables for indomethacin (Table 1) and phenytoin (Table 2), included in this chapter.

The choice of these two drugs is clinically relevant and not arbitrary. Indomethacin is a widely used NSAID, including in obstetric practice, where its administration in women of reproductive age is common. Phenytoin, on the other hand, is an antiepileptic drug frequently prescribed for seizure management in women of reproductive age and, when necessary, during pregnancy despite its teratogenic risks (Harden et al., 2009; Tomson et al., 2011). Clinical co-administration may occur in scenarios where pregnant or non-pregnant women require both seizure management and anti-inflammatory or tocolytic treatment.

From a pharmacokinetic perspective, indomethacin is primarily metabolized by CYP2C9 (Lucas, 2016; Dodick, 2004), while phenytoin is a potent inducer of CYP2C9 and CYP3A4 and is metabolized mainly by CYP2C9 and CYP2C19 (Pacifici, 2013; FDA, 2022). This induction can accelerate indomethacin metabolism, potentially reducing its systemic exposure and therapeutic efficacy. Therefore, this drug pair

represents a mechanistically plausible and clinically relevant case study of enzyme-mediated DDI (Ke, 2018), justifying their evaluation through PBPK modeling.

The age range selected for this study was 20-45 years, in 5-year intervals, covering earlier to later reproductive age. This choice reflects the period of highest clinical relevance to pregnancy, enabling the assessment of the impact of age and physiological state (pregnant vs. non-pregnant) on the PK of indomethacin and phenytoin.

Table 1. Predicted physicochemical properties of indomethacin.

Physicochemical Properties	Value	Reference
Log P	4.27	[a]
Ionization constant	4.5	ADMET Predictor v.10.3
Molecular Weight (g/mol)	357.8	[a]
Water Solubility (mg/mL)	0.04	ADMET Predictor v.10.3
Diff. Coeff. ($\text{cm}^2/\text{s} \cdot 10^5$)	0.71	ADMET Predictor v.10.3
Pe_{eff} ($\text{cm}/\text{s} \cdot 10^4$)	6.17	ADMET Predictor v.10.3
BBB penetration	Low (97%)	ADMET Predictor v.10.3

[a] drugbank.ca; <http://www.drugbank.ca/drugs/DB00328>

pKa, Ionization constant; Diff. Coeff, Differential Coefficient; Pe_{eff}, Effective human jejunal permeability; BBB, blood-brain barrier.

Table 2. Predicted physicochemical properties of phenytoin.

Physicochemical Properties	Value	Reference
Log P	2.21	Poulin et al.,2000
Ionization constant	8.249	ADMET Predictor v.10.3
Molecular Weight (g/mol)	252.27	Stella VJ et al.,1998
Water Solubility (mg/mL)	0.04	Chiang et al.,2013
Diff. Coeff. (cm²/s·10⁵)	0.86	ADMET Predictor v.10.3
Pe_{eff} (cm/s·10⁴)	2.925	ADMET Predictor v.10.3
BBB penetration	High (99%)	ADMET Predictor v.10.3

pKa, Ionization constant; Diff. Coeff, Differential Coefficient; Pe_{eff}, Effective human jejunal permeability; BBB, blood-brain barrier.

2.2 PBPK model development

PBPK models were built for indomethacin (victim drug) and phenytoin (perpetrator drug). In DDI studies, the victim drug refers to the compound whose PKs are altered by the co-administered drug, while the perpetrator drug is the one responsible for causing this alteration, usually through induction or inhibition of the metabolizing enzymes. Initial parameterization included physicochemical data (molecular weight, pKa, logP, and solubility), along with absorption, metabolism, and excretion properties. ADMET Predictor™ v10.3 was used to estimate missing parameters and ensure internal consistency of inputs, while MedChem Designer™ v10.0 supported molecular characterization and structure preparation.

Physiological conditions were represented using non-pregnant and pregnant population models generated with the GastroPlus™ v9.8.3 Physiological Pregnancy Model module. No manual changes were made to physiological or enzymatic parameters beyond those inherently provided by the pregnancy model within the software.

2.3 Model validation

Validation of the PBPK models was performed by comparison with published clinical data. For phenytoin, predictions were evaluated against the model of Rodriguez-Vera et al. (2023), which incorporated clinical data from Caraco et al. (2001). For indomethacin, validation was based on the work of Alqahtani and Kaddoumi (2015), who used clinical data from Rytting et al. (2014).

Validation included a visual inspection of the plots of the plasma concentration profile as well as the calculation of observed/predicted ratio to establish confidence between the PBPK models. The PBPK models were therefore validated.

2.4 Simulated dosing regimens

Dosing regimens were defined according to FDA-approved labeling for each drug to ensure clinical representativeness. Indomethacin (victim) was simulated at 50 mg administered q24h, q12h, and q8h, over 240 hours (10 days). Phenytoin (perpetrator) was simulated under two regimens: 100 mg q8h for 168 and 240 hours, and 300 mg q24h for 168 and 240 hours. The simulation windows were defined to reflect steady-state and clinically relevant induction conditions. Indomethacin has a short half-life (4.5h), reaches PK steady state within 1 day, but a 10-day course was simulated to reflect typical treatment durations (7-14 days) and to ensure that the inducer's effect was established. For phenytoin, steady state is usually reached after 7-10 days, therefore, 168 h (7 days) and 240 h (10 days) were chosen to represent early and near-maximal steady-state conditions. All simulations were conducted at steady state, ensuring that the evaluation of co-administration effects reflected PK equilibrium.

2.5 Drug–drug interaction (DDI) study

The interaction study considered indomethacin as the victim drug and phenytoin as the perpetrator. This choice is justified by the well-documented enzyme-inducing potential of phenytoin on hepatic metabolic pathways (CYP2C9 and UGT2B7),

which are involved in indomethacin clearance (Nakajima et al., 1998; Alqahtani et al., 2015; Rodriguez-Vera et al., 2023). Induction of these enzymes by phenytoin could reduce systemic exposure to indomethacin, potentially compromising therapeutic efficacy. Metabolism-mediated DDIs may occur either through induction, leading to decreased exposure, or inhibition, leading to increased exposure and higher risk of adverse effects (Lee et al., 2024). The DDI study was conducted exclusively in non-pregnant models, as attempts to extend the simulations to pregnant populations resulted in technical errors within the software. Therefore, results are presented exclusively for non-pregnant models.

3. Results and Discussion

The initial PBPK models for both indomethacin and phenytoin were developed to represent a healthy, fasting, 30-year-old American woman weighing 60 kg. These baseline models served as the reference framework for all subsequent simulations.

Model validation was confirmed through visual inspection of the simulated plasma concentration-time profiles compared with observed data (Figure 7 for Indomethacin; Figure 8 for Phenytoin), and by calculating the Predicted/Observed ratios for PK parameters, which are summarized in (Table 3 for Indomethacin and Table 4 for Phenytoin).

These results demonstrated that the models adequately reproduced clinical PKs. Based on these validated standard models, extrapolations were then performed to different physiological conditions.

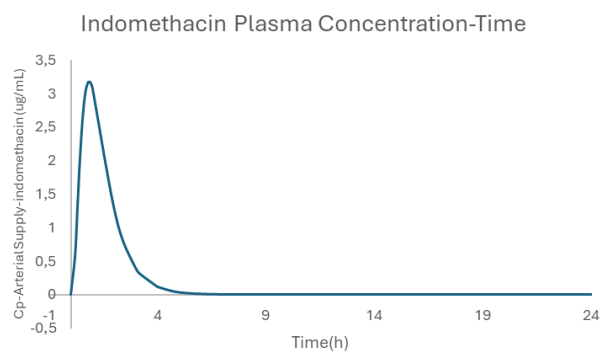


Figure 7. Simulated plasma concentration-time curve for indomethacin in the standard PBPK model representing a healthy, fasting, 30-year-old American woman (60 kg) after a single oral dose of 50 mg.

Table 3. Validation of the PBPK model for indomethacin through comparison of observed PK parameters with simulated values.

Pharmacokinetic Parameters	Observed Value	Predicted Value	Predicted/Observed ratio
Fa (%)	ND	99.999	ND
FDp (%)	ND	99.999	ND
F (%)	90	84.067	ND
Cmax (µg/mL)	3.2201	3.1069	0.9649
Tmax (h)	0.9945	0.88	0.8849
AUC0-inf (µg*h/mL)	9.1981	5.2693	0.5729
AUC0-t (µg*h/mL)	8.5983	5.2693	0.6129
Cmax liver (µg/mL)	ND	0.28866	ND

ND: Not Determined.

Table 4. Validation of the PBPK model for phenytoin through comparison of observed PK parameters with simulated values.

Pharmacokinetic Parameters	Observed Value	Predicted Value	Predicted/Observed ratio
Fa (%)	ND	93.406	ND
FDp (%)	ND	93.406	ND
F (%)	ND	91.733	ND
Cmax (µg/mL)	3.25	3.2334	0.9949
Tmax (h)	2.9125	4.68	1.6069
AUC0-inf (µg*h/mL)	125.62	98.047	0.7805
AUC0-t (µg*h/mL)	121.13	97.106	0.8017
Cmax liver (µg/mL)	ND	2.2604	ND

ND: not determined.

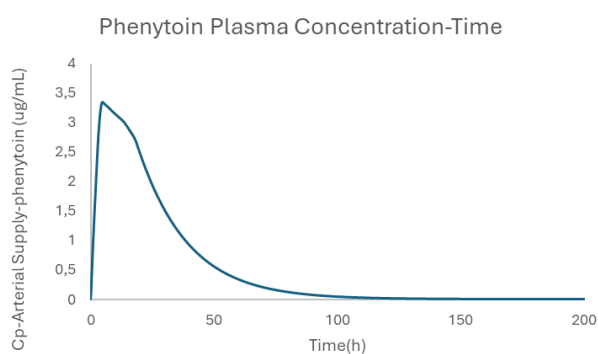


Figure 8. Simulated plasma concentration-time curve for phenytoin in the standard PBPK model representing a healthy, fasting, 30-year-old American woman (60 kg) after a single oral dose of 300 mg.

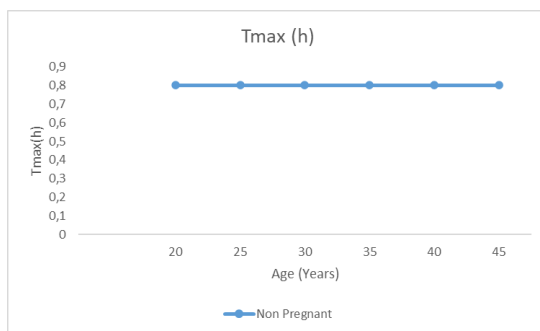
Although some deviations were observed for some PK parameters, they remained within the commonly accepted two-fold range for PBPK model validation, supporting the predictive performance of the developed models.

3.1 Influence of age on pharmacokinetic parameters

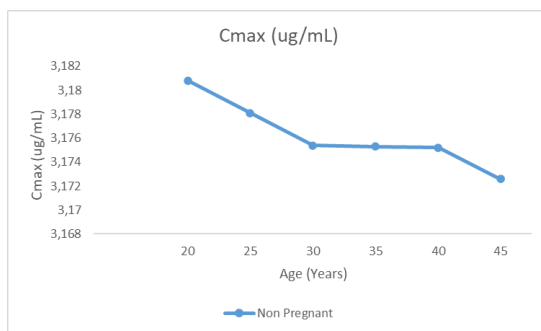
3.1.1 Indomethacin: impact of age (20–45 years) on pharmacokinetics

Within the indomethacin analysis, the effect of age on pharmacokinetic parameters was investigated in non-pregnant women. The results of this evaluation are presented graphically in Figures 9a) to 9h).

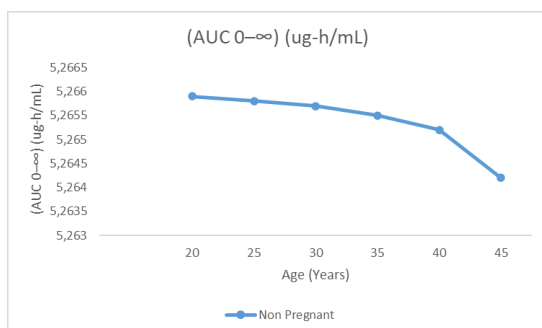
(a)



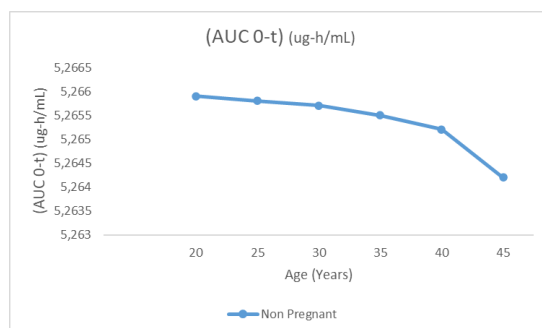
(b)



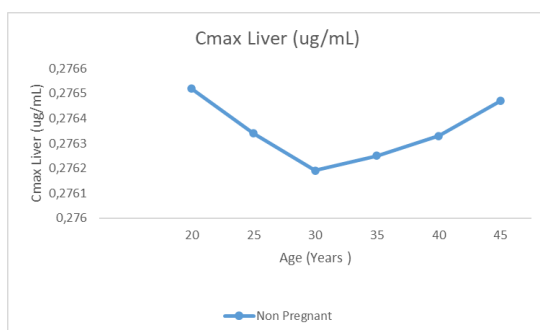
(c)



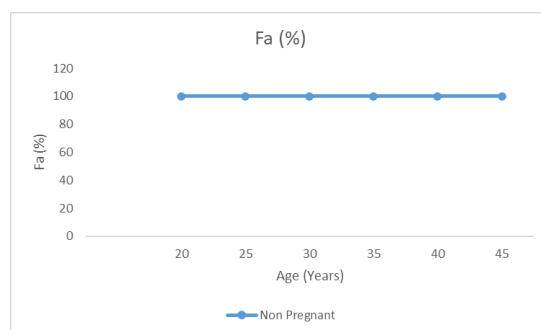
(d)



(e)



(f)



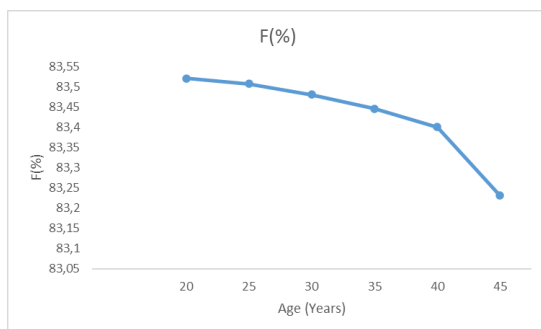
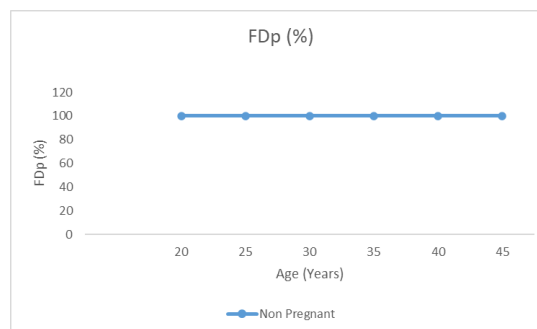
(g)**(h)**

Figure 9. Simulated PK parameters of indomethacin and non-pregnant women ages 20 to 45 years. The panels illustrate differences between different ages for: (a) T_{max} (h), (b) C_{max} ($\mu\text{g/mL}$), (c) $AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$), (d) AUC_{0-t} ($\mu\text{g}\cdot\text{h/mL}$), (e) liver C_{max} ($\mu\text{g/mL}$), (f) fraction absorbed F_a (%), (g) oral bioavailability F (%), and (h) fraction dissolved in the intestine FDp (%).

The impact of age on the pharmacokinetics of indomethacin in non-pregnant women revealed overall stability across the adult age range of 20 to 45 years, with only minimal variations detected. T_{max} (Figure 9a), remained constant at approximately 0.8 h in all age groups, indicating the absorption rate of indomethacin is not affected by age. Similarly, C_{max} (Figure 9b) showed only a slight progressive reduction with advancing age, while hepatic C_{max} fluctuated modestly without pharmacokinetic or clinical relevance. Systemic exposure, expressed by $AUC_{0-\infty}$ and AUC_{0-t} , also displayed a gradual but very small decline with age, paralleled by a comparable tendency in overall oral bioavailability ($F\%$). In contrast, the fraction absorbed ($F_a\%$) and the fraction dissolved ($FDp\%$) remained stable at 100%, confirming that dissolution and intestinal absorption do not represent limiting factors for indomethacin exposure.

Although the decreases observed in C_{max} , AUC and $F\%$ suggest a minor reduction in systemic exposure with age, the magnitude of these changes is extremely small and unlikely to have clinical implications. This absence of clinically relevant variability

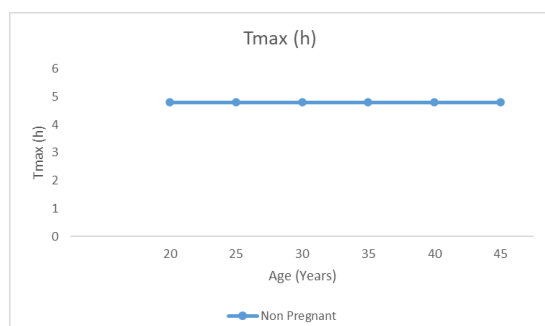
indicates that dose adjustments or additional monitoring are not necessary in adult non-pregnant women. Such stability is consistent with what would be expected in this age group, as women between 20 and 45 years generally maintain preserved hepatic and renal function, without the decline in metabolic capacity that typically occurs in the elderly (Mangoni & Jackson, 2004; Schmucker, 2001). Likewise, enzymatic activity of CYP2C9 and UGT2B7, the main enzymes involved in indomethacin clearance, remains relatively constant throughout adulthood.

The limited impact of age can also be explained by the physicochemical characteristics of indomethacin, which ensure efficient solubility and absorption, thereby minimizing the influence of age-related physiological differences on drug exposure (Davies & Anderson, 1997). From a clinical perspective, these findings are reassuring, as indomethacin is frequently used in obstetric practice for the management of preterm labor, even though pregnant women are rarely included in clinical trials. The similarity of pharmacokinetic tendencies across adult age groups supports the adequacy of extrapolating data obtained in non-pregnant women to broader populations, reinforcing the robustness of indomethacin pharmacokinetics (Ke et al., 2018; van der Graaf et al., 2018).

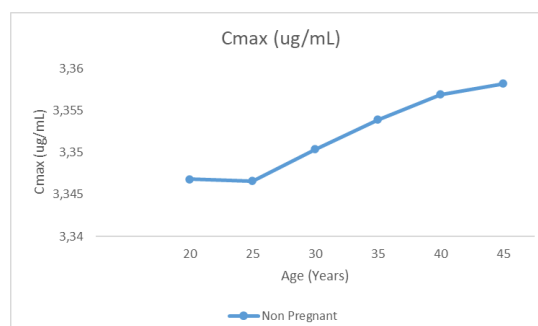
3.1.2 Phenytoin: impact of age (20–45 years) on pharmacokinetics

Within the phenytoin analysis, the effect of age on PK parameters was investigated in non-pregnant women. The results of this evaluation are presented graphically in Figures 10 a) to 10 h).

