Successful extrapolation of paracetamol exposure from adults to infants after oral administration of a paediatric aqueous suspension is highly dependent on the study dosing conditions

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Extrapolating drug exposure from adults to infants

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Abstract

Extending licensed drug use to the pediatric population has become an essential part of the drug development process. Nonetheless ethical concerns limit clinical testing in paediatric populations and data collected from oral bioavailability and food effect studies in adults are often extrapolated to the target paediatric (sub)populations. However, based on published information, food effects on drug absorption in infants may not be adequately evaluated by data collected in adults. In the present study, a physiologically based pharmacokinetic (PBPK) approach for modeling paracetamol suspension data collected in adults was proposed with the ultimate aim to investigate whether extrapolation to infants is substantially affected by the dosing conditions applied to adults. The development of the PBPK model for adults was performed using GastroPlus™ and, after scaling to infants considering physiological, anatomical, and drug clearance changes, extrapolation of the different dosing conditions was performed by applying dosing conditions dependent changes on the paracetamol gastric emptying process. Successful predictions of observed plasma concentration levels in infants were achieved when extrapolating from fasted and infant-formula-fed conditions data. Data collected following the reference meal appeared less useful for simulating paracetamol suspension performance in infants. The proposed methodology deserves further evaluation using high-quality clinical data both in adults and in infants.

Introduction

Extending licensed drug use to the paediatric populations has become an essential part of the drug development process to ensure appropriate dosing, efficacy and safety from birth to adulthood (1,2). As in adults, the oral route of administration is preferred from birth to adolescence and bioavailability studies are required to ensure suitable drug exposure and drug pharmacokinetics (PK) following the administration of the age-appropriate dosage form. However, ethical concerns and recruitment issues limit clinical testing in these vulnerable age groups more than in adults (3–5).

Physiologically based pharmacokinetic (PBPK) modelling could be a useful tool to drastically decrease the need for performing clinical studies in paediatric populations and, therefore, largely eliminate relevant concerns. Based on the ability to create PBPK physiologies representative of various human developmental stages, PBPK modeling utilization in paediatrics can facilitate drug performance predictions prior to testing in a clinical setting and guide drug formulation development (3,5). Additionally, to date, PBPK modeling has proven valuable as a tool to gain mechanistic understanding of physiological and drug parameters governing oral absorption processes across various paediatric age ranges (3,6–9). Interestingly, however, only a few of these studies use multi-compartmental representation of the gastro-intestinal (GI) tract, while implementing age-dependent physiological and anatomical changes to investigate different dosing and prandial conditions in the target population (3,8).

PBPK model development procedure to extrapolate adult data to paediatric populations employs a stepwise workflow, beginning by building a validated adult disposition model, followed by the development and validation of an adult absorption model, and, ultimately, the extrapolation to the paediatric population of interest (10). A recent draft guidance by the US Food and Drug Administration (FDA) proposed the use of age-specific meals and quantities for the investigation of populations receiving specific meals, e.g., infant formula for infants, without specification of an exact quantity (11). Although several studies in adults have employed infant formula or soft foods (e.g., applesauce, yoghurt, and fruit puree), the age-adjusted meal quantities simulate drug product administration with small amounts of food to facilitate drug formulation dosing and improve acceptability, rather than investigate the potential impact of dosing conditions on drug product performance (3,12). A recent study in healthy adults revealed reduced early exposure of paracetamol and ibuprofen, after administration under conditions simulating the fed state infants and toddlers (1-24 months) compared

to the administration under conditions simulating the fasted or fed state conditions in adults, as suggested by the current regulatory guidelines (9,11,13,14).

This manuscript describes and evaluates a PBPK modeling approach for extrapolation of drug exposure form adults to infants with view to the different conditions that can be used to inform the modeling process. The first objective was to propose a PBPK approach for modeling the recently collected paracetamol paediatric suspension data in adults under fasted and fed state conditions (13,14), and, under conditions mimicking dosing to infants (9). The second objective was to investigate if extrapolation to infants was substantially affected by the dosing conditions applied to adults. Both objectives were achieved by using the PBPK modeling platform GastroPlus™ V9.7.