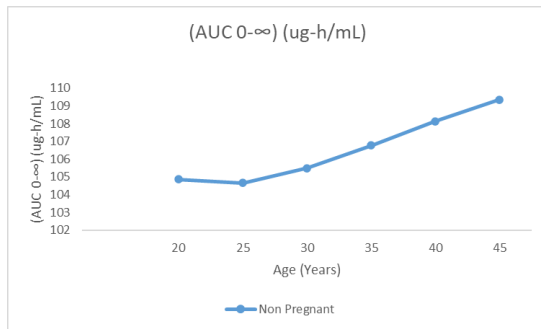
(a)



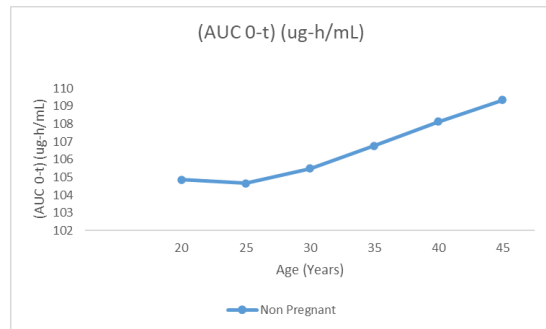
(b)



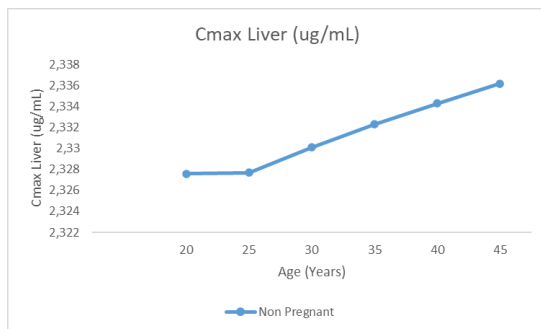
(c)



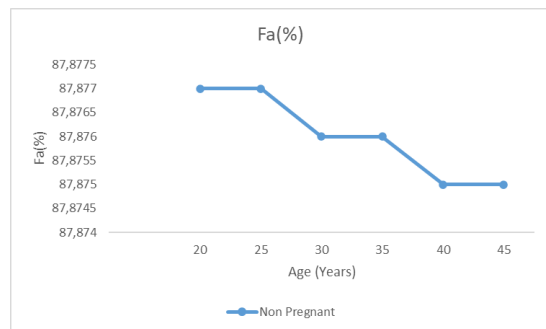
(d)



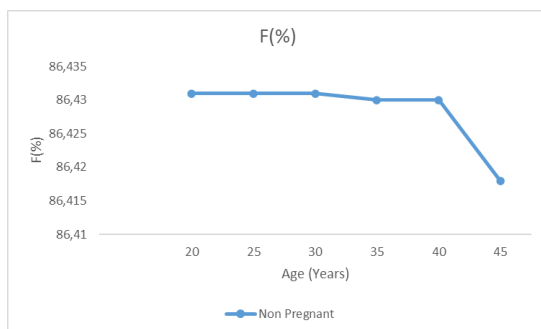
(e)



(f)



(g)



(h)

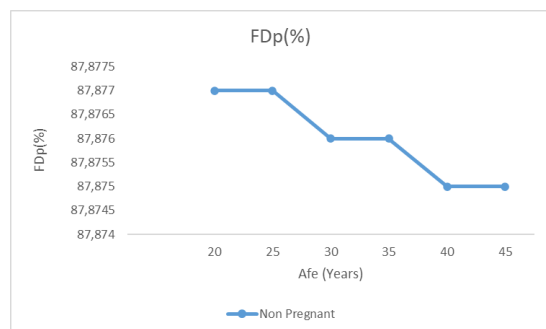


Figure 10. Simulated PK parameters of phenytoin non-pregnant women ages 20 to 45 years. The panels illustrate differences between different ages for: (a) T_{max} (h), (b) C_{max} ($\mu\text{g/mL}$), (c) $AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$), (d) AUC_{0-t} ($\mu\text{g}\cdot\text{h/mL}$), (e) liver C_{max} ($\mu\text{g/mL}$), (f) fraction absorbed F_a (%), (g) oral bioavailability F (%), and (h) fraction dissolved in the intestine FDp (%).

The analysis of the PK of phenytoin across the ages 20 to 45 years in non-pregnant women revealed overall stability, with only modest variations observed in the simulated parameters. T_{max} (Figure 10 a) remained constant at approximately 4.8 h across all ages, indicating that the absorption rate of phenytoin is not influenced by age.

In contrast to indomethacin, both C_{max} and systemic exposure (AUC_{0-t} and $AUC_{0-\infty}$) showed a slight increase with advancing age. C_{max} rose from about 3.345 $\mu\text{g/mL}$ at 20 years to nearly 3.36 $\mu\text{g/mL}$ at 45 years, while AUC values exhibited a similar upward tendency. This suggests that systemic exposure to phenytoin becomes marginally higher in older women within this age range. Hepatic C_{max} followed the same pattern, with modest increases across age groups, reinforcing the idea that distribution and hepatic exposure rise slightly with age. Although these changes are small, they may be attributed to age-associated reductions in hepatic clearance or plasma protein binding, processes known to evolve gradually throughout adulthood but becoming more clinically relevant in elderly populations (Rowland & Tozer, 2011; Bauer, 2014).

Absorption-related parameters remained essentially stable. F_a and FDp were close to 87.87% and unchanged across ages, while bioavailability (F) showed a nearly flat profile with only a minimal decline at 45 years. These results indicate that dissolution and absorption are not limiting steps for phenytoin exposure in this age range, and that overall bioavailability remains preserved.

The differences observed in C_{max} and AUC, though detectable, are of small magnitude and unlikely to have clinical consequences in women aged 20-45 years. This is consistent with what would be expected physiologically, as hepatic enzyme

activity, including CYP2C9 and CYP2C19, which are primarily responsible for phenytoin metabolism, remains relatively stable during adulthood (Mangoni & Jackson, 2004; Schmucker, 2001).

These findings indicate that in non-pregnant women between 20 and 45 years, the pharmacokinetics of phenytoin remain highly predictable and stable, with only marginal age-related increases in systemic exposure. These small changes are not clinically relevant and do not necessitate dose adjustments, reinforcing the robustness of phenytoin's pharmacokinetic profile across the different adult ages.

3.2 Influence of dosing regimens on pharmacokinetics

3.2.1 Indomethacin: comparison between dosing regimens (AUC, C_{max}, Fa)

The PKs of indomethacin were investigated under different dosing regimens to assess the impact of the frequency of administration on drug disposition. The analysis included multiple 50 mg doses administered at 24-, 12- and 8-hour intervals, allowing comparison of how dosing frequency influences systemic exposure and PK parameters C_{max}, AUC and Fa% (Table 5.)

Table 5. Simulated pharmacokinetic parameters (C_{max}, AUC_{0-∞}, Fa%) of indomethacin under different dosing regimens in the standard PBPK model.

Dose	C _{max} (ug/mL)	AUC _{0-∞} (ug-h/mL)	Fa (%)
50 mg	3.22	5.26	100
50 mg q24h for 240h	3.22	52.6	100
50 mg q12h for 240h	3.22	105	100
50 mg q8h for 240h	3.22	158	100

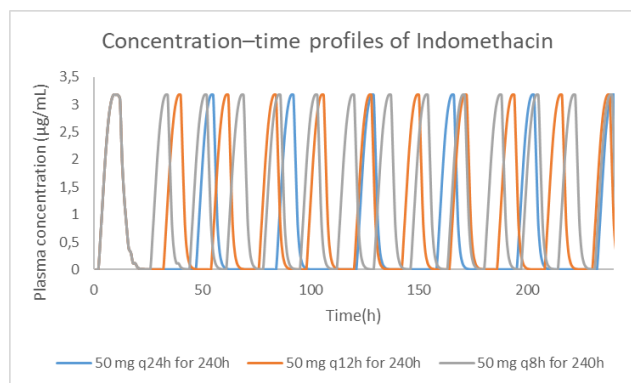


Figure 11. Simulated plasma concentration-time profiles of indomethacin in the standard PBPK model (healthy, fasting, 30-year-old woman, 60 kg) under different dosing regimens: 50 mg q24h, 50 mg q12h, and 50 mg q8h over 240 hours.

The PKs of indomethacin were investigated under different dosing regimens to evaluate the influence of administration frequency on systematic exposure.

The results demonstrated that C_{max} remained unchanged across regimens at about 3.2 µg/mL, indicating that the peak plasma concentration following each dose is independent of the dosing interval. This suggests that absorption and distribution processes are consistent with published data showing rapid and complete absorption of indomethacin (Davies & Anderson, 1997; Pacifici, 2013).

In contrast, AUC increased with more frequent administration, rising from 5.26 µg·h/mL after a single dose to 105 µg·h/mL after 50 mg every 12 h for 240 h. This increase does not reflect incomplete elimination or drug accumulation, since indomethacin has a relatively short half-life approximately 4-5 h and plasma concentrations return to near zero before the next dose is given (Davies & Anderson, 1997). Instead, the higher AUC is explained by the greater total amount of drug administered over the same time frame when dosing intervals are shortened, resulting in proportionally greater systemic exposure.

The Fa% remained constant at 100% across all regimens, confirming complete absorption and supporting previous reports of high oral bioavailability of indomethacin (Ogilvie et al., 2012).

The concentration-time curves (Figure 7) further illustrate these findings, all regimens produced sharp peaks and complete decline to baseline prior to the next administration, whereas 12 h and 8 h dosing resulted in more frequent peaks with shorter gaps between doses.

Overall, these results indicate that shortening the dosing interval increases systemic exposure AUC without altering C_{max} or Fa%. Clinically, this suggests that more frequent dosing may sustain therapeutic levels for longer periods, although the increased exposure could also elevate the risk of concentration-dependent adverse effects, reinforcing the need to balance efficacy and safety when selecting dosing regimens.

3.2.2 Phenytoin: comparison between dosing regimens (AUC, C_{max} , Fa)

The PKs of phenytoin were investigated under different dosing regimens, with the purpose of evaluating the effect of administration frequency on drug disposition. The analysis compared two different regimens, 100 mg administered every 8 hours and 300 mg administered once daily, each simulated over 168h (7days) and 240h (10 days). This analysis allowed assessment of how dosing regimen influences systemic exposure and PK parameters.

Table 6. Simulated pharmacokinetic parameters (C_{max} , $AUC_{0-\infty}$, Fa%) of phenytoin under different dosing regimens in the standard PBPK model

Dose	C_{max} (ug/mL)	$AUC_{0-\infty}$ (ug-h/mL)	Fa%
300 mg	3.3504	105.5	87.876
300 mg q24h for 168h	5.8315	762.86	87.706
100 mg q8h for 168h	5.7866	876.68	99.902
300 mg q24h for 240h	5.8356	1091.8	87.695
100 mg q8h for 240h	5.7903	1255	99.906

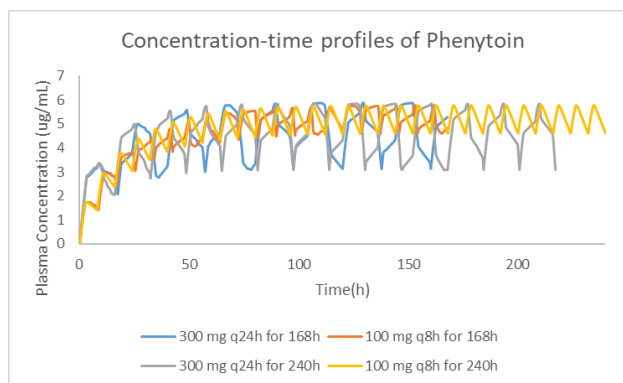


Figure 12. Simulated plasma concentration-time profiles of indomethacin in the standard PBPK model (healthy, fasting, 30-year-old woman, 60 kg) under different dosing regimens 300 mg q24h for 168 h, 100 mg q8h for 168 h, 300 mg q24h for 240 h, and 100 mg q8h for 240 h.

The PKs of phenytoin were evaluated under different dosing regimens to evaluate the influence of administration frequency and treatment duration on systemic exposure.

After a single 300 mg dose, the C_{max} was 3.35 µg/mL and the AUC was 105.5 µg·h/mL, with a Fa% of 87.9%. When the same dose was administered once daily for 168 h, C_{max} increased to 5.83 µg/mL, while the AUC rose markedly to 762.9 µg·h/mL. Extending treatment to 240 h further increased the AUC to 1091.8 µg·h/mL, whereas C_{max} remained stable, suggesting consistent peak concentrations across prolonged therapy.

More frequent administration with 100 mg q8h produced a similar C_{max} about 5.8 µg/mL, but systemic exposure was higher than with the once-daily regimen, with an AUC of 876.7 µg·h/mL at 168 h and 1255 µg·h/mL at 240 h. This indicates that by dividing the total daily dose into smaller, more frequent administrations increase overall exposure, even though peak concentrations are not substantially altered.

Unlike indomethacin, phenytoin exhibited a clear accumulative effect during repeated dosing. This is explained by its long elimination half-life (approximately 22 h in adults) and non-linear kinetics. At therapeutic concentrations, metabolism via CYP2C9 and CYP2C19 approaches saturation, resulting in dose- and time-

dependent accumulation until steady-state is achieved, typically after 7-10 days of continuous administration (Richens & Dunlop, 1975; Bialer, 1992; Perucca, 2006; Patsalos et al., 2008).

This PK profile explains the progressive increase in AUC and higher C_{max} values observed with prolonged regimens (240h).

The Fa% remained high, close to 100% for 100 mg doses and close to 88% for 300 mg, consistent with saturable absorption at higher single doses (Bialer, 1992; Perucca, 2006).

Overall, these results demonstrate that phenytoin undergoes PK accumulation, with systemic exposure (AUC) and C_{max} progressively increasing during multiple dosing. Clinically, this highlights the need for careful drug monitoring, as small adjustments in regimen can lead to disproportionate increases in plasma concentrations due to the drug's narrow therapeutic index (Richens & Dunlop, 1975; Patsalos et al., 2008).

3.3 Influence of pregnancy on pharmacokinetics

3.3.1 Effect of pregnancy on indomethacin pharmacokinetics

The impact of pregnancy on the PK of indomethacin was assessed by comparing pregnant and non-pregnant populations across the ages of 20 to 45 years. This analysis aimed to characterize how gestational physiological transformations influence systemic exposure and other PK parameters. Given the extensive maternal alterations during pregnancy, differences in drug disposition were anticipated. The results are presented in the following tables.

Table 7. Simulated pharmacokinetic parameters of indomethacin in 20-year-old pregnant and non-pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
50 mg	Non Pregnant	0.8	3.1808	5.2659	5.2659	0.27652	99.999	83.521	99.999
50 mg	Pregnant	0.9	2.9928	5.0089	5.0089	0.25949	100	82.871	100
50 mg q24h for 240h	Non Pregnant	0.8	3.1808	52.659	52.659	0.27652	99.999	83.521	99.999
50 mg q24h for 240h	Pregnant	168.9	2.9929	50.089	50.089	0.2595	100	82.874	100
50 mg q12h for 240h	Non Pregnant	108.8	3.1808	105.32	105.32	0.27652	99.999	83.521	99.999
50 mg q12h for 240h	Pregnant	228.9	2.9929	100.18	100.18	0.2595	100	82.878	100
50 mg q8h for 240h	Non Pregnant	224.8	3.1811	157.98	157.98	0.27652	99.999	83.521	99.999
50 mg q8h for 240h	Pregnant	232.9	2.9932	150.27	150.27	0.25952	100	82.881	100

Table 8. Simulated pharmacokinetic parameters of indomethacin in 25-year-old pregnant and non-pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
50 mg	Non Pregnant	0.8	3.1781	5.2658	5.2658	0.27634	99.999	83.507	99.999
50 mg	Pregnant	0.9	2.9908	5.0089	5.0089	0.25933	100	82.867	100
50 mg q24h for 240h	Non Pregnant	0.8	3.1781	52.658	52.658	0.27634	99.999	83.507	99.999
50 mg q24h for 240h	Pregnant	120.9	2.9907	50.088	50.088	0.25934	100	82.863	100
50 mg q12h for 240h	Non Pregnant	108.8	3.1781	105.32	105.32	0.27634	99.999	83.507	99.999
50 mg q12h for 240h	Pregnant	228.9	2.9907	100.18	100.18	0.25934	100	82.86	100
50 mg q8h for 240h	Non Pregnant	192.8	3.1784	157.97	157.97	0.27634	99.999	83.507	99.999
50 mg q8h for 240h	Pregnant	232.9	2.9908	150.26	150.26	0.25936	100	82.857	100

Table 9. Simulated pharmacokinetic parameters of indomethacin in 30-year-old pregnant and non-pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
50 mg	Non Pregnant	0.8	3.1754	5.2657	5.2657	0.27619	99.999	83.481	99.999
50 mg	Pregnant	0.9	2.9884	5.0086	5.0086	0.25919	100	82.83	100
50 mg q24h for 240h	Non Pregnant	0.8	3.1754	52.657	52.657	0.27619	99.999	83.481	99.999
50 mg q24h for 240h	Pregnant	216.9	2.9885	50.086	50.086	0.2592	100	82.834	100
50 mg q12h for 240h	Non Pregnant	24.8	3.1754	105.31	105.31	0.27619	99.999	83.481	99.999
50 mg q12h for 240h	Pregnant	228.9	2.9885	100.17	100.17	0.2592	100	82.837	100
50 mg q8h for 240h	Non Pregnant	152.8	3.1754	157.97	157.97	0.27619	99.999	83.481	99.999
50 mg q8h for 240h	Pregnant	232.9	2.9888	150.26	150.26	0.25922	100	82.84	100

Table 10. Simulated pharmacokinetic parameters of indomethacin in 35-year-old pregnant and non-pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
50 mg	Non Pregnant	0.8	3.1753	5.2655	5.2655	0.27625	99.999	83.446	99.999
50 mg	Pregnant	0.9	2.9885	5.0085	5.0085	0.25925	100	82.807	100
50 mg q24h for 240h	Non Pregnant	96.8	3.1753	52.654	52.654	0.27625	99.999	83.446	99.999
50 mg q24h for 240h	Pregnant	120.9	2.9885	50.084	50.084	0.25926	100	82.804	100
50 mg q12h for 240h	Non Pregnant	48.8	3.1753	105.31	105.31	0.27625	99.999	83.446	99.999
50 mg q12h for 240h	Pregnant	228.9	2.9884	100.17	100.17	0.25926	100	82.797	100
50 mg q8h for 240h	Non Pregnant	152.8	3.1753	157.96	157.96	0.27625	99.999	83.446	99.999
50 mg q8h for 240h	Pregnant	232.9	2.9885	150.25	150.25	0.25928	100	82.794	100

Table 11. Simulated pharmacokinetic parameters of indomethacin in 40-year-old pregnant and non pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
50 mg	Non Pregnant	0.8	3.1752	5.2652	5.2652	0.27633	99.999	83.4	99.999
50 mg	Pregnant	0.9	2.9882	5.0081	5.0081	0.25934	100	82.747	100
50 mg q24h for 240h	Non Pregnant	0.8	3.1752	52.652	52.652	0.27633	99.999	83.4	99.999
50 mg q24h for 240h	Pregnant	144.9	2.9882	50.081	50.081	0.25934	100	82.75	100
50 mg q12h for 240h	Non Pregnant	228.8	3.1752	105.3	105.3	0.27633	99.999	83.4	99.999
50 mg q12h for 240h	Pregnant	228.9	2.9883	100.16	100.16	0.25934	100	82.754	100
50 mg q8h for 240h	Non Pregnant	232.8	3.1752	157.95	157.95	0.27633	99.999	83.4	99.999
50 mg q8h for 240h	Pregnant	232.9	2.9886	150.24	150.24	0.25936	100	82.757	100

Table 12. Simulated pharmacokinetic parameters of indomethacin in 45-year-old pregnant and non-pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
50 mg	Non Pregnant	0.8	3.1726	5.2642	5.2642	0.27647	99.999	83.231	99.999
50 mg	Pregnant	0.9	2.986	5.0071	5.0071	0.25949	100	82.586	100
50 mg q24h for 240h	Non Pregnant	72.8	3.1726	52.642	52.642	0.27647	99.999	83.231	99.999
50 mg q24h for 240h	Pregnant	216.9	2.9859	50.07	50.07	0.25949	100	82.583	100
50 mg q12h for 240h	Non Pregnant	216.8	3.1726	105.28	105.28	0.27647	99.999	83.231	99.999
50 mg q12h for 240h	Pregnant	228.9	2.9858	100.14	100.14	0.2595	100	82.579	100
50 mg q8h for 240h	Non Pregnant	128.8	3.1729	157.93	157.93	0.27647	99.999	83.231	99.999
50 mg q8h for 240h	Pregnant	232.9	2.986	150.21	150.21	0.25952	100	82.576	100

The comparison of PK parameters between pregnant and non-pregnant women revealed consistent differences across all dosing regimens and age groups. In non-pregnant women, T_{max} was slightly higher, while C_{max} , C_{max} in the liver, AUC_{0-t} , $AUC_{0-\infty}$, and F% were also greater compared to pregnant women. In contrast, Fa% and FDp% were higher in pregnant women. These results reflect the profound physiological and biochemical changes that occur during pregnancy and highlight their impact on indomethacin pharmacokinetics.

The increase Fa% and FDp% observed in pregnant women are in line with pregnancy-associated gastrointestinal adaptations. Progesterone-mediated slowing of gastric emptying and intestinal motility prolongs gastrointestinal transit time, providing additional time for drug dissolution and absorption. Furthermore, the elevation in gastric pH during pregnancy may favor the solubilization of acidic compounds such as indomethacin, a Biopharmaceutics Classification System (BCS) class II drug with low solubility and high permeability (Halpern et al., 2019; Philipson, 1977). Together, these changes can enhance the Fa% despite delayed gastric emptying. The slightly lower T_{max} in pregnant women compared with non-pregnant women is consistent with this compensatory effect (Pinheiro & Stika, 2020).