Methods

PK data collection

Initially, a thorough search at PubMed was performed (completed March 2020) for previously published plasma data after intravenous administration (bolus and infusion) and oral administration (solution and suspension forms) of paracetamol to adults and paediatrics. Data that had been collected after administration of liquids containing excipients influencing the product performance, from an unspecified product, after co-administration with drug(s) influencing the GI physiology, and/or by employing paediatrics without age stratification were excluded from further consideration. The Statelova *et al.* (2020) study in adults was used as the basis for extrapolation to paediatrics. In addition to that study (9), a total of 23 paracetamol PK studies met the search criteria, with 15 studies in adults and 8 in paediatrics. From the adult studies, 12 studies reported i.v. paracetamol administration (15–24) and 2 studies reported oral administration of paracetamol solutions in the fasted state (19,21). From the 8 paediatric studies, 5 reported i.v. administration in infants (1 month – 2 years), children and adolescents (2 - 18 years) (25,26) and 2 studies reported oral administration in infants (1 month-2 years) or infants and young children (3-36 months) (27,28). From the 23 studies retrieved from literature, plasma concentration-time profiles and respective standard deviations (SD) or standard errors of the means (SEM) were digitized using the WebPlotDdigitizer software V4.1 (Ankit Rohatgi, 2017). Along with the reported plasma levels as a function of time, extracted information also included drug dosing conditions, drug products, and demographics of the study population, i.e., number of study subjects, age, gender, body height, body weight, and race. For the Statelova *et al.* study (2020) (9), in addition to the published mean plasma concentrations and demographics, individual data were also available.

Modeling strategy

The PBPK model for paracetamol was developed using the GastroPlus™ software platform (V. 9.7, Simulations Plus, Lancaster, CA, USA). The model development strategy employed a “middle-out” approach (29), whereby model parameterization was guided by clinical observations in humans (Figure 1). As part of the applied “learn-confirm-apply” approach (30), the model was built and refined using *in vivo* data sets and, then it was verified using external data sets before applying/extrapolating to infants. As a first step, a disposition model for healthy adults was developed and optimized according to clinical studies after i.v. drug administration reported in literature (16), followed by verification with external clinical datasets not used for the model development (15,18). After gaining certainty in the disposition model, oral absorption in adults was described using the Advanced

Compartmental Absorption and Transit (ACAT™) model within the GastroPlus™ platform for liquid drug formulations i.e., solution and suspension. For the paracetamol suspension formulation, different prandial and dosing conditions were modeled and relevant parameters were adjusted according to data observed in adults (9). The model was scaled to different paediatric age groups for which clinical data following intravenous drug dosing was available to confirm the scaling of drug disposition across ages. Finally, different dosing and prandial conditions for the administration of the paediatric suspension were extrapolated from adults to infants and compared to data observed in this paediatric population.

Adult model

A full PBPK model for adults was established for paracetamol using the data listed in Table I. Human physiologies matching to each simulated study demographics (age, body weight, gender, body-mass-index) were created using the Population Estimates for Age-Related (PEAR) Physiology module within GastroPlus™ (6,43,44). Within the PEAR™ physiology module, after selecting the subject demographics, blood flows, organ and tissue sizes, as well as tissue composition are adjusted based on literature (6,43,44). A default physiology for a healthy American adult 30-year-old male with a body weight of 70 kg was used when the simulated study lacked reporting of the demographics. A study reported by Clements *et al.* investigated the i.v. administration of paracetamol at 5 mg/kg and 20 mg/kg doses covering the range of the typical paracetamol dose-strengths, e.g. 15 mg/kg (16). Additionally, the study has been used successfully for building paracetamol PBPK models in literature and the study report allowed for reliable extraction of the datapoints (32,33,45). Therefore, based on data sets from the study by Clements *et al.* (16), clearance (CL) and volume of distribution at steady state (Vss) were estimated via non-compartmental analysis performed with the PKPlus™ module within GastroPlus™ and were used as benchmark values for CL and Vss in healthy adults. Within the current modeling development, Vss was derived from the tissue partitioning coefficient values (Kp) for perfusion-limited tissues estimated using the Rogers, Roland, Lukacova method (6,38). The predicted Vss value was adjusted to match the benchmark value from clinical observations (Table I). The *in vivo* clearance was scaled to *in vitro* clearance for each enzyme contributing to drug metabolism using a retrograde stepwise routine (46) as briefly explained in the following text (exact calculations are provided as Supplementary Information). Based on the extensive liver metabolism of the drug and the literature reports indicating insignificant paracetamol metabolism in the gut and kidney (16,22,47,48), the total clearance was considered to originate from the liver. Hence, the benchmark total paracetamol clearance after i.v. administration was used for the estimation of the *in vivo* unbound intrinsic hepatic clearance according to the well-stirred clearance model (49). Based on the hepatic

metabolism contributions of isoenzymes of the Cytochrome P-450 (CYP), UDP-glucuronosyltransferase (UGT), and cytosolic sulfotransferases (SULT) enzyme families, *in vivo* intrinsic clearance values per isoenzyme were calculated (7,16,32). These were further employed to determine *in vitro* drug-metabolizing enzyme parameters (Table I) (7,32,33). The disposition model was verified with data from reported i.v. studies of paracetamol (External Datasets) that were not utilized for the model development.

The ACAT™ model describes the drug dissolution, precipitation, and luminal absorption during drug transfer through the nine compartments of the GI-tract within the model, i.e. stomach, duodenum, two jejunum, three ileum, and colon compartments (50,51). Each compartment is characterized by a physiology-adjusted small-intestinal (SI) length, radius, specific absorption factor (ASF), intraluminal fluid volumes and composition, and transit times. Human effective permeability of paracetamol ($P_{eff,man}$) was estimated from the *in vitro* apparent permeability in Caco-2 cells ($P_{app,Caco2}$) employing atenolol as a calibrator (7,34,35), Eq. 1.

$$logP_{eff,man} = 0.6795 \times logPapp, Caco2 - 0.3036 \quad \text{Eq. 1}$$

Oral solution data from literature were used as confirmation that the estimated permeability predicted paracetamol oral absorption (19,21). The software's default gastric transit time (GTT) value of 0.1 h and 1st order gastric emptying (GE) kinetics were employed for the solution; GTT for 1st order emptying kinetics represents the mean gastric transit time value (MGTT) defined as the GE half-life divided by the natural logarithm of 2.

Modeling under different dosing conditions

The exploratory relative bioavailability study by Statelova *et al*. was performed in healthy adult male volunteers and included three study arms to investigate suitable dosing conditions to evaluate the performance of paediatric suspensions for administration in infants (1 month-2 years), i.e., paracetamol paediatric suspension (Panadol®) (9). The human physiology used for the modeling represented the average values of 78 kg, 28 years of age and BMI of 20.23 kg/m^2 as reported in the study by Statelova *et al*. (mentioned throughout the text as "population representative"). A single dose of 1000 mg was administered on a crossover basis under different dosing conditions. In particular, the investigated dosing conditions included administration of the paediatric drug formulation under fasted conditions, fed conditions as proposed by current regulatory guidelines for adults (30 min after the start of the consumption of the reference meal) (13,14) and conditions mimicking dosing in infants

where the drug formulation was administered during infant formula consumption, i.e. infant-formula-fed conditions (9).

Model parameters were adjusted to capture the performance of the paediatric formulation as observed in adults, e.g., adjustment of GTT as GE and arrival of paracetamol in the SI were associated to paracetamol appearance in the systemic circulation (52). Due to the multiple-peak phenomenon observed for Panadol® under fasted conditions, an empirical modeling strategy was employed following "mixed multiple dosing" (MMD) of the suspension to verify that gastric emptying events were responsible for the observed profile shape (and not other absorption factors). Multiple GI-physiologies were created and applied using alternating rapid (GTT 0.1 h or 0.25 h) and slow GTT (10 h) values starting at different timepoints after drugs administration within the performed simulation; the multiple GI-physiologies and the different GTT introduced were adjusted (fitted) to simulate the observed discontinuous GE of the suspension under fasted conditions. As the goal was to extrapolate the model to infants, a compromise was made for a single GE process for fasted state modeling.