However, despite enhanced absorption, systemic exposure was consistently lower in pregnant women, as reflected by reduced C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, and F%. Several mechanisms contribute to this reduction. First, pregnancy is characterized by marked hemodynamic and distributional changes, including a 40% increase in plasma volume and total body water, which expand the volume of distribution of hydrophobic and protein-bound drugs such as indomethacin (Hyttén, 1985; Halpern et al., 2019). This dilutional effect decreases peak plasma concentrations. Secondly, pregnancy decreases serum albumin and α 1-acid glycoprotein levels, reducing protein binding and increasing the free fraction of indomethacin. While the unbound concentration drives pharmacological effect, lower protein binding also decreases total plasma concentrations, which explains the reduced C_{max} and AUCs in pregnant women despite higher Fa% (Bardy et al., 1990; Hebert et al., 2009; Buxton, 2017).

Also, pregnancy substantially alters hepatic metabolism. Indomethacin undergoes extensive hepatic clearance, primarily through CYP2C9-mediated oxidation and UGT2B7-mediated glucuronidation. Both CYP2C9 and UGT enzymes are upregulated in pregnancy, leading to enhanced metabolic clearance. Additionally, increased activity of CYP3A4 during pregnancy may further contribute to the accelerated metabolism of indomethacin (Isoherranen & Thummel, 2013; Ke et al., 2018; Pariente et al., 2016; Pinheiro & Stika, 2020). These enzymatic changes reduce

the extent of first pass and systemic exposure, thereby explaining the lower F% and overall AUCs observed in pregnant women. Following this tendency, simulated hepatic exposure ($C_{\max}$ liver) was higher in non-pregnant women, reflecting reduced intrinsic clearance outside pregnancy.

Finally, enhanced renal clearance during pregnancy, driven by increased renal plasma flow and glomerular filtration rate, may contribute to the lower systemic exposure of indomethacin or its metabolites. Upregulation of renal transporters such as P-gp and OATs may further accelerate renal elimination beyond filtration alone (Odutayo & Hladunewich, 2012; Hebert et al., 2008).

All together, these results demonstrate that while the gastrointestinal environment in pregnancy can increase absorption (higher Fa% and FDp%), the dominant physiological changes in distribution, protein binding, metabolism, and renal clearance reduce systemic exposure to indomethacin (lower C_{max}, AUCs, and F%). This mismatch between absorption and systemic exposure has important clinical implications, as standard dosing may result in subtherapeutic concentrations in pregnant patients. Given the narrow therapeutic index of indomethacin in obstetric use, careful dose optimization and therapeutic monitoring may be warranted to ensure efficacy while minimizing adverse effects.

3.3.2. Effect of pregnancy on phenytoin pharmacokinetics

The impact of pregnancy on the PK of phenytoin was evaluated by comparing pregnant and non-pregnant women across the age range of 20 to 45 years. This analysis sought to characterize how gestational physiological adaptations affect systemic exposure and other PK parameters. Considering the extensive maternal physiological alterations that occur during pregnancy, differences in drug disposition were anticipated. The results are presented in the following tables.

Table 13. Simulated pharmacokinetic parameters of phenytoin in 20 year old pregnant and non pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
300 mg	Non Pregnant	4.8	3.3468	104.86	104.86	2.3276	87.877	86.431	87.877
300 mg	Pregnant	5.2408	3.5711	105.01	105	2.4827	90.949	89.529	90.949
300 mg q24 for 168h	Non Pregnant	148.5	5.8023	757.94	695.25	3.9827	87.708	86.32	87.704
300 mg q24h for 168h	Pregnant	148.96	5.8894	757.56	698.81	4.0471	90.791	89.431	90.787
100 mg q8h for 168h	Non Pregnant	162.6	5.7517	870.91	774.5	3.9712	99.903	98.334	99.896
100 mg q8h for 168h	Pregnant	162.7	5.5498	838.4	749.26	3.8389	99.928	98.437	99.922
300 mg q24h for 240h	Non Pregnant	220.5	5.8061	1084.8	1022	3.9851	87.697	86.315	87.694
300 mg q24h for 240h	Pregnant	220.8	5.8918	1084.1	1025.3	4.049	90.781	90.778	90.781
100 mg q8h for 240h	Non Pregnant	234.6	5.7552	1246.7	1150.1	3.9738	99.906	98.334	99.901
100 mg q8h for 240h	Pregnant	234.7	5.5523	1199.9	1110.7	3.8405	99.931	98.448	99.927

Table 14 Simulated pharmacokinetic parameters of phenytoin in 25 year old pregnant and non pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
300 mg	Non Pregnant	4.8	3.3466	104.66	104.66	2.3277	87.877	86.431	87.877
300 mg	Pregnant	5.2404	3.5704	104.73	104.73	2.4824	90.95	89.53	90.95
300 mg q24 for 168h	Non Pregnant	148.5	5.7933	756.36	694.01	3.9769	87.709	86.321	87.704
300 mg q24h for 168h	Pregnant	148.96	5.877	755.41	697.09	4.0391	90.792	89.431	90.787
100 mg q8h for 168h	Non Pregnant	162.6	5.797	869.05	773.11	3.964	99.903	99.896	99.896
100 mg q8h for 168h	Pregnant	162.7	5.5352	835.97	747.41	3.8295	99.928	98.435	99.922
300 mg q24h for 240h	Non Pregnant	220.5	5.797	1082.5	1020.1	3.9793	87.697	86.316	87.694
300 mg q24h for 240h	Pregnant	220.9	5.8794	1081	1022.6	4.041	90.782	89.428	90.779
100 mg q8h for 240h	Non Pregnant	234.6	5.7439	1244	1147.9	3.9665	99.906	98.345	99.901
100 mg q8h for 240h	Pregnant	234.7	5.5376	1196.4	1107.7	3.8312	99.931	98.446	99.927

Table 15. Simulated pharmacokinetic parameters of phenytoin in 30 year old pregnant and non pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
300 mg	Non Pregnant	4.8	3.3504	105.5	105.5	2.3301	87.876	86.431	87.876
300 mg	Pregnant	5.2408	3.5756	105.73	105.73	2.4858	90.049	89.529	90.949
300 mg q24 for 168h	Non Pregnant	148.5	5.8315	762.86	699.2	4.0025	87.706	86.319	87.702
300 mg q24h for 168h	Pregnant	148.96	5.9224	763.1	703.31	4.0693	90.789	89.429	90.784
100 mg q8h for 168h	Non Pregnant	162.6	5.7866	876.68	778.92	3.9948	99.902	98.334	99.896
100 mg q8h for 168h	Pregnant	162.7	5.5875	844.63	754.09	3.8644	99.928	98.437	99.921
300 mg q24h for 240h	Non Pregnant	220.5	5.8356	1091.8	1028.1	4.005	87.695	86.314	87.692
300 mg q24h for 240h	Pregnant	220.9	5.8918	1084.1	1025.3	4.049	90.781	89.427	90.778
100 mg q8h for 240h	Non Pregnant	234.6	5.7903	1255	1157.1	3.9975	99.906	98.346	99.901
100 mg q8h for 240h	Pregnant	324.7	5.5902	1208.2	1118.2	3.8662	99.931	98.449	99.931

Table 16. Simulated pharmacokinetic parameters of phenytoin in 35 year old pregnant and non pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
300 mg	Non Pregnant	4.8	3.3539	106.78	106.78	2.3323	87.876	86.43	87.876
300 mg	Pregnant	5.2408	3.5814	107.27	107.27	2.4892	90.948	89.531	90.948
300 mg q24 for 168h	Non Pregnant	148.5	5.8897	772.89	707.12	4.0412	87.702	86.316	87.698
300 mg q24h for 168h	Pregnant	148.96	5.9921	775.09	712.89	4.1157	90.784	89.426	90.78
100 mg q8h for 168h	Non Pregnant	162.6	5.8576	888.47	787.79	4.0423	99.902	98.336	99.895
100 mg q8h for 168h	Pregnant	162.7	5.6688	858.13	764.38	3.9188	99.927	98.437	99.921
300 mg q24h for 240h	Non Pregnant	220.5	5.8943	1106.3	1040.4	4.0441	87.691	86.311	87.687
300 mg q24h for 240h	Pregnant	221	5.9951	1109.3	1047.1	4.118	90.774	89.422	90.771
100 mg q8h for 240h	Non Pregnant	234.6	5.8618	1271.9	1171.1	4.0454	99.905	98.348	99.9
100 mg q8h for 240h	Pregnant	234.7	5.6719	1228.3	1134.4	3.9209	99.931	98.449	99.936

Table 17. Simulated pharmacokinetic parameters of phenytoin in 40 year old pregnant and non pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
300 mg	Non Pregnant	4.8	3.3569	108.14	108.14	2.3343	87.875	86.43	87.875
300 mg	Pregnant	5.2408	3.5866	108.88	108.88	2.4925	90.948	89.528	90.948
300 mg q24 for 168h	Non Pregnant	148.5	5.951	783.51	715.43	4.0822	87.698	86.314	87.694
300 mg q24h for 168h	Pregnant	148.96	6.0651	787.7	722.9	4.1644	90.78	89.423	90.775
100 mg q8h for 168h	Non Pregnant	162.6	5.9326	900.96	797.1	4.0926	99.901	98.337	99.895
100 mg q8h for 168h	Pregnant	162.7	5.7543	872.35	775.13	3.976	99.927	98.439	99.92
300 mg q24h for 240h	Non Pregnant	220.5	5.9561	1121.6	1053.4	4.0854	87.686	86.308	87.683
300 mg q24h for 240h	Pregnant	221	6.0687	1127.4	1062.6	4.1671	90.769	89.419	90.766
100 mg q8h for 240h	Non Pregnant	234.6	5.9374	1289.9	1185.9	4.0961	99.905	98.349	99.9
100 mg q8h for 240h	Pregnant	234.7	5.7579	1248.7	1151.4	3.9784	99.93	98.452	99.935

Table 18. Simulated pharmacokinetic parameters of phenytoin in 45 year old pregnant and non pregnant women.

Dose	Status	Tmax (h)	Cmax (ug/mL)	AUC 0-∞ (ug-h/mL)	AUC 0-t (ug-h/mL)	CMax Liver (ug/mL)	Fa %	F %	FDp %
300 mg	Non Pregnant	4.8	3.3582	109.36	109.36	2.3362	87.875	86.418	87.875
300 mg	Pregnant	5.2408	3.5896	110.32	110.32	2.4955	90.047	89.518	90.947
300 mg q24 for 168h	Non Pregnant	148.5	6.0059	793.11	722.86	4.1201	87.695	86.3	87.69
300 mg q24h for 168h	Pregnant	148.96	6.1295	798.9	731.7	4.2085	90.776	89.41	90.771
100 mg q8h for 168h	Non Pregnant	162.6	6.0004	912.24	805.42	4.1394	99.901	98.325	99.894
100 mg q8h for 168h	Pregnant	162.7	5.8302	884.98	784.57	4.0282	99.926	98.428	99.919
300 mg q24h for 240h	Non Pregnant	220.5	6.0116	1135.4	1065	4.1238	87.682	86.295	87.679
300 mg q24h for 240h	Pregnant	221	6.1337	1143.6	1076.3	4.2116	90.764	89.406	90.761
100 mg q8h for 240h	Non Pregnant	234.6	6.0058	1306.2	1199.2	4.1433	99.904	98.338	99.899
100 mg q8h for 240h	Pregnant	234.7	5.8343	1266.9	1166.3	4.031	99.93	98.44	99.925

The comparison of phenytoin PKs between pregnant and non-pregnant women showed consistent profiles across all age groups and dosing regimens. In pregnant women, T_{max} was slightly longer than in non-pregnant women, while systemic exposure parameters (C_{max} , AUC_{0-t} and $AUC_{0-\infty}$) exhibited variable patterns, sometimes higher in pregnant and other times in non-pregnant women. C_{max} liver was marginally higher in pregnant women, although the difference was minimal. In

contrast, Fa% and FDP% were consistently higher in non-pregnant women, as was overall oral bioavailability (F%).

The delayed T_{max} in pregnant women is consistent with the physiological effects of progesterone, which slows gastric emptying and intestinal motility, prolonging the time required to reach peak plasma concentrations (Pineiro & Stika, 2020; Halpern et al., 2019). The observation that Fa% and FDP% were higher in non-pregnant women indicates that gastrointestinal conditions in pregnancy, including elevated gastric pH and changes in bile salt secretion, may reduce the efficiency of phenytoin absorption (Philipson, 1977; Halpern et al., 2019).

Despite this reduction in absorption efficiency, C_{max} and AUCs were not consistently lower in pregnant women but showed variability. This variability can be explained by opposing physiological influences during pregnancy. On one hand, decreased albumin and α 1-acid glycoprotein concentrations increase the free fraction of highly protein-bound drugs such as phenytoin, lowering plasma concentrations (Bardy et al., 1990; Hebert et al., 2009; Buxton, 2017). On the other hand, altered hepatic metabolism may either increase or decrease clearance depending on the relative contribution of specific enzymes. Phenytoin is mainly metabolized by CYP2C9 and CYP2C19, and pregnancy is associated with increased CYP2C9 activity but decreased CYP2C19 activity (Isoherranen & Thummel, 2013; Ke et al., 2018; Tornio & Backman, 2018). The balance between these opposing enzymatic changes likely explains the inconsistent differences in systemic exposure observed between groups. The slightly higher hepatic C_{max} in pregnant women aligns with these metabolic adaptations, reflecting increased enzyme activity within the liver.

In addition, pregnancy is characterized by increased renal plasma flow and glomerular filtration rate, which enhances renal clearance (Oduyayo & Hladunewich, 2012; Cunningham et al., 2018). Upregulation of renal transporters such as P-gp further contributes to altered elimination (Hebert et al., 2008). These changes may reduce systemic exposure of phenytoin metabolites, thereby reinforcing the lower bioavailability (F%) observed in pregnant women compared with non-pregnant women.

Taken together, these results highlight that pregnancy modifies phenytoin pharmacokinetics through a combination of delayed absorption, reduced absorption efficiency, altered protein binding, enzyme-specific metabolic changes, and enhanced renal clearance. The net effect is a longer T_{max} , reduced $Fa\%$, $FDp\%$ and $F\%$, and variable systemic exposure across dosing regimens. Given the narrow therapeutic index of phenytoin, these results underscore the importance of therapeutic drug monitoring in pregnant patients to ensure both efficacy and safety

3.4 Drug–drug interaction (DDI) evaluation

3.4.1 Impact of co-administration on indomethacin pharmacokinetics in non-pregnant women

To assess the potential PK interaction between phenytoin and indomethacin, phenytoin was considered as the perpetrator and indomethacin as the victim drug. Indomethacin undergoes extensive hepatic metabolism, primarily via cytochrome P450 isoenzyme CYP2C9 and by glucuronidation through UGT2B7 (Nakajima et al., 1998; Alqahtani et al., 2015). Phenytoin is a well-documented inducer of multiple metabolic enzymes, such as CYP2C9, CYP2C19 and UGT2B7, which can accelerate the clearance of co-administered substrates (Perucca, 2006; Patsalos et al., 2008). In the co-administration of both drugs, induction is expected to decrease indomethacin systemic exposure, reflected by reductions in AUC and C_{max} .

Accordingly, simulations in non-pregnant women were performed to quantify these changes and evaluate the clinical relevance of the interaction under baseline physiological conditions.

Table 19. Pharmacokinetic parameters of indomethacin in a 20-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

	Tmax (h)	Cmax (ug/mL)	AUC(0-inf) (ng-h/mL)	AUC(0-t) (ng-h/mL)	Fa (%)	FDp (%)	F (%)
Indomethacin 50 mg q8h - baseline	200.8	3.181	158000	158000	100	100	83.52
Indomethacin 50 mg q8h- Phenytoin 100 mg q8h - DDI	24.8	3.181	158000	158000	100	100	83.52
Indomethacin-ratio	0.124	1	1	1	1	1	1
Indomethacin 50 mg q8h- baseline	16.88	3.182	158000	158000	100	100	83,2
Indomethacin 50 mg q8h - Phenytoin 300 mg q24h - DDI	8.8	3.181	158000	158000	100	100	83,2
Indomethacina-ratio	0.521	1	1	1	1	1	1

Table 20. Pharmacokinetic parameters of indomethacin in a 25-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

	Tmax (h)	Cmax (ug/mL)	AUC(0-inf) (ng-h/mL)	AUC(0-t) (ng-h/mL)	Fa (%)	FDp (%)	F (%)
Indomethacin 50 mg q8h - baseline	88.8	3.178	158000	158000	100	100	83.51
Indomethacin 50 mg q8h- Phenytoin 100 mg q8h - DDI	24.8	3.178	158000	158000	100	100	83.51
Indomethacin-ratio	0.279	1	1	1	1	1	1
Indomethacin 50 mg q8h- baseline	104.8	3.178	158000	158000	100	100	83.51
Indomethacin 50 mg q8h - Phenytoin 300 mg q24h - DDI	8.8	3.178	158000	158000	100	100	83.51
Indomethacina-ratio	0.084	1	1	1	1	1	1

Table 21. Pharmacokinetic parameters of indomethacin in a 30-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

	Tmax (h)	Cmax (ug/mL)	AUC(0-inf) (ng-h/mL)	AUC(0-t) (ng-h/mL)	Fa (%)	FDp (%)	F (%)
Indomethacin 50 mg q8h - baseline	72.8	3.176	158000	158000	100	100	83.48
Indomethacin 50 mg q8h - Phenytoin 100 mg q8h - DDI	24.8	3.176	158000	158000	100	100	83.48
Indomethacin-ratio	0.341	1	1	1	1	1	1
Indomethacin 50 mg q8h - baseline	200.8	3.176	158000	158000	100	100	83.48
Indomethacin 50 mg q8h - Phenytoin 300 mg q24h - DDI	8.8	3.176	158000	158000	100	100	83.48
Indomethacin-ratio	0.044	1	1	1	1	1	1

Table 22. Pharmacokinetic parameters of indomethacin in a 35-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

	Tmax (h)	Cmax (ug/mL)	AUC(0-inf) (ng-h/mL)	AUC(0-t) (ng-h/mL)	Fa (%)	FDp (%)	F (%)
Indomethacin 50 mg q8h - baseline	72.8	3.176	158000	158000	100	100	83.45
Indomethacin 50 mg q8h - Phenytoin 100 mg q8h - DDI	24.8	3.176	158000	158000	100	100	83.45
Indomethacin-ratio	0.341	1	1	1	1	1	1
Indomethacin 50 mg q8h - baseline	64.8	3.176	158000	158000	100	100	83.45
Indomethacin 50 mg q8h - Phenytoin 300 mg q24h - DDI	8.8	3.176	158000	158000	100	100	83.45
Indomethacin-ratio	0.136	1	1	1	1	1	1

Table 23. Pharmacokinetic parameters of indomethacin in a 40-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

	Tmax (h)	Cmax (ug/mL)	AUC(0-inf) (ng-h/mL)	AUC(0-t) (ng-h/mL)	Fa (%)	FDp (%)	F (%)
Indomethacin 50 mg q8h - baseline	48.8	3.176	158000	158000	100	100	83.4
Indomethacin 50 mg q8h - Phenytoin 100 mg q8h - DDI	24.8	3.176	158000	158000	100	100	83.4
Indomethacin-ratio	0.508	1	1	1	1	1	1
Indomethacin 50 mg q8h - baseline	152.8	3.176	158000	158000	100	100	83.4
Indomethacin 50 mg q8h - Phenytoin 300 mg q24h - DDI	8.8	3.176	158000	158000	100	100	83.4
Indomethacin-ratio	0.058	1	1	1	1	1	1

Table 24. Pharmacokinetic parameters of indomethacin in a 45-year-old female simulation under drug–drug interaction (DDI) with phenytoin.