For simulations of paracetamol dosing under postprandial conditions, the hepatic blood flow was increased by one third of the baseline hepatic blood flow, to mimic the increase splanchnic blood flow observed after food consumption (50). By switching the prandial conditions option to "fed conditions", the luminal conditions within the simulated adult physiology were adjusted to the fed state e.g., bile salt increased as a function of fat content in the meal, pH increase in the gastric compartment, and prolongation of GE. To simulate different prandial conditions within GastroPlus™ V 9.7, in addition to a single default fed options for fed conditions applied in previous software versions, the "user-defined meal" option allows for flexibility in adapting the GTT as a function of the caloric content and the bile salt level adjustment according to the percentage of fat of the selected meal. Simulations were performed according to the software-proposed values for the different prandial conditions (referred to as "default settings or conditions" simulation throughout the manuscript). Under fed conditions, the total caloric content of the meal was 990 kcal with 60 % derived from fats, while under infant-formula-fed conditions the total caloric content was 520 kcal with 43 % fat content (9). Within the present model development, adjustments were undertaken based on *in vivo* observations for parameters that changed as a function of the meal texture and formulation type, e.g., following the solid-liquid reference meal the GE process followed 1st order kinetics, although incomplete mixing of the suspension led to shorter paracetamol GTT compared to typically reported GE times for similar meals, or GE times were prolonged and GE followed zero order kinetics when administered with the infant formula (liquid homogeneous) (9). It should be noted that under zero order GE kinetics the GTT value to be entered in the software represents the time for drug gastric emptying to complete.

Moreover, population simulations were performed for the refined settings for the three dosing conditions using a virtual population with similar demographics to the study by Statelova *et al*. (9). Under each type of dosing conditions, eight virtual male healthy subjects were generated using the PEAR™ module of the modeling platform with age range 21-48 years, body weight range 60-100 kg, and BMI range 20-28 kg/m^2. Simulation were performed over 10 h. Software default variability was employed for all parameters (44), except for the GTT values employed under both postprandial conditions, for which no variability could be included based on software limitations.

Paediatric model scaling

Paediatric physiologies were generated for each paediatric study using the PEAR physiology module within the modeling platform (44), i.e., a mean population representative according to the study demographics (25,27,28). The generation of virtual subjects (using the PEAR physiology module) accounted for maturation and development changes occurring from birth to adolescence, i.e., body and tissue sizes, blood parameters, tissue compositions, as well as hepatic CYP-enzyme abundances based on data from an exhaustive literature review (6,43,44,53). The microsomal protein per gram liver tissue was assumed to be independent of age (44), while age-adjusted scaling factors for enzymes of the UGT and SULT families were extracted from literature to scale the adult baseline abundances incorporated in the systemic adult parameters within the simulation platform (32,33,53,54). The presented scaling approach has been shown to lead to successful modeling of paracetamol metabolism across different ages following intravenous drug administration (33). Clinical observations in infants and in children and adolescents after i.v. administration were used to verify the present disposition model in paediatrics (25).

Oral absorption in infants under different dosing conditions

Relevant to oral absorption modeling as a function of age, the change of PEAR physiologies accounts for developmental changes in the paediatric GI tract within the ACAT™ physiology, such as GI-segments length and transit times, and accounts for some of the age-dependent factors that can influence paracetamol bioavailability. For the extrapolation to infants and evaluation of the usefulness of the three dosing conditions applied in the study by Statelova *et al.*, adjusted parameters from the adult paediatric suspension model were scaled to infants and applied to paediatric simulations. In the dataset described in (27), 5 infants with a mean age of four months (2 - 6 months) were dosed with a target dose of 15 mg/kg Calpol® suspension (dose administered 19.6 mg/kg), while in the dataset reported in (28) the paracetamol dose 12.14 mg/kg was given to infants and young children with a

mean age of ten months (range: 3 - 36 months). As in adults, the performance of the software default settings was evaluated during the infant model development, i.e., default settings for the fasted state and "user-defined meal" settings using zero and first order kinetics, for a solid-liquid meal and liquid homogeneous meal, respectively. As a next step, extrapolated parameters based on the refined adult model according to the study by Statelova *et al*. were used as input for the simulations in infants, with detailed description of the extrapolation rationale for the three different conditions described in the following paragraph (9).