	Tmax (h)	Cmax (ug/mL)	AUC(0-inf) (ng-h/mL)	AUC(0-t) (ng-h/mL)	Fa (%)	FDp (%)	F (%)
Indomethacin 50 mg q8h - baseline	48.8	3.173	158000	158000	100	100	83.23
Indomethacin 50 mg q8h - Phenytoin 100 mg q8h - DDI	24.8	3.173	158000	158000	100	100	83.23
Indomethacin-ratio	0.508	1	1	1	1	1	1
Indomethacin 50 mg q8h - baseline	112.8	3.173	158000	158000	100	100	83.23
Indomethacin 50 mg q8h - Phenytoin 300 mg q24h - DDI	8.88	3.173	158000	158000	100	100	83.23
Indomethacin-ratio	0.079	1	1	1	1	1	1

The evaluation of the PK interaction between phenytoin as the perpetrator and indomethacin as the victim drug, demonstrated that co-administration did not result in alterations in the PK profile of indomethacin. In non-pregnant women, the parameters C_{max} , T_{max} , AUC_{0-t} , $AUC_{0-\infty}$, as well as the oral bioavailability parameters Fa%, FDp% and F% remained comparable to those that resulted from control conditions without phenytoin, (indomethacin baseline).

$C_{\max}$ values of indomethacin were unchanged in the presence of phenytoin, indicating that peak plasma systemic exposure was not affected by the concomitant drug. Likewise, $T_{\max}$ did not show relevant changes, suggesting that the absorption kinetics of indomethacin are not influenced by phenytoin. The AUC parameters, which reflect total systemic exposure, were also consistent with the control conditions (indomethacin baseline), supporting the absence of an inhibitory or inductive effect of phenytoin on the overall clearance of indomethacin.

Although indomethacin is partially metabolized by the enzyme CYP2C9, the same enzyme responsible for phenytoin metabolism and induction, the unchanged systemic exposure implies that this metabolic pathway was not significantly affected to the point of altering indomethacin disposition. Previous studies have shown that indomethacin undergoes extensive glucuronidation via UGT2B7, in addition to oxidative metabolism (Nakajima et al., 1998; Alqahtani et al., 2015). These routes likely remain dominant and may compensate for any potential CYP2C9 induction by phenytoin, thereby preventing meaningful pharmacokinetic changes. Furthermore, phenytoin, while recognized as a strong inducer of CYP2C9 and CYP3A4 (Perucca, 2006; Zaccara et al., 2014), may not reach an induction threshold sufficient to impact indomethacin clearance under the simulated conditions.

The absence of significant differences suggests that the concomitant use of indomethacin and phenytoin is unlikely to require dose adjustment or additional monitoring in non-pregnant women (Rodriguez et al., 2023). Nevertheless, it should be emphasized that these conclusions are based on simulations, and further clinical studies are warranted to confirm the lack of interaction in real-world scenarios, particularly in populations with altered metabolisms.

3.5 Integrative Discussion

The integrated analysis of the present simulations highlights the contrasting PK behaviors of indomethacin and phenytoin, the differential impact of age and pregnancy, and the limited drug-drug interaction. Together, these results illustrate

the clinical complexities of dosing in women of reproductive age and highlight the utility of PBPK modeling as a mechanistic framework of prediction.

One of the central findings of this analysis is the difference in linearity between the two compounds. Indomethacin exhibited dose-proportional kinetics across all different regimens, with $C_{\max}$ values remaining stable at about 3.2 $\mu\text{g/mL}$ and $\text{Fa\%}$ close to 100%, while systemic exposure (AUC) increased proportionally with dosing frequency (from about 53 $\mu\text{g}\cdot\text{h/mL}$ at q24h to about 158 $\mu\text{g}\cdot\text{h/mL}$ at q8h over 240 h). This proportionality confirms that absorption is not rate-limiting and clearance mechanisms are not saturated in these therapeutic range. Clinically, this predictability facilitates straightforward dose adjustments based on total daily dose. These results are consistent with clinical pharmacokinetic reports describing indomethacin as a drug with near-complete oral bioavailability and dose-proportional kinetics (Dodick, 2004; Lucas, 2016). This linear behavior also means that small dosing errors are unlikely to produce disproportionate shifts in systemic exposure, a property that confers a relatively wide margin of predictability compared with drugs exhibiting nonlinear kinetics. From a clinical management perspective, such proportionality simplifies both acute and chronic therapeutic use.

In contrast, phenytoin revealed classical nonlinear kinetics, with evidence of dose-dependent accumulation and AUC rising disproportionately between 168 h and 240h simulations showed 763 $\mu\text{g}\cdot\text{h/mL}$ vs. 1092 $\mu\text{g}\cdot\text{h/mL}$ under q24h dosing, while $C_{\max}$ values remained relatively stable at about 5.8 $\mu\text{g/mL}$. This reflects saturation of CYP2C9- and CYP2C19-mediated metabolism within therapeutic concentrations, consistent with the known Michaelis-Menten elimination of phenytoin (Rodriguez-Vera et al., 2023; Gupta & Tripp, 2023). Clinically, this finding reinforces the narrow therapeutic index of phenytoin and the need for therapeutic drug monitoring (TDM), as even small increments in daily dose may lead to marked increases in plasma concentration and toxicity (Pennell, 2003; Tomson et al., 2011).

Unlike indomethacin, where linearity supports predictable accumulation, the saturable metabolism of phenytoin introduces a level of uncertainty that can only be managed with regular monitoring. This explains why, despite long-standing

clinical use, phenytoin remains one of the most challenging AEDs to manage during pregnancy and beyond.

Regarding age-related variability, both compounds showed only minor and clinically irrelevant changes in systemic exposure across the 20–45-year range. Indomethacin exhibited slight decreases in oral bioavailability with advancing age, while phenytoin showed modest increases in $C_{\max}$ and AUC in older age groups. These alterations, however, were negligible compared to changes induced by the pregnancy status. This contrast emphasizes that chronological age within the reproductive window is not a major determinant of PK variability, whereas pregnancy physiology emerges as the dominant factor influencing drug disposition in women of reproductive age.

This finding is reassuring for clinicians, as it supports that therapeutic management of these drugs in adult women does not need to be tailored to chronological age. Instead, resources and monitoring efforts should be concentrated on pregnancy-related variability, which is the primary factor of altered exposure.

The impact of pregnancy was more pronounced and mechanistically diverse. For indomethacin, pregnancy consistently reduced systemic exposure, with AUC and F% values lower in pregnant women despite higher Fa% and FDp%. For instance, bioavailability decreased during pregnancy, reflecting that the net effect of decreased albumin binding, induction of CYP2C9 and UGT2B7, and increased glomerular filtration (Anderson, 2005; Pariente et al., 2016). Although gastrointestinal absorption may be favored by slower gastric emptying and higher gastric pH (Halpern et al., 2019), the systemic exposure was nonetheless reduced. These findings align with clinical observations demonstrating lower indomethacin AUC in pregnant women (Rytting et al., 2014; Alqahtani and Kaddoumi, 2015). From a therapeutic perspective, this reduction may compromise efficacy in indications such as preterm labor suppression or ductus arteriosus closure.

This illustrates a common paradox in pregnancy pharmacology, although physiological changes could theoretically improve drug absorption, the prevailing outcome is a reduction in systemic exposure driven by enhanced clearance

mechanisms. This complexity emphasizes the limitations of empirical dose adjustments and the value of PBPK models in providing mechanistic guidance.

For phenytoin, pregnancy introduced heterogeneous and regimen-dependent changes. $T_{\max}$ was modestly prolonged, while AUC and $C_{\max}$ values fluctuated depending on the dosing schedule, in some simulations, exposure was reduced, in others it remained comparable, and occasionally it increased. This variability reflects the interference of opposing mechanisms such as reduced albumin concentrations leading to lower total concentrations but higher unbound fraction, induction of CYP2C9 and CYP3A4 accelerating clearance, relative inhibition of CYP2C19 decreasing alternative metabolism, and enhanced renal clearance (Tomson & Battino, 2012; Harden et al., 2009). The net result is an unpredictable balance that varies by individual and dosing context. Clinically, this reinforces the guideline consensus that phenytoin dosing during pregnancy must be individualized through regular TDM to avoid both subtherapeutic exposure and maternal toxicity (Pennell, 2003; Tomson et al., 2011).

Finally, the DDI simulation analysis revealed no clinically significant reduction in indomethacin exposure during co-administration with phenytoin in non-pregnant women. For example, $C_{\max}$ and AUC remained the same as the baseline values despite phenytoin's known inductive effects on CYP2C9 and CYP3A4 (Pacifici, 2013; FDA, 2022). A possible explanation is that indomethacin clearance relies substantially on glucuronidation via UGT2B7, which may compensate for increases in oxidative metabolism. These findings are consistent with Rodriguez-Vera et al. (2023), who also reported minimal interaction under simulated conditions. Nonetheless, the inability to extend these simulations to pregnant populations due to technical limitations leaves open the possibility that gestational physiology could reveal interactions showing differences comparing to baseline conditions.

This limitation highlights the need for future modelling studies that specifically integrate pregnancy modules for DDI prediction. Given that both phenytoin and indomethacin are commonly used in women of reproductive age, the clinical importance of this gap cannot be underestimated.

Taken together, these results establish several overarching insights. Indomethacin demonstrates predictable, linear kinetics, but is sensitive to pregnancy-induced reductions in systemic exposure that may compromise therapeutic efficacy. Phenytoin exhibits nonlinear, saturable kinetics, with interindividual and gestational variability that requires individualized management and TDM. While age alone contributes minimally, pregnancy physiology dominates PK variability, reinforcing the importance of studying this population specifically rather than extrapolating from non-pregnant data. Finally, although the simulated DDI between phenytoin and indomethacin appeared clinically negligible in non-pregnant women, pregnancy could alter this balance, warranting further evaluation. Collectively, the findings underscore the utility of PBPK modeling to mechanistically dissect complex scenarios, providing a foundation for risk assessment and potential guidance in populations historically excluded from clinical trials.

3.6 Study limitations

Although this study provides valuable insights into the PKs of indomethacin and phenytoin under various physiological and dosing conditions, several limitations must be acknowledged to contextualize these results and frame their applicability.

A first limitation relates to the fact that the drug-drug interaction simulations, were restricted to non-pregnant women. Technical constraints in the pregnancy module prevented the evaluation of co-administration scenarios under gestational conditions. This omission is critical, as the physiological changes of pregnancy including increased metabolic capacity, altered protein binding, and enhanced renal clearance could modify the magnitude of induction exerted by phenytoin on indomethacin metabolism. The absence of such simulations limits the ability to draw firm conclusions about the clinical significance of this interaction in the population of highest interest.

A second limitation concerns the parameterization of the models. Several inputs were derived from *in silico* predictions using ADMET Predictor™ and MedChem Designer™, which, despite being widely validated tools, cannot fully substitute experimental data. Uncertainties in solubility, permeability, or enzyme fraction

contributions could propagate through the model, particularly in sensitive scenarios such as nonlinear phenytoin kinetics. While predicted/observed ratios during validation fell within conventional acceptance criteria, the absence of direct experimental data for some parameters reduces the certainty of extrapolations, especially in pregnancy where physiological variability is high.

A third limitation is the use of a standardized reference profile a healthy, fasting, 30-year-old, 60 kg woman as the baseline for all simulations. Although this approach aligns with PBPK modeling practice, it does not capture the diversity of clinical populations, where variations in body weight, ethnicity, comorbidities, and nutritional status significantly influence drug disposition. Consequently, while the models offer mechanistic plausibility, their predictive power outside the defined scenarios is inherently constrained.

The lack of pharmacogenomic representation also constitutes an important limitation. Both CYP2C9 and CYP2C19 play central roles in phenytoin metabolism and contribute to indomethacin clearance, both enzymes exhibit highly polymorphic variants with substantial interethnic frequency differences. The omission of genotype-driven variability restricts the ability of the models to capture the full spectrum of patient responses, particularly given the known clinical consequences of poor versus extensive metabolizer phenotypes.

Finally, the limited availability of pregnancy-specific PK data restricted external validation of the models. While non-pregnant simulations reproduced clinical data with acceptable accuracy, the paucity of studies in pregnant women remains a major barrier to confirmatory validation. This limitation reflects a broader structural gap in clinical pharmacology, as pregnant women are systematically underrepresented in clinical research (van der Graaf et al., 2018; Blehar et al., 2013). As such, while the current findings are mechanistically plausible, they cannot be directly translated into dosing recommendations without further prospective confirmation.

3.7 Future perspectives

The limitations identified in this work provide a blueprint for future research directions aimed at enhancing the predictive capacity and clinical applicability of PBPK models in pregnancy.

A first priority should be the implementation of DDI simulations in pregnancy models, once the technical constraints of the software are resolved. Evaluating phenytoin's inductive effect on CYP2C9 and UGT2B7 in pregnant physiology, particularly during the second and third trimesters, would clarify whether systemic exposure to indomethacin is reduced to a clinically significant extent in this population.

The integration of pharmacogenomics represents a critical next step. Incorporating allele frequency distributions for CYP2C9, CYP2C19, and UGT2B7 into virtual trial simulations would allow stratification of exposure predictions by metabolizer phenotypes. These integrations would not only increase predictive robustness but also identify patient subgroups at higher risk of underexposure or toxicity, aligning PBPK modeling with precision medicine efforts.

Expanding the scope of analysis to include PK/PD modeling would also strengthen clinical translatability. By linking PK parameters AUC, C_{max} , trough concentrations to pharmacodynamic endpoints such as seizure suppression for phenytoin or inhibition of uterine contractions for indomethacin, the models could inform therapeutic targets more directly. This approach would go beyond exposure-based assessment to clinically meaningful predictions of efficacy and safety.

Another promising direction involves simulating real-world clinical conditions, including fed versus fasted states, partial adherence, obesity, renal or hepatic impairment, and the presence of multiple concomitant medications. Given the high prevalence of comorbidities and polypharmacy in obstetric and neurological practice, extending PBPK models to these contexts would improve their external validity and clinical relevance.

Finally, prospective clinical validation remains essential. Observational studies in pregnant women, using model-informed sparse sampling strategies, could provide

the data necessary to refine and confirm the predictions of PBPK models. Collaborative efforts involving academic, pharmaceutical industry, and regulatory agencies are crucial to perform such studies and to establish PBPK modeling as a recognized decision-support tool. By bridging the translational gap, these initiatives could contribute to safer and more effective drug use in pregnancy, a population historically excluded from clinical trials but in urgent need of evidence-based dosing recommendations.

4. Conclusion

This thesis developed and validated PBPK models for both indomethacin and phenytoin, allowing for a comprehensive assessment of their PK profiles under clinically relevant conditions. The models were consistent with published *in vitro* and *in vivo* data providing a robust framework to explore the influence of age, dosing regimens, pregnancy and DDIs. The analysis demonstrated that the difference in ages between 20- and 45-years results in only minor effects on the PK of both drugs. These variations, are unlikely to be clinically relevant, as they do not appear to translate into changes in efficacy or toxicity that would justify dose adjustment or additional monitoring.

Regarding dosing regimens, indomethacin showed linear PK, with systemic exposure increasing proportionally to dosing frequency. In contrast, phenytoin exhibited non-linear kinetics, as expected, with fractionated regimens leading to higher overall exposure than once daily administration. These results highlight the need for careful consideration of regimen choice, especially for drugs with narrow therapeutic indices such as phenytoin.

Pregnancy significantly altered the PK profiles of both drugs. For indomethacin, pregnancy-related changes led to reduced systemic exposure. In contrast,

phenytoin exhibited variable exposure across regimens, reflecting the opposing influence of enzyme induction and inhibition during pregnancy. These results highlight the complexity of pregnancy pharmacology and reinforce the need for therapeutic drug monitoring and individualized dose adjustment in pregnant patients.

Regarding the DDI study, in which phenytoin acted as the perpetrator drug and indomethacin as the victim, showed no clinically relevant alterations in the PK of indomethacin in non-pregnant women. Although both drugs undergo metabolism primarily via CYP2C9, systemic exposure to indomethacin remained unchanged upon co-administration. This outcome may be explained by the contribution of alternative elimination pathways, such as UGT-mediated glucuronidation, which can compensate for potential CYP2C9 induction by phenytoin. Consequently, the absence of a significant interaction indicates that phenytoin-induced enzymatic effects are not sufficient to reduce indomethacin under the simulated conditions. These results suggest a low risk of reduced efficacy or increase toxicity for indomethacin when co-administered with phenytoin in non-pregnant populations, although clinical confirmation would still be required.

In conclusion, this work showcases PBPK modeling as a powerful, innovation-driven approach to de-risk dosing decisions, particularly in populations where clinical data are sparse (e.g., pregnancy). By mechanistically integrating physiological change with drug properties and regimen design, the models provide actionable insights to support dose selection, TDM strategies, and safer pharmacotherapy, while establishing a scalable in silico foundation that can be readily adapted to additional drugs, special populations, and prospective “virtual trials” in future studies.

References

- Abdelsayed, M., & Sokolov, S. (2013). Voltage-gated sodium channels: Pharmaceutical targets via anticonvulsants to treat epileptic syndromes. *Channels*, 7(3), 146–152. <https://doi.org/10.4161/CHAN.24380>,
- Abramovici, A., Cantu, J., & Jenkins, S. M. (2012). Tocolytic Therapy for Acute Preterm Labor. *Obstetrics and Gynecology Clinics of North America*, 39(1), 77–87. <https://doi.org/10.1016/j.ogc.2011.12.003>
- Aldridge, A., Bailey, B., & Neims, A. H. (1981, October). *The disposition of caffeine during and after pregnancy* - PubMed. Semin Perinatol. <https://pubmed.ncbi.nlm.nih.gov/7302604/>
- Alkema, L., Chou, D., Hogan, D., Zhang, S., Moller, A. B., Gemmill, A., Fat, D. M., Boerma, T., Temmerman, M., Mathers, C., & Say, L. (2016). Global, regional, and national levels and trends in maternal mortality between 1990 and 2015, with scenario-based projections to 2030: A systematic analysis by the un Maternal Mortality Estimation Inter-Agency Group. *The Lancet*, 387(10017), 462–474. [https://doi.org/10.1016/S0140-6736\(15\)00838-7](https://doi.org/10.1016/S0140-6736(15)00838-7)
- Anderson, G. D. (2005). Pregnancy-induced changes in pharmacokinetics: A mechanistic-based approach. *Clinical Pharmacokinetics*, 44(10), 989–1008. <https://doi.org/10.2165/00003088-200544100-00001>,
- Arfè, A., Scotti, L., Varas-Lorenzo, C., Nicotra, F., Zambon, A., Kollhorst, B., Schink, T., Garbe, E., Herings, R., Straatman, H., Schade, R., Villa, M., Lucchi, S., Valkhoff, V., Romio, S., Thiessard, F., Schuemie, M., Pariente, A., Sturkenboom, M., & Corrao, G. (2016). Non-steroidal anti-inflammatory drugs and risk of heart failure in four European countries: nested case-control study. *BMJ (Clinical Research Ed.)*, 354, i4857. <https://doi.org/10.1136/BMJ.I4857>,
- Argikar, U. A., Cloyd, J. C., Birnbaum, A. K., Leppik, I. E., Conway, J., Kshirsagar, S., Oetting, W. S., Klein, E. C., & Remmel, R. P. (2006). Paradoxical urinary phenytoin metabolite (S)/(R) ratios in CYP2C19*1/*2 patients. *Epilepsy Research*, 71(1), 54–63. <https://doi.org/10.1016/j.eplepsyres.2006.05.015>
- AZ, C., BD, M., & EC, W. (2012). Specific safety and tolerability considerations in the use of anticonvulsant medications in children. *Drug, Healthcare and Patient Safety*, 4, 39. <https://doi.org/10.2147/DHPS.S28821>
- Bajpai, M., Roskos, L. K., Shen, D. D., & Levy, R. H. (1996, December). *Roles of cytochrome P4502C9 and cytochrome P4502C19 in the stereoselective metabolism of phenytoin to its major metabolite*. Drug Metab Dispos. <https://pubmed.ncbi.nlm.nih.gov/8971149/>
- Bergen, D. C. (2009). Pharmacokinetics of phenytoin: reminders and discoveries. *Epilepsy Currents*, 9(4), 102–104. <https://doi.org/10.1111/J.1535-7511.2009.01307.X>
- Bernard, S., Neville, K. A., Nguyen, A. T., & Flockhart, D. A. (2006). Interethnic Differences in Genetic Polymorphisms of CYP2D6 in the U.S. Population: Clinical Implications. *The Oncologist*, 11(2), 126–135. <https://doi.org/10.1634/THEONCOLOGIST.11-2-126>,
- Bogen, D. L., Perel, J. M., Helsel, J. C., Hanusa, B. H., Romkes, M., Nukui, T., Friedman, C. R., & Wisner, K. L. (2013). Pharmacologic evidence to support clinical decision making for

peripartum methadone treatment. *Psychopharmacology*, 225(2), 441–451. <https://doi.org/10.1007/S00213-012-2833-7>,