A recent meta-analysis of GE as a function of age revealed that food type rather than age determined GE across ages (55). Therefore, under the assumption that no age dependent GTT changes would occur under fasted conditions, the GTT value found to appropriately describe the fasted state was inherited directly from the refined adult PBPK model. For the fed conditions and the infant-formula-fed conditions, the average paracetamol meal-dependent GE rate was estimated as a function of the type (solid-liquid *vs*. liquid homogeneous meal) and the caloric content of the meal. For this purpose, the caloric content of the meal given to adults was divided by the adjusted GTT values employed for the fed and infant-formula-fed conditions found to best describe paracetamol appearance in the systemic circulation (Eq. 2). Subsequently, fed GTT values for infants were estimated based on the caloric content of the recommended formula amounts for the age of interest and the paracetamol meal-dependent GE (Eq. 3). Different recommended meal calories reported for infant formula were selected for the infant group with a mean age four months and for the infant/young children group with a mean age ten months, i.e., 140 kcal and 170 kcal, respectively (3).

$$Average\ Paracetamol\ Gastric\ emptying\ rate_{adults,Meal} = \frac{Caloric\ content\ (meal\ based)}{Paracetamol\ GTT(meal\ based)} \quad \text{Eq. 2}$$

$$Paracetamol\ GTT_{infants,meal} = \frac{Meal\ caloric\ content\ recommended\ for\ age\ needs}{Average\ Paracetamol\ Gastric\ emptying\ rate_{adult,Meal}} \quad \text{Eq. 3}$$

where Paracetamol GTT represents the MGTT for a first order GE process (solid-liquid meal) and total GTT for a zero order GE process (Infant formula).

In addition to the single simulations, population simulations were performed for the two infant study groups, matching the demographics from each study (27,28) under the three dosing conditions employing the adjusted GTT values. Software limitations to parameter variability incorporation (GTT) is as described for the adult population simulations.

Model performance evaluation

For population representative and population simulations, (mean) predicted and observed PK parameters describing total drug exposure, peak exposure, and time to reach peak exposure (area under the plasma concentration vs. time curve (AUC), Cmax, and Tmax, respectively) were compared using the predicted *vs.* observed fold difference ($FD_{pred/obs}$). The predicted concentration-time profiles from population representative simulations and mean predicted plasma concentration-time profiles from the population simulations were evaluated by the average fold error (AFE) and the absolute average fold error ($AAFE$) calculated using Equation 4 (Eq. 4) and Equation 5 (Eq. 5), respectively.

$$AFE = 10^{\left(\frac{1}{n}\sum \log\left(\frac{PREDi}{OBSi}\right)\right)} \quad \text{Eq. 4}$$

$$AAFE = 10^{\left(\frac{1}{n}\sum \left|\log\left(\frac{PREDi}{OBSi}\right)\right|\right)} \quad \text{Eq. 5}$$

where n denotes the number of observed sampling points, PREDi and OBSi denote the predicted and observed plasma concentration, respectively, at the sampling time point i.

Additionally, for the population simulations, 90 % confidence intervals (CI), and probability contours (10 %, 25%, 50 %, 75 %, 90 %, 95 % and 100 %) including 5th and 95th percentiles were evaluated.

AFE values indicate the trend of the simulated data to underpredict (AFE < 1) or overpredict (AFE > 1) the observed plasma concentrations, while an $AAFE$ value close to unity signifies the precision of the simulations. Predictions resulting in $FD_{pred/obs}$ and $AAFE$ values less than two are considered adequate (56), while stricter evaluation criteria for $FD_{pred/obs}$ between 0.5-1.5 for and $AAFE$ below 1.5 indicate a successful prediction (57).

Parameter sensitivity analysis

Parameter sensitivity analysis (PSA) was performed according to a one-factor-at-a-time approach to understand the uncertainties of the parameters employed within the refined adult oral absorption model developed and evaluated in the present investigation regarding drug-related parameters, i.e., drug solubility, permeability, particle size radius, as well as physiological parameters, i.e., GTT. The investigation was performed with a population representative matching the mean demographic parameters of the clinical study by Statelova *et al*., i.e. 28-year-old male with a 78 kg bodyweight (9). Additionally, PSA was run for physiological, drug-dependent, and dosing parameters contributing to model uncertainty for infants under fasted, fed, and infant-formula-fed conditions using a physiology

297 matching the mean infant representative in Hopkins *et al*. (27). Physiological parameters included SI
298 radius and length, GTT, SITT, and gastric and duodenal pH, while drug-dependent parameters as
299 permeability, bile salt solubilization ratio, diffusion coefficient, reference solubility, and particle size
300 were investigated as drug-dependent parameters. Finally, the influence of dose strength and dosing
301 volume were simulated to explore the influence of trial conditions. Under fed and infant-formula fed
302 conditions, PSA was performed additionally regarding the caloric content of the paediatric meal
303 administered to the infants (Table SI, Supplementary Information). The extent to which paracetamol
304 PK and PK parameters are influenced by the selected parameter range was evaluated.

Results

Adult model performance

The developed disposition model for adults was able to adequately describe paracetamol disposition in the i.v. study used for modeling development when paracetamol was administered at a low dose 5 mg/kg, i.e., 350 mg ($AAFE$ 1.045) and high dose of 20 mg/kg, i.e., 1400 mg ($AAFE$ 1.080) (Figure 2A and 2B, respectively). External datasets used for model verification from two studies (15) simulated the observed data acceptably ($AAFE$ 1.131) for predictions at low paracetamol dose of 500 mg paracetamol and for predictions at high paracetamol dose of 1000 mg paracetamol ($AAFE$ 1.212), as shown in Figures 2C and 2D, respectively. Predicted clearance and Vss values were within observed ranges reported in the literature (Table SII, Supplementary Information). In addition, the disposition model was found to simulate all clinical study data following i.v. administration reported in literature with reasonable accuracy, as shown in Figure S1 and Table SIII in the Supplementary Information. The effective permeability value for humans scaled from Caco-2 apparent permeability experiments was in line with reported permeability ranges (45,58,59). Using the default GastroPlus™ settings for oral solution including a GTT of 0.1 h, the developed model achieved satisfactory prediction of paracetamol exposure after oral administration of 1000 mg solution in healthy adults in two different clinical studies [$AAFE$ 1.088, Figure 3A (19) and $AAFE$ 1.275, Figure 3B (21)]; thus confirming the suitability of the permeability value applied (Table SIII, Supplementary Information).

Modeling under different dosing conditions

The default settings for fasted and fed conditions utilizing the user-defined meal option for defining the meal specific caloric (reference meal 990 kcal and infant formula 520 kcal) and lipid (reference meal 60 % and infant formula 43 %) content failed to describe the data observed for the paracetamol suspension administered to healthy adults (Figure 4A, 4C, 4E). Consequently, adjustments of the GTT values for fasted, fed, and infant-formula-fed conditions were undertaken to match data observed *in vivo* (Figure 4B, 4D, 4F). Results herein are presented for the population representative from the Statelova et al. clinical study (9), while results for population simulations including mean profiles and their respective 90 % CIs, 5th and 95th percentiles and probability contours are reported in the Supplementary Information in Figure S3 and S3-1 and the mean predicted PK parameters and their respective $FD_{pred/obs}$ values are presented in Table SIV. Due to the multiple peak phenomena observed under fasted conditions in adults, drug performance was better described when multiple GE events were fitted using the MMD dosing available in the software (AFE 0.941 / $AAFE$ 1.052, Figure S2). However, for the purposes of extrapolation to infants, a compromise was made for a single GE process