Borges, S., Desta, Z., Jin, Y., Faouzi, A., Robarge, J. D., Philips, S., Nguyen, A., Stearns, V., Hayes, D., Rae, J. M., Skaar, T. C., Flockhart, D. A., & Li, L. (2010). Composite functional genetic and comedication CYP2D6 activity score in predicting tamoxifen drug exposure among breast cancer patients. *Journal of Clinical Pharmacology*, 50(4), 450–458. <https://doi.org/10.1177/0091270009359182>,

Bousman, C. A., Stevenson, J. M., Ramsey, L. B., Sangkuhl, K., Hicks, J. K., Strawn, J. R., Singh, A. B., Ruaño, G., Mueller, D. J., Tsermpini, E. E., Brown, J. T., Bell, G. C., Leeder, J. S., Gaedigk, A., Scott, S. A., Klein, T. E., Caudle, K. E., & Bishop, J. R. (2023). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants. *Clinical Pharmacology and Therapeutics*, 114(1), 51–68. <https://doi.org/10.1002/CPT.2903>,

Claesen, M., Moustafa, M. A., Adline, J., Vandervorst, D., & Poupaert, J. H. (1982). Evidence for an arene oxide-NIH shift pathway in the metabolic conversion of phenytoin to 5-(4-hydroxyphenyl)-5-phenylhydantoin in the rat and in man. *Drug Metab Dispos.*, 10(6), 667–671. <https://pubmed.ncbi.nlm.nih.gov/6130920/>

Craig, S. (2005). Phenytoin poisoning. *Neurocritical Care*, 3(2), 161–170. <https://doi.org/10.1385/NCC:3:2:161>,

Crane, P., & Wiegand, T. J. (2023). Phenytoin. *Encyclopedia of Toxicology, Fourth Edition: Volume 1-9*, 7, V7-563-V7-567. <https://doi.org/10.1016/B978-0-12-824315-2.00741-7>

Cuttle, L., Munns, A. J., Hogg, N. A., Scott, J. R., Hooper, W. D., Dickinson, R. G., & Gillam, E. M. (2000, August). *Phenytoin metabolism by human cytochrome P450: involvement of P450 3A and 2C forms in secondary metabolism and drug-protein adduct formation*. *Drug Metab Dispos.* <https://pubmed.ncbi.nlm.nih.gov/10901705/>

Davis-Floyd, R., Gutschow, K., & Schwartz, D. A. (2020). Pregnancy, Birth and the COVID-19 Pandemic in the United States. *Medical Anthropology: Cross Cultural Studies in Health and Illness*, 39(5), 413–427. <https://doi.org/10.1080/01459740.2020.1761804>,

Dickmann, L. J., & Isoherranen, N. (2013). Quantitative prediction of CYP2B6 induction by estradiol during pregnancy: Potential explanation for increased methadone clearance during pregnancy. *Drug Metabolism and Disposition*, 41(2), 270–274. <https://doi.org/10.1124/dmd.112.047118>

Dixon CM, Colthup PV, Serabjit-Singh CJ, Kerr BM, Boehlert CC, Park GR, & Tarbit MH. (1995). Multiple forms of cytochrome P450 are involved in the metabolism of ondansetron in humans. *Drug Metab Dispos.*, 23(11), 1225–1230. <https://pubmed.ncbi.nlm.nih.gov/8591723/>

Dodick, D. W. (2004). Indomethacin-responsive headache syndromes. *Current Pain and Headache Reports*, 8(1), 19–26. <https://doi.org/10.1007/S11916-004-0036-6>,

Dougherty, C. (2021). Indomethacin-Induced Headache: Causing the Problem You Are Trying to Solve. *Current Pain and Headache Reports*, 25(11). <https://doi.org/10.1007/S11916-021-00986-9>,

Duggan, D. E., Hogans, A. F., Kwan, K. C., & McMahon, F. G. (1972, June). *The metabolism of indomethacin in man*. *J Pharmacol*. <https://pubmed.ncbi.nlm.nih.gov/4555898/>

Engeland, A., Bjørge, T., Klungsøyr, K., Hjellvik, V., Skurtveit, S., & Furu, K. (2018). Trends in prescription drug use during pregnancy and postpartum in Norway, 2005 to 2015. *Pharmacoepidemiology and Drug Safety*, 27(9), 995–1004. <https://doi.org/10.1002/PDS.4577>,

FDA. (2009). *Indocin*® I.V. (indomethacin for injection). https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/018878s027lbl.pdf

FDA. (2016). *HIGHLIGHTS OF PRESCRIBING INFORMATION*. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/016059s100lbl.pdf

Fritz, S., Lindner, W., Roots, I., Frey, B. M., & Küpfer, A. (1987). Stereochemistry of aromatic phenytoin hydroxylation in various drug hydroxylation phenotypes in humans. *J Pharmacol Exp Ther*, 241(2), 615–622. <https://pubmed.ncbi.nlm.nih.gov/3572817/>

Fuller, K. L., Wang, Y. Y., Cook, M. J., Murphy, M. A., & D'Souza, W. J. (2013). Tolerability, safety, and side effects of levetiracetam versus phenytoin in intravenous and total prophylactic regimen among craniotomy patients: A prospective randomized study. *Epilepsia*, 54(1), 45–57. <https://doi.org/10.1111/J.1528-1167.2012.03563.X>,

Harden, C. L., Hopp, J., Ting, T. Y., Pennell, P. B., French, J. A., Hauser, W. A., Wiebe, S., Gronseth, G. S., Thurman, D., Meador, K. J., Koppel, B. S., Kaplan, P. W., Robinson, J. N., Gidal, B., Hovinga, C. A., Wilner, A. N., Vazquez, B., Holmes, L., Krumholz, A., ... Le Guen, C. (2009). Practice parameter update: Management issues for women with epilepsy - Focus on pregnancy (an evidence-based review): Obstetrical complications and change in seizure frequency: Report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and American Epilepsy Society. *Neurology*, 73(2), 126–132. <https://doi.org/10.1212/WNL.0B013E3181A6B2F8>,

Harden, C. L., Meador, K. J., Pennell, P. B., Allen Hauser, W., Gronseth, G. S., French, J. A., Wiebe, S., Thurman, D., Koppel, B. S., Kaplan, P. W., Robinson, J. N., Hopp, J., Ting, T. Y., Gidal, B., Hovinga, C. A., Wilner, A. N., Vazquez, B., Holmes, L., Krumholz, A., ... Le Guen, C. (2009). Management issues for women with epilepsy - Focus on pregnancy (an evidence-based review): II. Teratogenesis and perinatal outcomes: Report of the Quality Standards Subcommittee and Therapeutics and Technology Subcommittee of the American Academy of Neurology and the American Epilepsy Society. *Epilepsia*, 50(5), 1237–1246. <https://doi.org/10.1111/J.1528-1167.2009.02129.X>,

Hauser, W., & Hesdorffer, D. (1990). *Epilepsy: frequency, causes, and consequences*.

Helleberg, L. (1981). Clinical Pharmacokinetics of Indomethacin. *Clinical Pharmacokinetics*, 6(4), 245–258. <https://doi.org/10.2165/00003088-198106040-00001>,

Hicks, J. K., Sangkuhl, K., Swen, J. J., Ellingrod, V. L., Müller, D. J., Shimoda, K., Bishop, J. R., Kharasch, E. D., Skaar, T. C., Gaedigk, A., Dunnenberger, H. M., Klein, T. E., Caudle, K. E., & Stingl, J. C. (2017). Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. *Clinical Pharmacology and Therapeutics*, 102(1), 37–44. <https://doi.org/10.1002/CPT.597>,

- Hoppmann, H. A., Peden, J. G., & Ober, S. K. (1991). *Central nervous system side effects of nonsteroidal anti-inflammatory drugs. Aseptic meningitis, psychosis, and cognitive dysfunction - PubMed*. 151(7), 1309–1313. <https://pubmed.ncbi.nlm.nih.gov/2064481/>
- Hornik, C. D., & Wade, K. C. (2024). Pharmacokinetics in Neonatal Medicine. *Fanaroff and Martin's Neonatal-Perinatal Medicine, 2-Volume Set: Diseases of the Fetus and Infant*, 786–801. <https://doi.org/10.1016/B978-0-323-93266-0.00047-7>
- Hussain, N., Mustafa, U., Davis, J., Thakkar, S., Ali, A. M., Mirrakhimov, A. E., Barbaryan, A., & Rowley, G. A. (2013). Indomethacin-related leukocytoclastic vasculitis: A case report and review of literature. *Case Reports in Dermatology*, 5(1), 33–37. <https://doi.org/10.1159/000348240>,
- Indomethacin*. (2025, August 5). <https://pubmed.ncbi.nlm.nih.gov/31643333/>
- Iorga, A., & Horowitz, B. Z. (2023). *Phenytoin Toxicity*. <https://pubmed.ncbi.nlm.nih.gov/29494051/>
- Jaffe, E. F., Lyerly, A. D., & Goldfarb, I. T. (2021). Advancing research in pregnancy during COVID-19: Missed opportunities and momentum in the US. *Med*, 2(5), 460–464. <https://doi.org/10.1016/j.medj.2021.04.019>
- Johnson, M. S., Jackson, D. L., & Schust, D. J. (2021). Endocrinology of Pregnancy. *Encyclopedia of Reproduction: Volume 1-6, Second Edition*, 2, 469–476. <https://doi.org/10.1016/B978-0-12-801238-3.64672-X>
- Katzuni, E., Shalev, E., & Gilad, G. (1984). Further study of the inhibition of premature labor by indomethacin Part II double-blind study. *Journal of Perinatal Medicine*, 12(1), 25–30. <https://doi.org/10.1515/JPME.1984.12.1.25>,
- Ke, A. B., Greupink, R., & Abduljalil, K. (2018). Drug dosing in pregnant women: Challenges and opportunities in using physiologically based pharmacokinetic modeling and simulations. *CPT: Pharmacometrics and Systems Pharmacology*, 7(2), 103–110. <https://doi.org/10.1002/PSP4.12274>,
- Kinobe, R. T., Parkinson, O. T., Mitchell, D. J., & Gillam, E. M. J. (2005). P450 2C18 catalyzes the metabolic bioactivation of phenytoin. *Chemical Research in Toxicology*, 18(12), 1868–1875. <https://doi.org/10.1021/TX050181O>,
- Komatsu, T., Yamazaki, H., Asahi, S., Gillam, E. M., Guengerich, F. P., Nakajima, M., & Yokoi, T. (2000, November). *Formation of a dihydroxy metabolite of phenytoin in human liver microsomes/cytosol: roles of cytochromes P450 2C9, 2C19, and 3A4 - PubMed*. Drug Metab Dispos. <https://pubmed.ncbi.nlm.nih.gov/11038165/>
- Kowalski, M. L., & Makowska, J. (2006). Use of nonsteroidal anti-inflammatory drugs in patients with aspirin hypersensitivity: Safety of cyclo-oxygenase-2 inhibitors. *Treatments in Respiratory Medicine*, 5(6), 399–406. <https://doi.org/10.2165/00151829-200605060-00005>,
- Leeder, J. S. (1998). Mechanisms of idiosyncratic hypersensitivity reactions to antiepileptic drugs. *Epilepsia*, 39(SUPPL. 7). <https://doi.org/10.1111/J.1528-1157.1998.TB01679.X>,
- Lucas, S. (2016). The Pharmacology of Indomethacin. *Headache*, 56(2), 436–446. <https://doi.org/10.1111/HEAD.12769>,

- Male, V. (2021). Are COVID-19 vaccines safe in pregnancy? *Nature Reviews Immunology*, 21(4), 200–201. <https://doi.org/10.1038/S41577-021-00525-Y>;SUBJMETA=1567,250,251,326,4130,596,631;KWRD=IMMUNIZATION,SARS-COV-2
- Martins, C., & Ferreira, A. O. (2015). *Utilização de modelos farmacocinéticos de base fisiológica em estudos toxicológicos*. [s.n.]. <http://hdl.handle.net/10284/5154>
- McGready, R., Stepniewska, K., Seaton, E., Cho, T., Cho, D., Ginsberg, A., Edstein, M. D., Ashley, E., Looareesuwan, S., White, N. J., & Nosten, F. (2003). Pregnancy and use of oral contraceptives reduces the biotransformation of proguanil to cycloguanil. *European Journal of Clinical Pharmacology*, 59(7), 553–557. <https://doi.org/10.1007/S00228-003-0651-X>,
- Meador, K., Reynolds, M. W., Crean, S., Fahrbach, K., & Probst, C. (2008). Pregnancy outcomes in women with epilepsy: A systematic review and meta-analysis of published pregnancy registries and cohorts. *Epilepsy Research*, 81(1), 1–13. <https://doi.org/10.1016/J.EPLEPSYRES.2008.04.022>,
- Miller, N. A., Reddy, M. B., Heikkinen, A. T., Lukacova, V., & Parrott, N. (2019). Physiologically Based Pharmacokinetic Modelling for First-In-Human Predictions: An Updated Model Building Strategy Illustrated with Challenging Industry Case Studies. *Clinical Pharmacokinetics*, 58(6), 727–746. <https://doi.org/10.1007/S40262-019-00741-9>,
- Mitchell, A. A., Gilboa, S. M., Werler, M. M., Kelley, K. E., Louik, C., & Hernández-Díaz, S. (2011). Medication use during pregnancy, with particular focus on prescription drugs: 1976–2008. *American Journal of Obstetrics and Gynecology*, 205(1), 51.e1–51.e8. <https://doi.org/10.1016/j.ajog.2011.02.029>
- Morrell, M. J. (2002). Epilepsy in women. *Am Fam Physician*, 66(8), 1489–1494. <https://pubmed.ncbi.nlm.nih.gov/12408423/>
- Nakajima, M., Yamanaka, H., Fujiwara, R., Katoh, M., & Yokoi, T. (2007). Stereoselective glucuronidation of 5-(4'-hydroxyphenyl)-5-phenylhydantoin by human UDP-glucuronosyltransferase (UGT) 1A1, UGT1A9, and UGT2B15: Effects of UGT-UGT interactions. *Drug Metabolism and Disposition*, 35(9), 1679–1686. <https://doi.org/10.1124/dmd.107.015909>
- Nalamachu, S., & Wortmann, R. (2014). Role of indomethacin in acute pain and inflammation management: A review of the literature. *Postgraduate Medicine*, 126(4), 92–97. <https://doi.org/10.3810/PGM.2014.07.2787>,
- National Center for Biotechnology Information. (2025a, August 6). *PubChem Compound Summary for CID 3715, Indomethacin*. <https://pubchem.ncbi.nlm.nih.gov/compound/Indomethacin>
- National Center for Biotechnology Information. (2025b, August 8). *PubChem Compound Summary for CID 1775, Phenytoin*. <https://pubchem.ncbi.nlm.nih.gov/compound/Phenytoin>
- National Toxicology Program, I. of E. H. S. (1992). *National Toxicology Program Chemical Repository Database*.

- Newborn, C. on F. and. (2003). Controversies Concerning Vitamin K and the Newborn. *Pediatrics*, 112(1), 191–192. <https://doi.org/10.1542/PEDS.112.1.191>
- Obach, R. S., Cox, L. M., & Tremaine, L. M. (2005). Sertraline is metabolized by multiple cytochrome P450 enzymes, monoamine oxidases, and glucuronyl transferases in human: An in vitro study. *Drug Metabolism and Disposition*, 33(2), 262–270. <https://doi.org/10.1124/dmd.104.002428>
- Owen, P. R. (1984). Prostaglandin synthetase inhibitors in the treatment of primary dysmenorrhea: Outcome trials reviewed. *American Journal of Obstetrics and Gynecology*, 148(1), 96–103. [https://doi.org/10.1016/S0002-9378\(84\)80039-3](https://doi.org/10.1016/S0002-9378(84)80039-3)
- Pacifici, G. M. (2013). Clinical pharmacology of indomethacin in preterm infants: Implications in patent ductus arteriosus closure. *Pediatric Drugs*, 15(5), 363–376. <https://doi.org/10.1007/S40272-013-0031-7>,
- Pennell, P. B. (2003). Antiepileptic drug pharmacokinetics during pregnancy and lactation. *Neurology*, 61(6 SUPPL. 2). https://doi.org/10.1212/WNL.61.6_SUPPL_2.S35,
- Pond, S. M., Kreek, M. J., Tong, T. G., Raghunath, J., & Benowitz, N. L. (1985). Altered methadone pharmacokinetics in methadone-maintained pregnant women. *The Journal of Pharmacology and Experimental Therapeutics*, 233(4), 1–6.
- Rane, A., Oelz, O., Frolich, J. C., Seyberth, H. W., Sweetman, B. J., Watson, J. T., Wilkinson, G. R., & Oates, J. A. (1978). Relation between plasma concentration of indomethacin and its effect on prostaglandin synthesis and platelet aggregation in man. *Clinical Pharmacology & Therapeutics*, 23(6), 658–668. <https://doi.org/10.1002/CPT1978236658>
- Richens, A. (1979). Clinical Pharmacokinetics of Phenytoin. *Clinical Pharmacokinetics*, 4(3), 153–169. <https://doi.org/10.2165/00003088-197904030-00001>
- Rytting, E., Nanovskaya, T. N., Wang, X., Vernikovskaya, D. I., Clark, S. M., Cochran, M., Abdel-Rahman, S. M., Venkataramanan, R., Caritis, S. N., Hankins, G. D. V., & Ahmed, M. S. (2014). Pharmacokinetics of indomethacin in pregnancy. *Clinical Pharmacokinetics*, 53(6), 545–551. <https://doi.org/10.1007/S40262-014-0133-6>,
- Samrén, E. B., Van Duijn, C. M., Koch, S., Hiilesmaa, V. K., Klepel, H., Bardy, A. H., Mannagetta, G. B., Deichl, A. W., Gaily, E., Granström, M. L., Meinardi, H., Grobbee, D. E., Hofman, A., Janz, D., & Lindhout, D. (1997). Maternal use of antiepileptic drugs and the risk of major congenital malformations: A joint European prospective study of human teratogenesis associated with maternal epilepsy. *Epilepsia*, 38(9), 981–990. <https://doi.org/10.1111/J.1528-1157.1997.TB01480.X>,
- Say, L., Chou, D., Gemmill, A., Tunçalp, Ö., Moller, A. B., Daniels, J., Gülmezoglu, A. M., Temmerman, M., & Alkema, L. (2014). Global causes of maternal death: A WHO systematic analysis. *The Lancet Global Health*, 2(6). [https://doi.org/10.1016/S2214-109X\(14\)70227-X](https://doi.org/10.1016/S2214-109X(14)70227-X)
- Scheinfeld, N. (2004). Impact of phenytoin therapy on the skin and skin disease. *Expert Opinion on Drug Safety*, 3(6), 655–665. <https://doi.org/10.1517/14740338.3.6.655>,
- Shearer, M. J. (2009). Vitamin K deficiency bleeding (VKDB) in early infancy. *Blood Reviews*, 23(2), 49–59. <https://doi.org/10.1016/J.BLRE.2008.06.001>,

- Smolina, K., Hanley, G. E., Mintzes, B., Oberlander, T. F., & Morgan, S. (2015). Trends and Determinants of Prescription Drug Use during Pregnancy and Postpartum in British Columbia, 2002–2011: A Population-Based Cohort Study. *PLOS ONE*, 10(5), e0128312. <https://doi.org/10.1371/JOURNAL.PONE.0128312>
- Spina, E., & Perugi, G. (2004, June). *Antiepileptic drugs: indications other than epilepsy* - PubMed. Epileptic Disord. <https://pubmed.ncbi.nlm.nih.gov/15246950/>
- Thorn, C. F., Whirl-Carrillo, M., Leeder, J. S., Klein, T. E., & Altman, R. B. (2012). PharmGKB summary: Phenytoin pathway. *Pharmacogenetics and Genomics*, 22(6), 466–470. <https://doi.org/10.1097/FPC.0B013E32834AEEDB>
- Thrombotic, C. (2016). *Indomethacin Capsules, USP 25 mg Rx only WARNING: RISK OF SERIOUS CARDIOVASCULAR AND GASTROINTESTINAL EVENTS*. <https://www.fda.gov/drugsatfda>
- Tomson, T., & Battino, D. (2012). Teratogenic effects of antiepileptic drugs. *The Lancet Neurology*, 11(9), 803–813. [https://doi.org/10.1016/S1474-4422\(12\)70103-5](https://doi.org/10.1016/S1474-4422(12)70103-5),
- Tomson, T., Battino, D., Bonizzoni, E., Craig, J., Lindhout, D., Sabers, A., Perucca, E., & Vajda, F. (2011). Dose-dependent risk of malformations with antiepileptic drugs: An analysis of data from the EURAP epilepsy and pregnancy registry. *The Lancet Neurology*, 10(7), 609–617. [https://doi.org/10.1016/S1474-4422\(11\)70107-7](https://doi.org/10.1016/S1474-4422(11)70107-7),
- Tornio, A., & Backman, J. T. (2018). Cytochrome P450 in Pharmacogenetics: An Update. *Advances in Pharmacology*, 83, 3–32. <https://doi.org/10.1016/BS.APHA.2018.04.007>,
- Tracy, T. S., Venkataramanan, R., Glover, D. D., & Caritis, S. N. (2005). Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. *American Journal of Obstetrics and Gynecology*, 192(2), 633–639. <https://doi.org/10.1016/j.ajog.2004.08.030>
- Van Calsteren, K., Gersak, K., Sundseth, H., Klingmann, I., Dewulf, L., Van Assche, A., & Mahmood, T. (2016). Position statement from the European Board and College of Obstetrics & Gynaecology (EBCOG): The use of medicines during pregnancy - Call for action. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, 201, 189–191. <https://doi.org/10.1016/j.ejogrb.2016.04.004>
- Ververs, F. F. T., Voorbij, H. A. M., Zwarts, P., Belitser, S. V., Egberts, T. C. G., Visser, G. H. A., & Schobben, A. F. A. M. (2009). Effect of cytochrome P450 2D6 genotype on maternal paroxetine plasma concentrations during pregnancy. *Clinical Pharmacokinetics*, 48(10), 677–683. <https://doi.org/10.2165/11318050-000000000-00000>,
- Wadelius, M., Darj, E., Frenne, G., & Rane, A. (1997). Induction of CYP2D6 in pregnancy. *Clinical Pharmacology and Therapeutics*, 62(4), 400–407. [https://doi.org/10.1016/S0009-9236\(97\)90118-1](https://doi.org/10.1016/S0009-9236(97)90118-1),
- Wan, E. Y. F., Yu, E. Y. T., Chan, L., Mok, A. H. Y., Wang, Y., Chan, E. W. Y., Wong, I. C. K., & Lam, C. L. K. (2023). Comparative Risks of Nonsteroidal Anti-inflammatory Drugs on Cardiovascular Diseases: A Population-Based Cohort Study. *Journal of Clinical Pharmacology*, 63(1), 126–134. <https://doi.org/10.1002/JCPH.2142>,

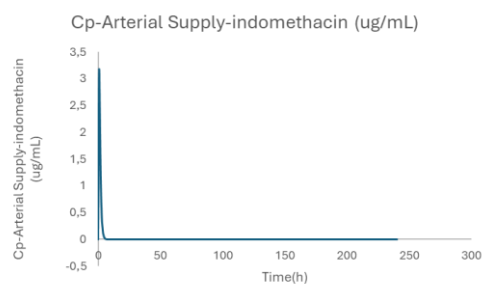
- Wang, H., & Tompkins, L. (2008). CYP2B6: New Insights into a Historically Overlooked Cytochrome P450 Isozyme. *Current Drug Metabolism*, 9(7), 598–610. <https://doi.org/10.2174/138920008785821710>,
- Wattanagoon, Y., Taylor, R., Moody, R., Ocheke, N., Looareesuwan, S., & White, N. (1987). Single dose pharmacokinetics of proguanil and its metabolites in healthy subjects. *British Journal of Clinical Pharmacology*, 24(6), 775–780. <https://doi.org/10.1111/J.1365-2125.1987.TB03245.X>,
- Weber, M., Kodjikian, L., Kruse, F. E., Zagorski, Z., & Allaire, C. M. (2013). Efficacy and safety of indomethacin 0.1% eye drops compared with ketorolac 0.5% eye drops in the management of ocular inflammation after cataract surgery. *Acta Ophthalmologica*, 91(1). <https://doi.org/10.1111/J.1755-3768.2012.02520.X>,
- Wiebe, S., Bellhouse, D. R., Fallahay, C., & Eliasziw, M. (1999). Burden of epilepsy: The Ontario Health Survey. *Canadian Journal of Neurological Sciences*, 26(4), 263–270. <https://doi.org/10.1017/S0317167100000354>,
- Wiqvist, N. (1975). Endogenous Prostaglandins in normal pregnancy and labor. *Pharmacology And Therapeutics: Part B: General And Systematic*, 1(2), 297–310. [https://doi.org/10.1016/0306-039X\(75\)90009-4](https://doi.org/10.1016/0306-039X(75)90009-4)
- Yamanaka, H., Nakajima, M., Hara, Y., Katoh, M., Tachibana, O., Yamashita, J., & Yokoi, T. (2005). Urinary excretion of phenytoin metabolites, 5-(4'-hydroxyphenyl)-5-phenylhydantoin and its O-glucuronide in humans and analysis of genetic polymorphisms of UDP-glucuronosyltransferases. *Drug Metabolism and Pharmacokinetics*, 20(2), 135–143. <https://doi.org/10.2133/dmpk.20.135>
- Zahn, C. A., Morrell, M. J., Collins, S. D., Labiner, D. M., & Yerby, M. S. (1998). Management issues for women with epilepsy: A review of the literature. *Neurology*, 51(4), 949–956. <https://doi.org/10.1212/WNL.51.4.949>,
- Zuckerman, H., Reiss, U., & Rubinstein, I. (1974). *Inhibition of human premature labor by indomethacin*. 44(6), 787–792. <https://pubmed.ncbi.nlm.nih.gov/4437814/>

List of Appendices

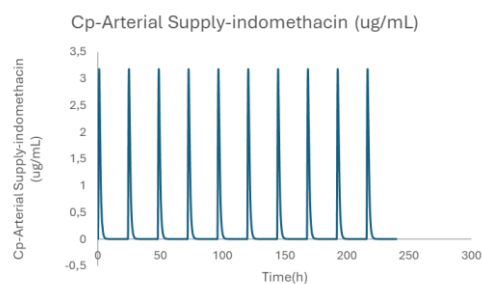
- Appendix A -Indomethacin simulations
- Appendix B- Phenytoin simulations

Appendix A – Indomethacin simulations

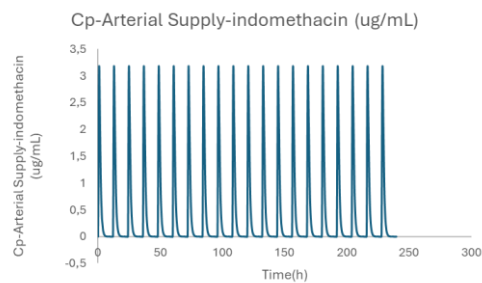
(1)



(2)



(3)



(4)

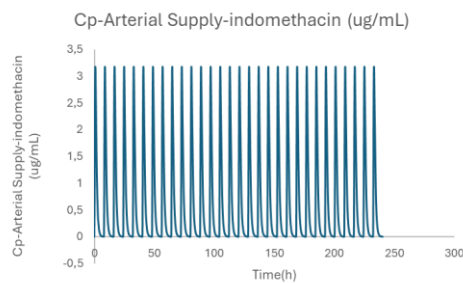
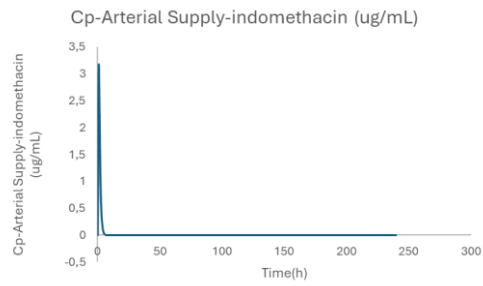
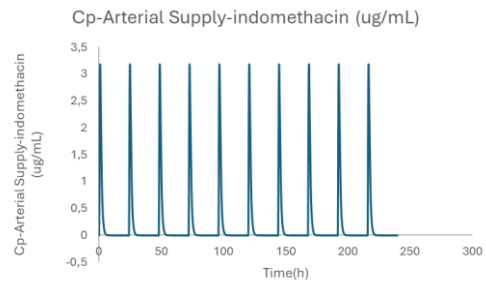


Figure A1. Simulated pharmacokinetic profile of indomethacin in a 20-year-old woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

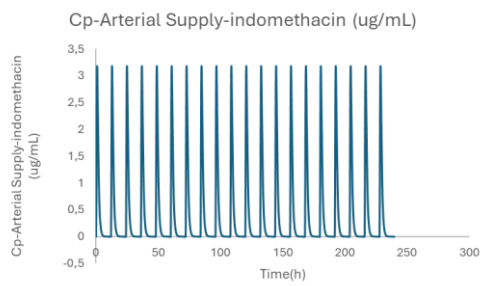
(1)



(2)



(3)



(4)

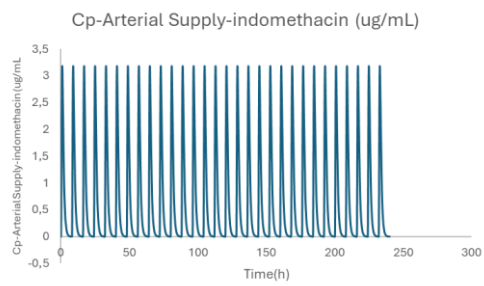
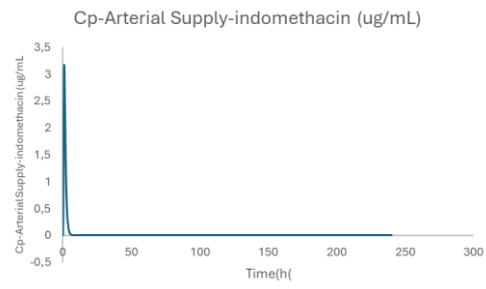
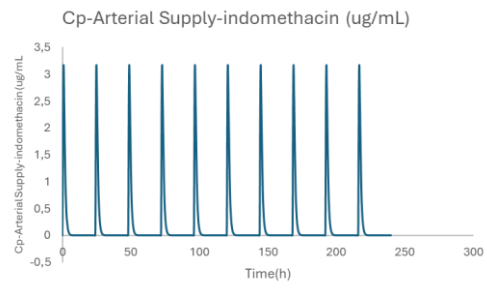


Figure A2. Simulated pharmacokinetic profile of indomethacin in a 25-year-old woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

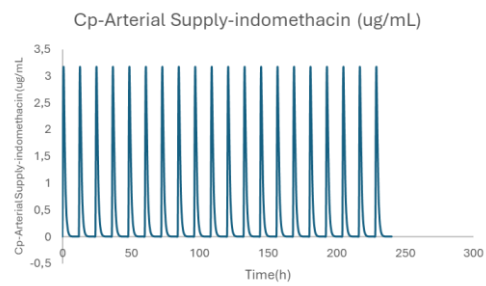
(1)



(2)



(3)



(4)

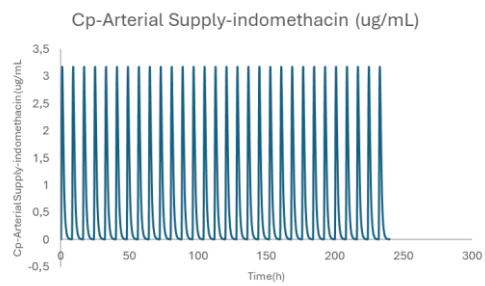
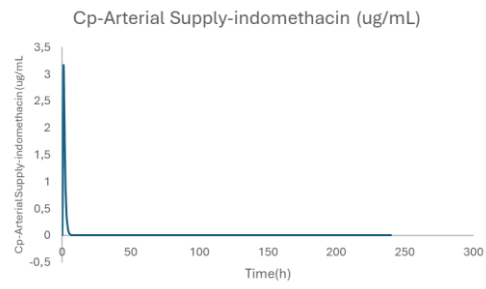
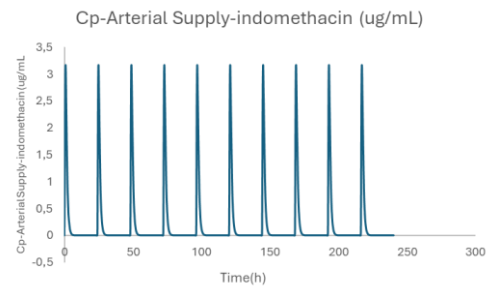


Figure A3. Simulated pharmacokinetic profile of indomethacin in a 30-year-old woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

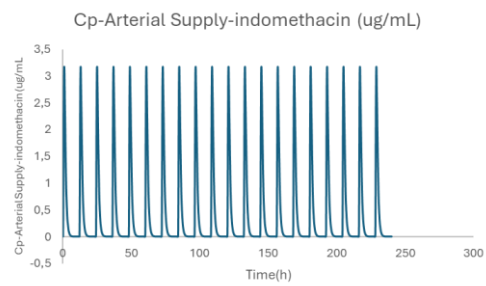
(1)



(2)



(3)



(4)

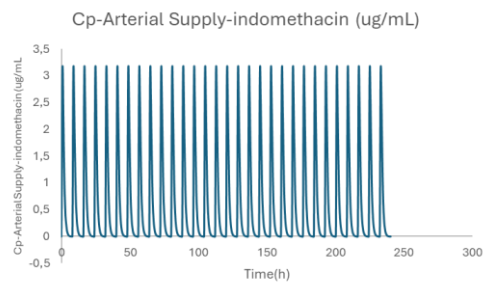
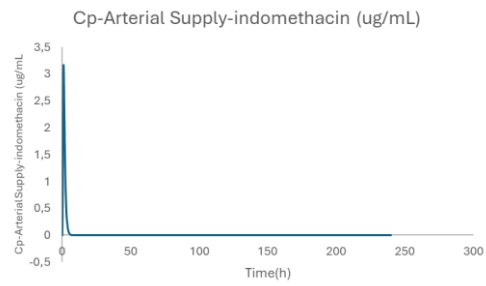
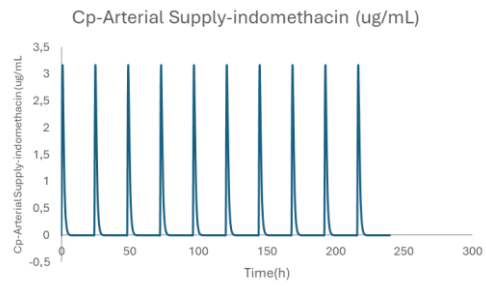


Figure A4. Simulated pharmacokinetic profile of indomethacin in a 35-year-old woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

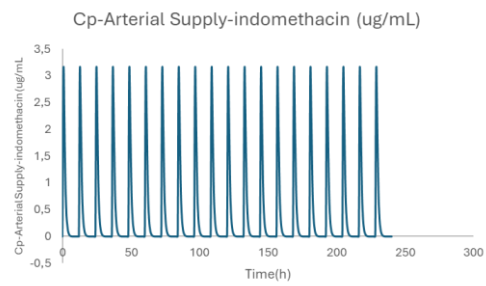
(1)



(2)



(3)



(4)

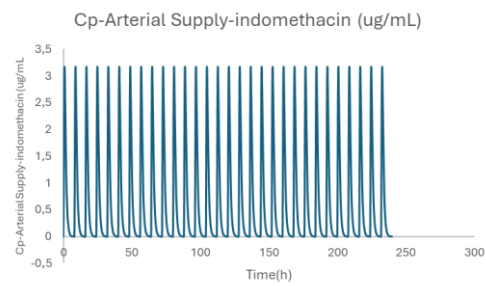
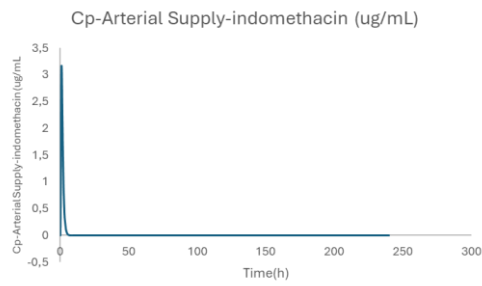
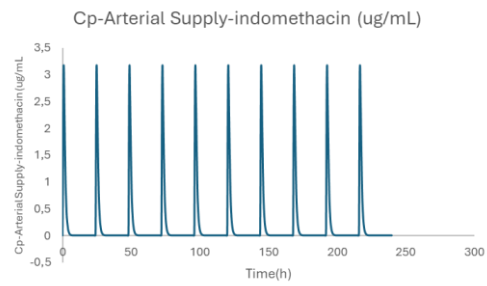


Figure A5. Simulated pharmacokinetic profile of indomethacin in a 40-year-old woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

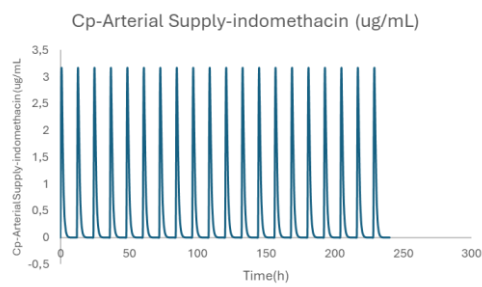
(1)



(2)



(3)



(4)

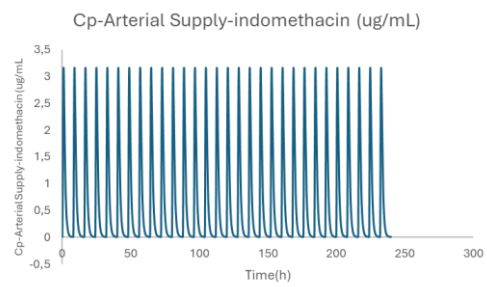
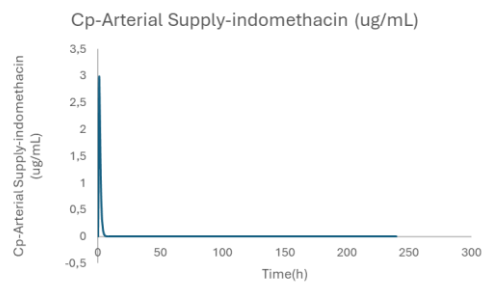
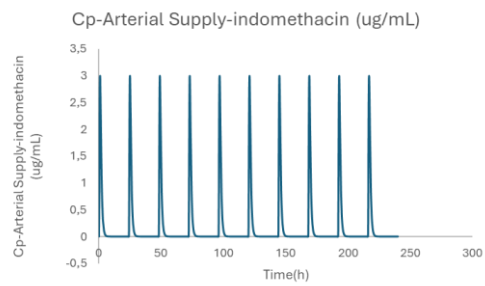


Figure A6. Simulated pharmacokinetic profile of indomethacin in a 45-year-old woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

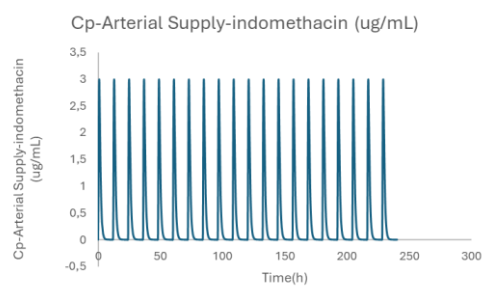
(1)



(2)



(3)



(4)

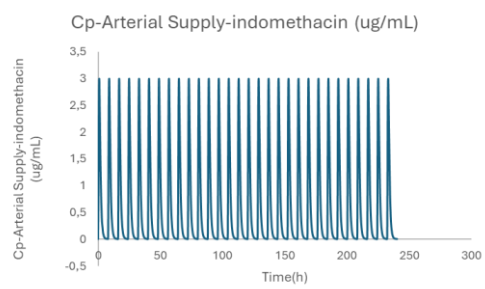
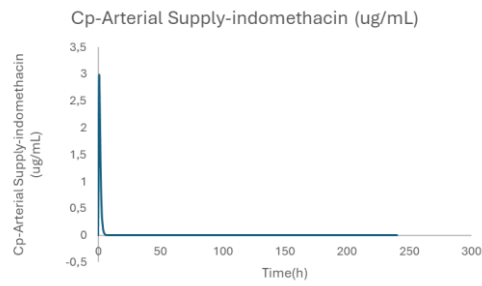
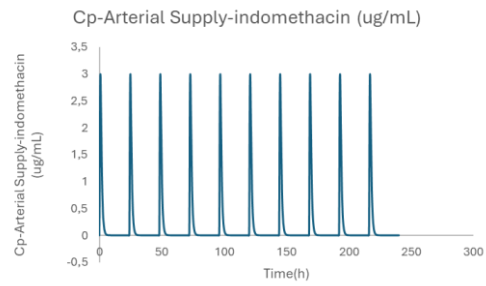


Figure A7. Simulated pharmacokinetic profile of indomethacin in a 20-year-old pregnant woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

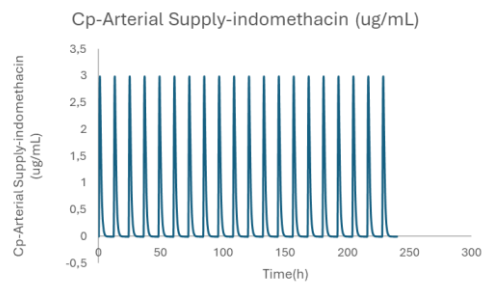
(1)



(2)



(3)



(4)

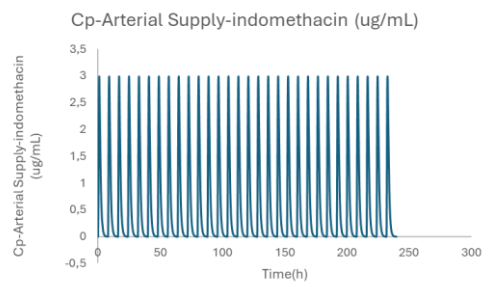
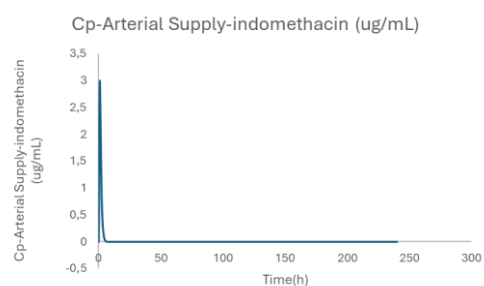
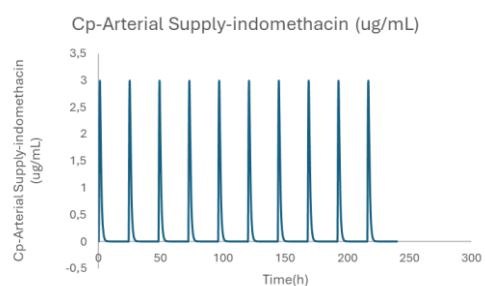


Figure A8. Simulated pharmacokinetic profile of indomethacin in a 25-year-old pregnant woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

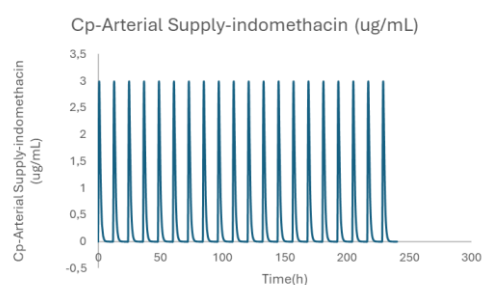
(1)



(2)



(3)



(4)

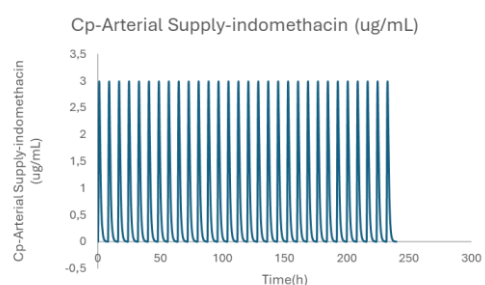
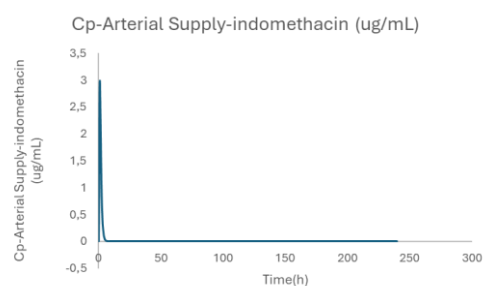
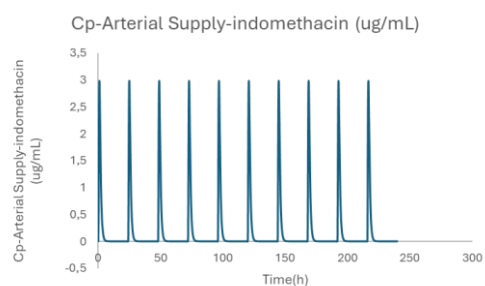


Figure A9. Simulated pharmacokinetic profile of indomethacin in a 30-year-old pregnant woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

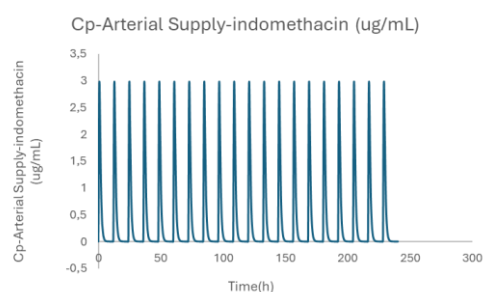
(1)



(2)



(3)



(4)

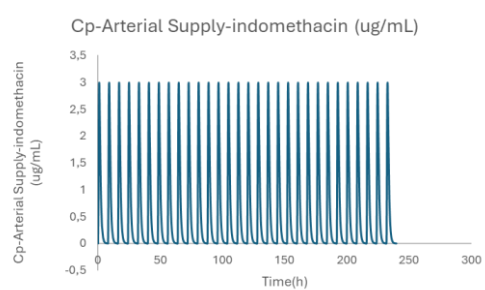
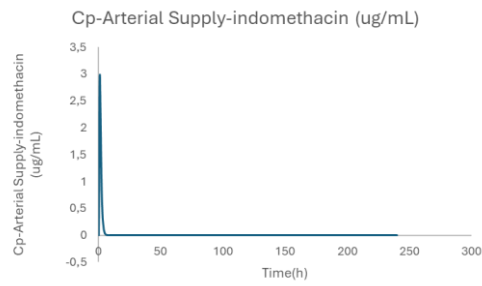
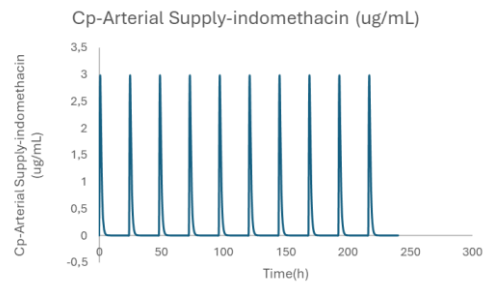


Figure A10. Simulated pharmacokinetic profile of indomethacin in a 35-year-old pregnant woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

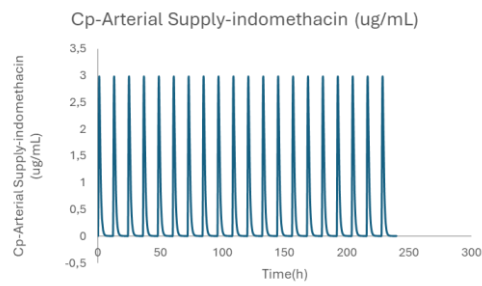
(1)



(2)



(3)



(4)

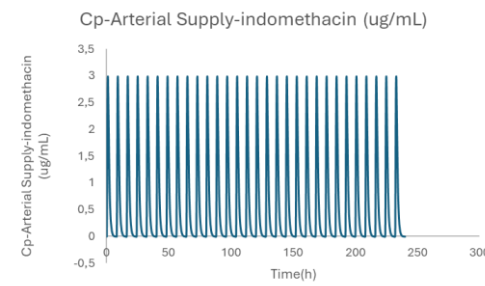
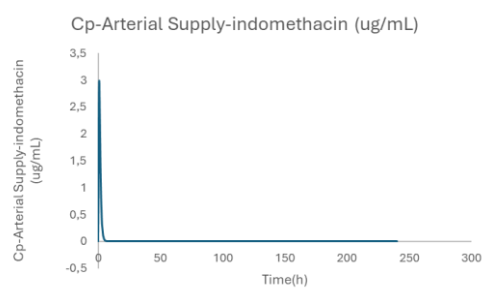
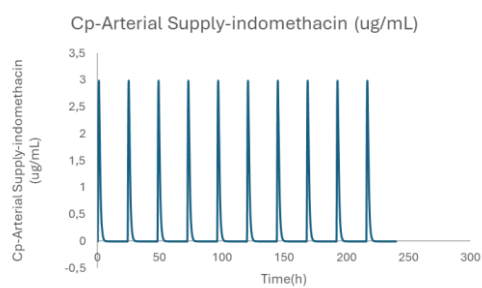


Figure A11. Simulated pharmacokinetic profile of indomethacin in a 40-year-old pregnant woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

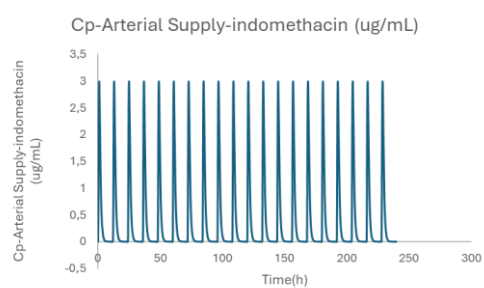
(1)



(2)



(3)



(4)

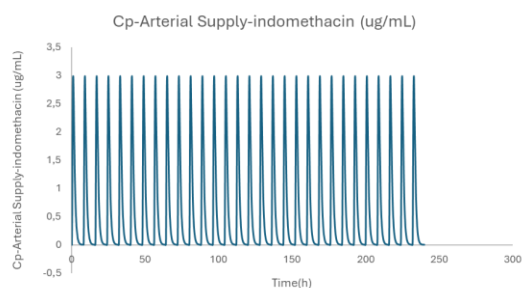
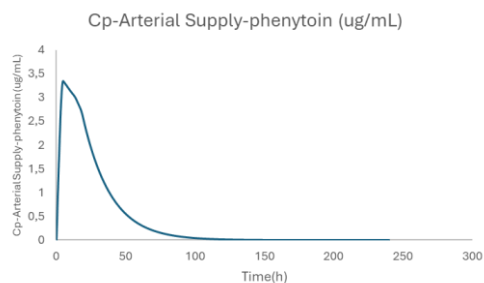


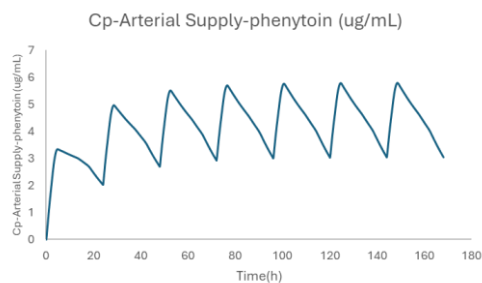
Figure A12. Simulated pharmacokinetic profile of indomethacin in a 45-year-old pregnant woman: (1) single 50 mg dose, (2) 50 mg q24h, (3) 50 mg q12h, (4) 50 mg q8h.

Appendix B- Phenytoin simulations

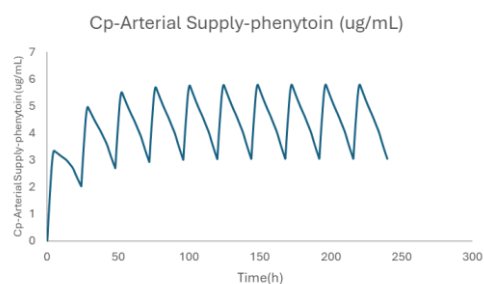
(1)



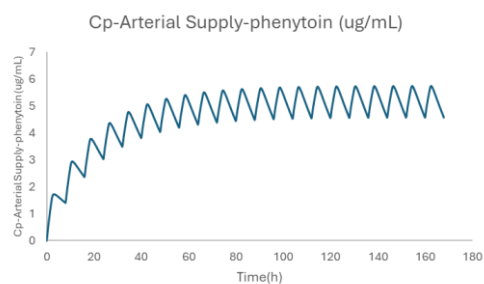
(2)



(3)



(4)



(5)

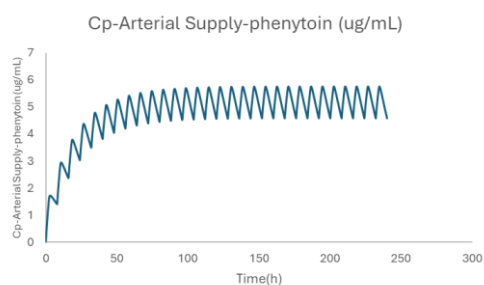
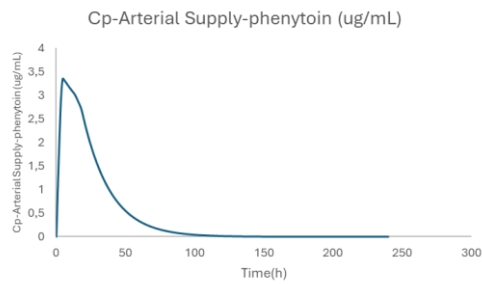
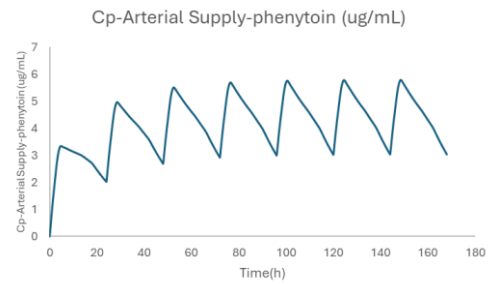


Figure B1. Simulated pharmacokinetic profile of phenytoin in a 20-year-old woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

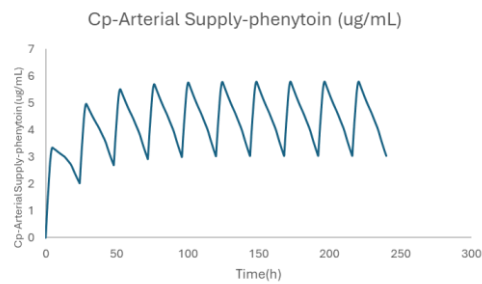
(1)



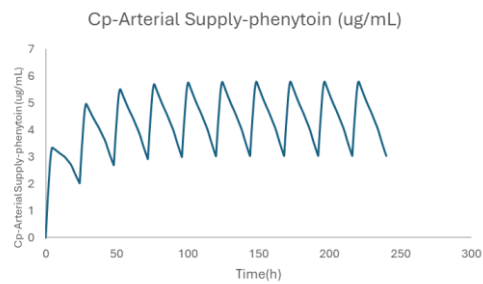
(2)



(3)



(4)



(5)

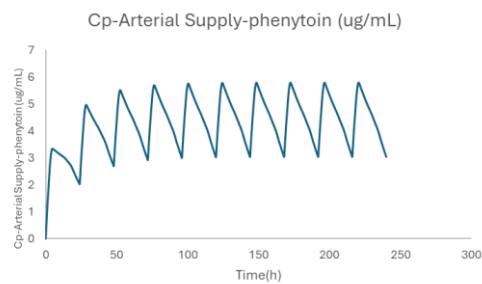
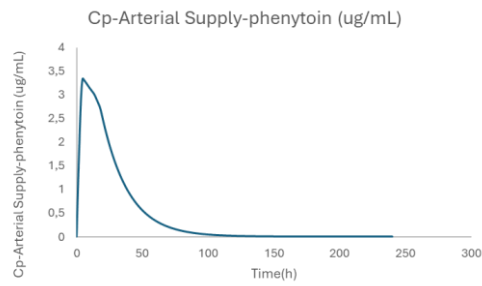
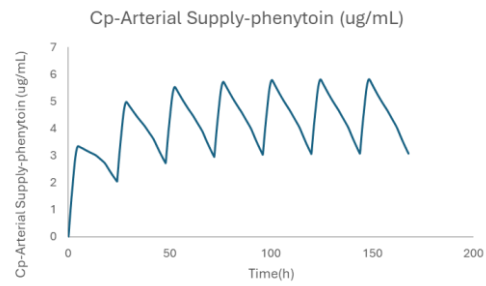


Figure B2. Simulated pharmacokinetic profile of phenytoin in a 25-year-old woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

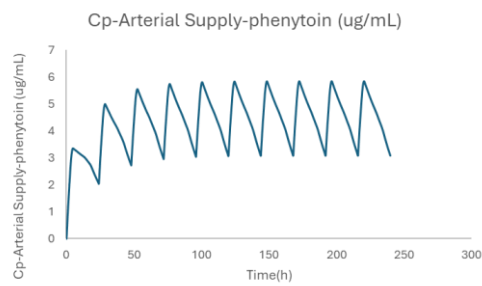
(1)



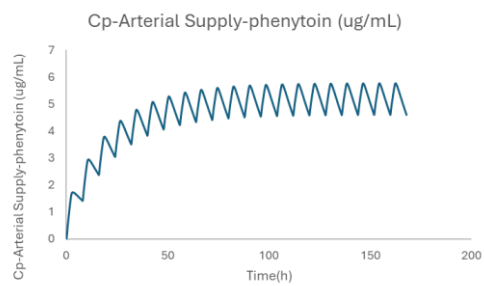
(2)



(3)



(4)



(5)

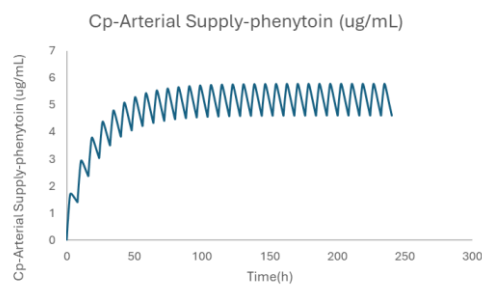
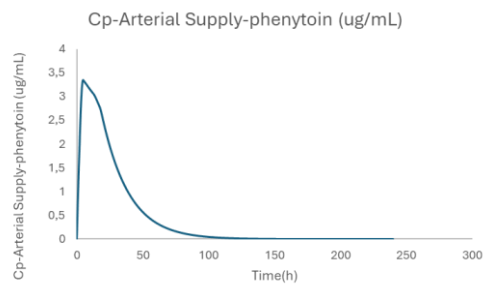
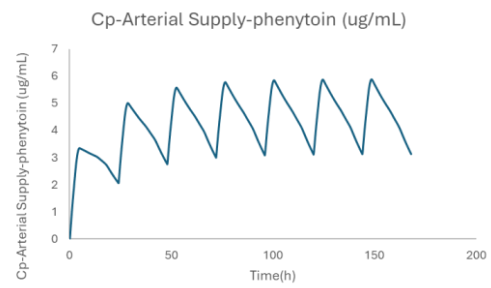


Figure B3. Simulated pharmacokinetic profile of phenytoin in a 30-year-old woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

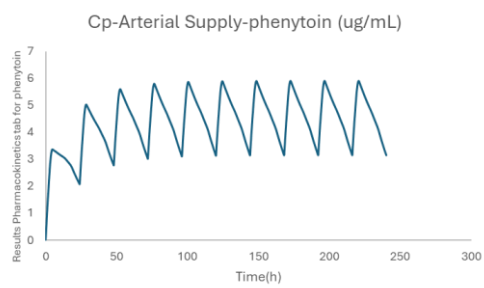
(1)



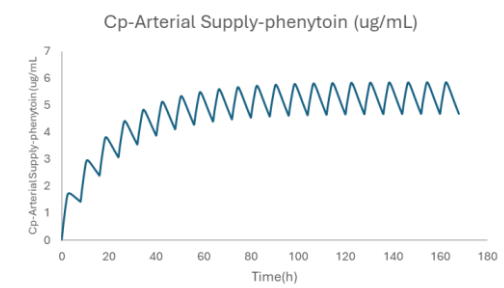
(2)



(3)



(4)



(5)

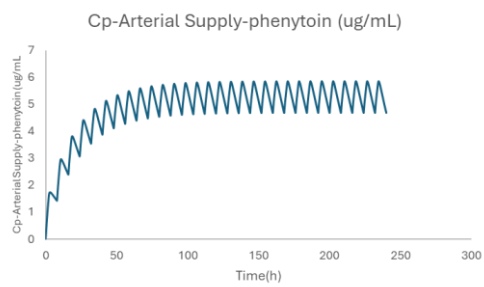
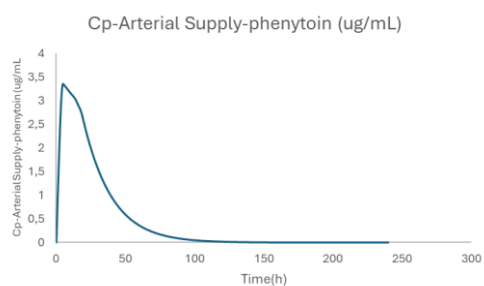
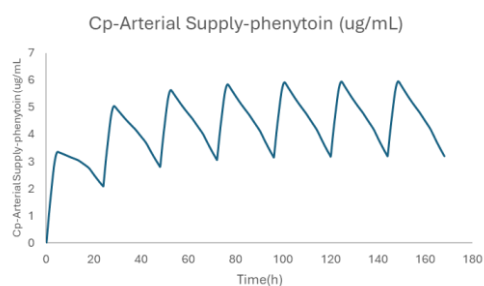


Figure B4. Simulated pharmacokinetic profile of phenytoin in a 35-year-old woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

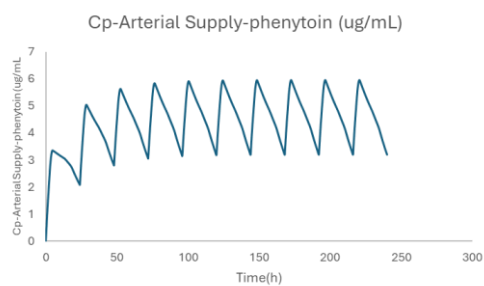
(1)



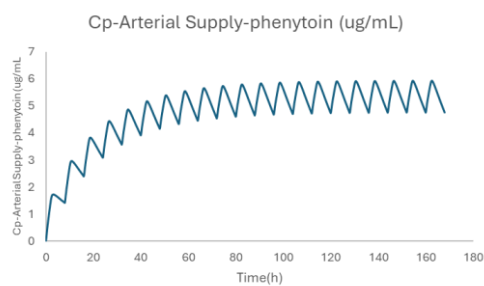
(2)



(3)



(4)



(5)

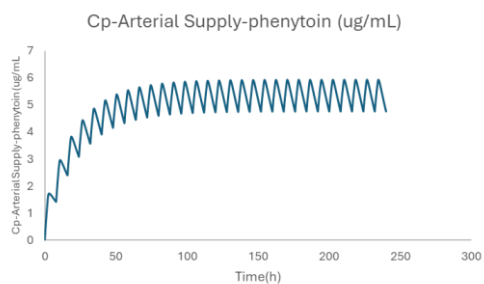
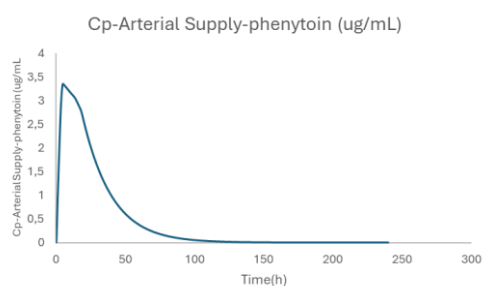
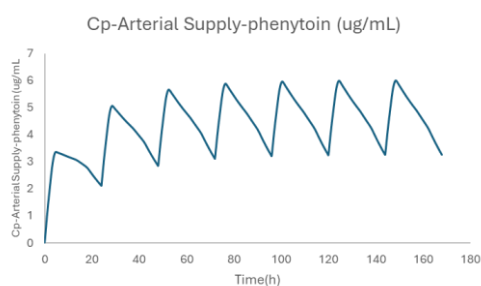


Figure B5. Simulated pharmacokinetic profile of phenytoin in a 40-year-old woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

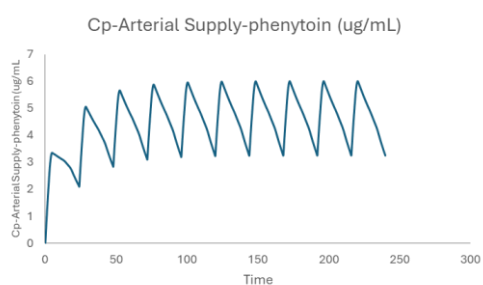
(1)



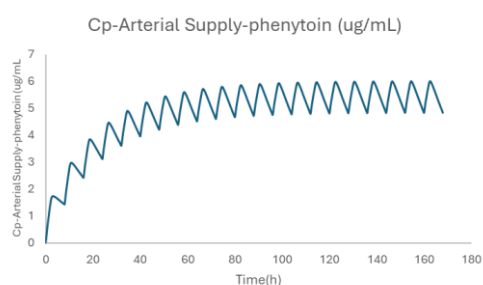
(2)



(3)



(4)



(5)

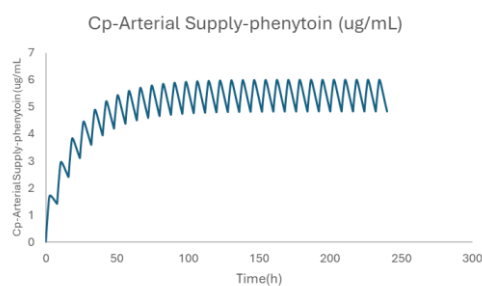
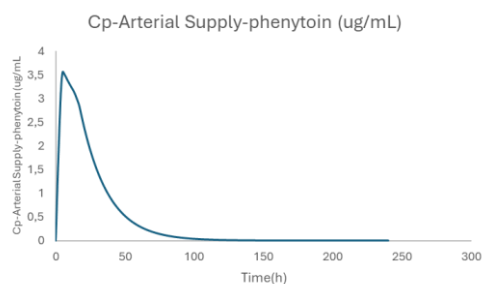
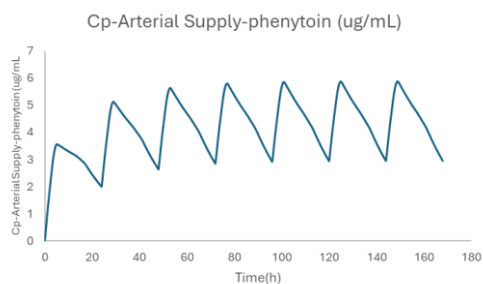


Figure B6. Simulated pharmacokinetic profile of phenytoin in a 45-year-old woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

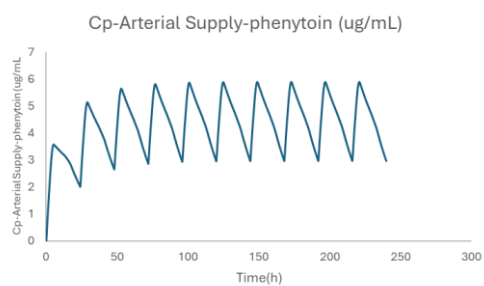
(1)



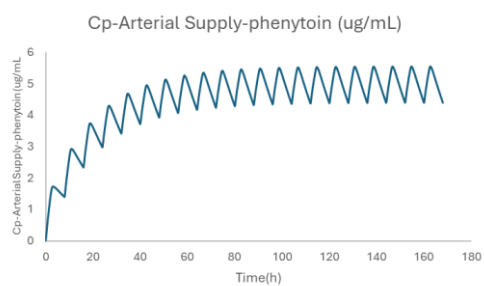
(2)



(3)



(4)



(5)

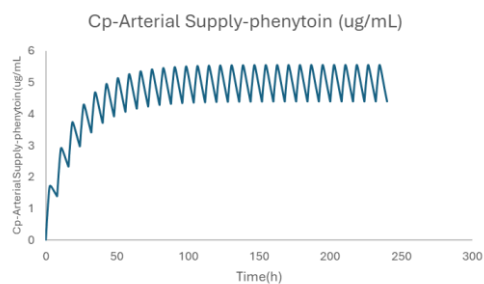
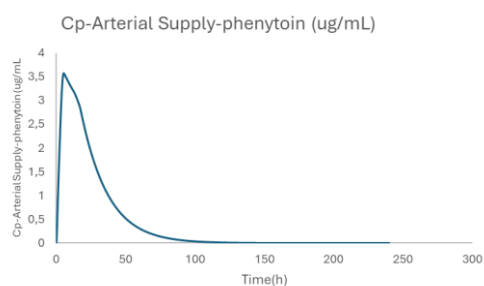
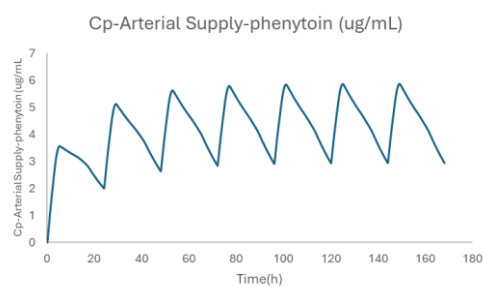


Figure B7. Simulated pharmacokinetic profile of phenytoin in a 20-year-old pregnant woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

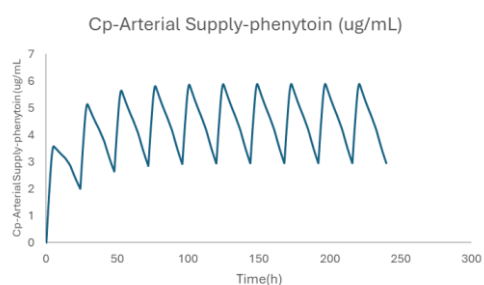
(1)



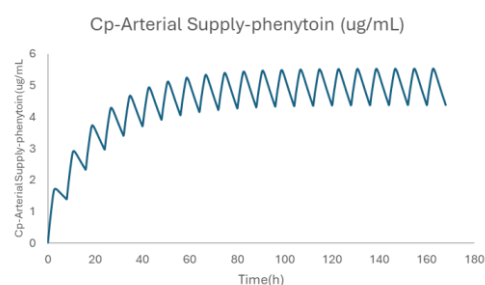
(2)



(3)



(4)



(5)

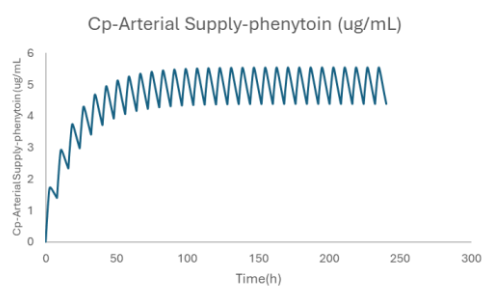
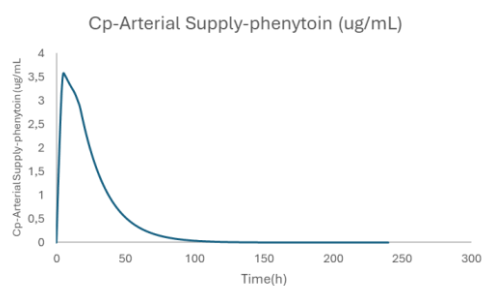
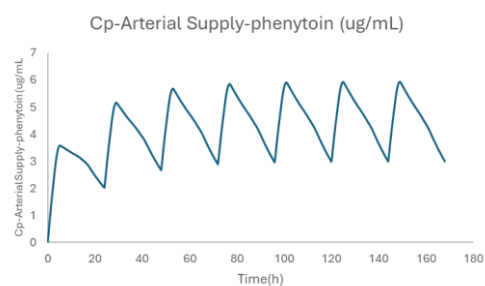


Figure B8. Simulated pharmacokinetic profile of phenytoin in a 25-year-old pregnant woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

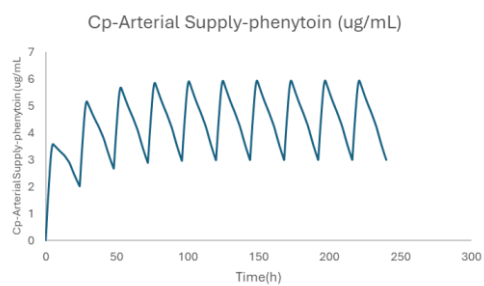
(1)



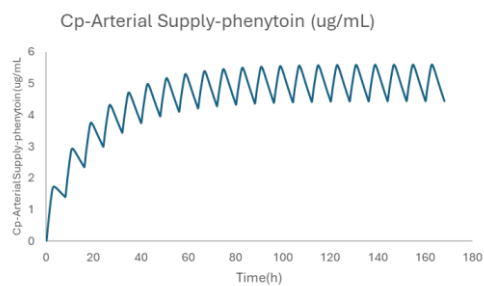
(2)



(3)



(4)



(5)

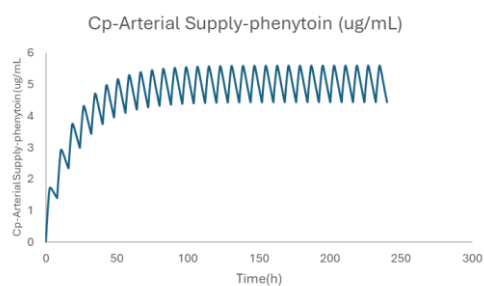
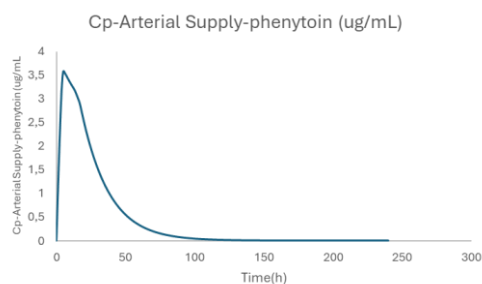
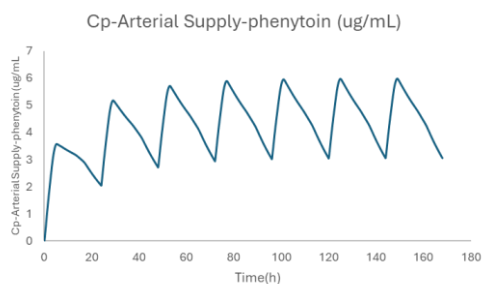


Figure B9. Simulated pharmacokinetic profile of phenytoin in a 30-year-old pregnant woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

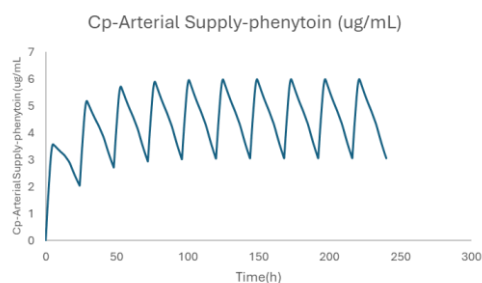
(1)



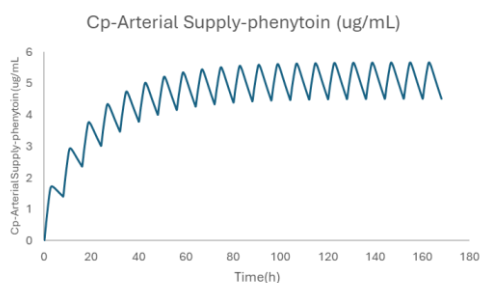
(2)



(3)



(4)



(5)

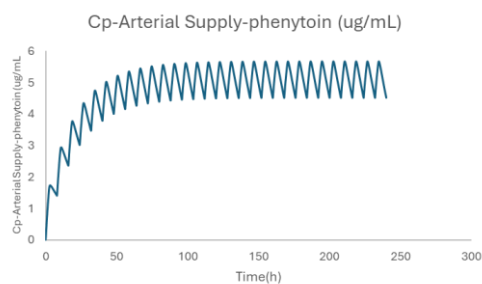
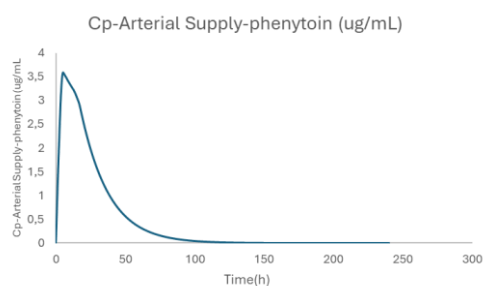
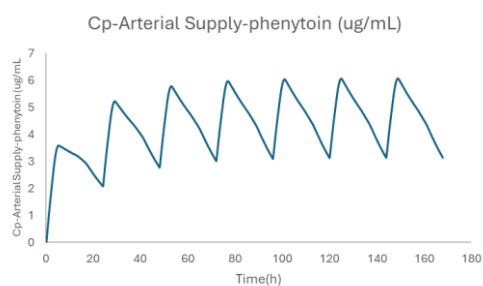


Figure B10. Simulated pharmacokinetic profile of phenytoin in a 35-year-old pregnant woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

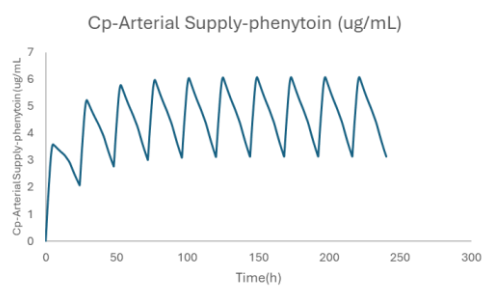
(1)



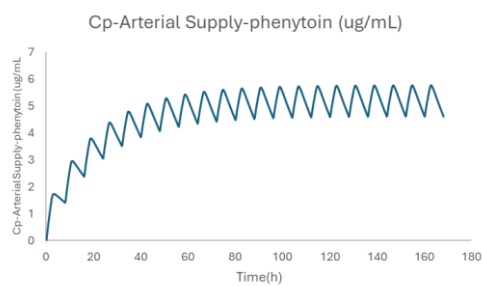
(2)



(3)



(4)



(5)

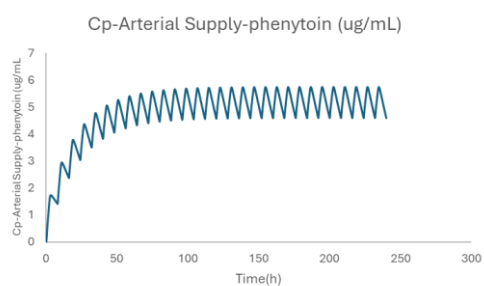
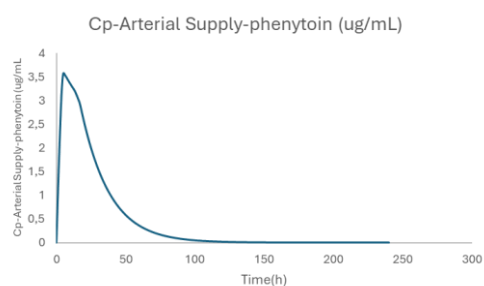
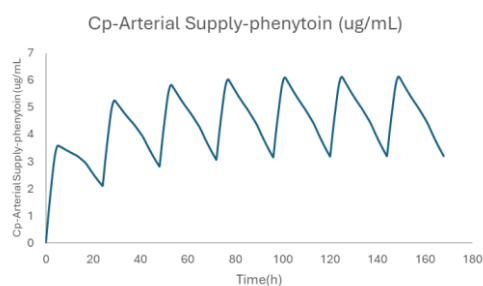


Figure B11. Simulated pharmacokinetic profile of phenytoin in a 40-year-old pregnant woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.

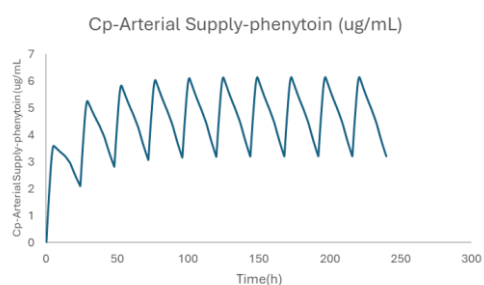
(1)



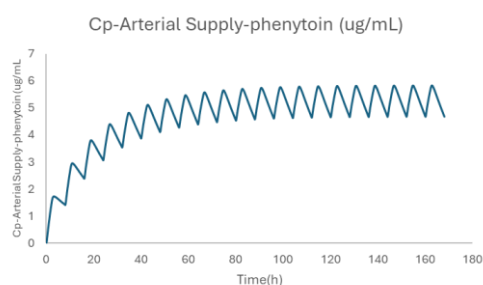
(2)



(3)



(4)



(5)

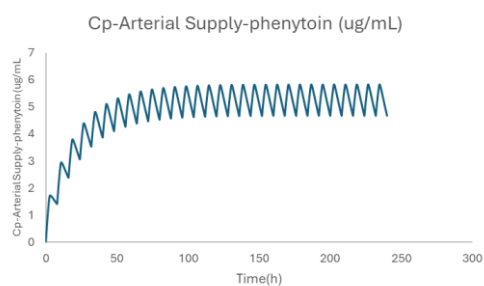


Figure B12. Simulated pharmacokinetic profile of phenytoin in a 45-year-old pregnant woman: (1) single 300 mg dose, (2) 300 mg q24h for 168 h, (3) 300 mg q24h for 240 h, (4) 100 mg q8h for 168 h, (5) 100 mg q8h for 240 h.