for fasted state modeling, employing an adjusted GTT of 0.75 h ($AAFE$ 1.200). In the fed state following the reference meal, the suspension was emptied faster than the proposed GE times for the reference meal, thus requiring an adjustment of the 3.43 h GTT proposed for the meal to 1.5 h. Simulations utilizing the adjusted GGT value indicated better predictions compared to predictions using default values for GTT, i.e., $AAFE$ 1.145 *vs* $AAFE$ 1.733, respectively. In line with typical GE kinetics of liquid meals (60), under infant-formula-fed conditions, mean plasma concentration-time profiles were well-described by a zero-order GE. As for the reference meal, GTT adjustments were needed, as default parameters underpredicted the delay observed with ($AAFE$ 1.059 *vs* $AAFE$ 1.873). For the population simulations, although the mean predictions successfully matched the observed data, individual measured plasma concentrations fell outside the 5th and 95th percentiles for the simulations (Figure S3, Supplementary Information). This was especially noticeable at early times (Figure S3A and C, Supplementary Information) for both fed conditions (reference meal and infant formula) and was attributed to the limitation of the platform to include any variability for the adjusted GTT values.

Scaling to paediatrics

Disposition

Disposition kinetics and clearance employing isoenzymes of the CYP, UGT, and SULT families could predict observed paracetamol levels following i.v. administration over 0.25 h at doses of 12.5 mg/kg or 19.6 mg/kg (7,32,33). The model scaling was suitable to predict reported concentrations for i.v. administration for a population representative of infants (male, mean age 4 months and 4 kg) and of a population representative of a mixed children and adolescents group (male, mean age 6 years old and 23 kg) (25). Simulations for population representative of infants were performed for a higher dose administered at 15 mg/kg ($AAFE$ 1.312, Figure 5A) and a lower dose administered at 12.5 mg/kg ($AAFE$ 1.081, Figure 5B). On the other hand, simulations for a population representative of the mixed group were adequate for a high dose of 15 mg/kg paracetamol ($AAFE$ 1.420, Figure 5C) and a lower dose of 12.5 mg/kg paracetamol ($AAFE$ 1.187, Figure 5D).

Oral absorption in infants

Clinical data in infants following oral administration of a liquid paracetamol formulation available from two datasets were used for the evaluation of the usefulness of the developed adult model to predict paracetamol exposure in infants (27,28). Initially, using the default software settings, simulation of paracetamol plasma profiles in infants were performed under the three different dosing conditions. Then, for the purpose of extrapolating the fed conditions and the infant-formula-fed conditions to

infants, adjusted GTT values for infants were calculated based on these values and on caloric needs of the population representative of each study (Eq. 2 and Eq. 3), presented in Table II.

All simulations performed for the infant population representative, i.e. 4 month-old infants according to (27), are presented in Figure 6. Fasted state simulations employing default software parameters (GTT 0.1 h) could not describe early drug exposure, as they underpredicted Tmax ($FD_{pred/obs}$ = 0.60) and overpredicted Cmax ($FD_{pred/obs}$ = 1.3), although the overall description of the postabsorptive phase was adequate ($AAFE$ 1.185). The fasted conditions extrapolated from the refined adult model (GTT 0.75 h) led to a better prediction of the Tmax and slight underprediction of Cmax ($FD_{pred/obs}$ = 0.90), capturing both the early and the overall exposure better than the default settings (Figure 6A *vs* 6B, Table III). Following first order GE kinetics of the reference meal (a solid-liquid meal) and a caloric content of 140 kcal (the caloric content of a meal for a 4-month-old infant), default simulations (GGT = 2.1 h) could not successfully describe the data observed ($AAFE$ 1.523, Figure 5C). Calculation of the adjusted GTT for infants resulted in a value of 0.21 h (Table II) and although the postabsorptive PK were captured ($AAFE$ 1.201) the early exposure was overpredicted (Figure 5D). Under infant-formula-fed conditions and following zero order GE kinetics (as in adults), default simulations (GGT = 2.1 h) inaccurately described the data observed ($AAFE$ 1.428, Figure 5C). However, when using the adjusted GTT value (1.21 h), successful predictions of both early exposure and total exposure were achieved ($AAFE$ 1.215, Figure 6F). Mean simulation profiles from the population simulations (n=25, age range 2-6 months) corroborated the observations from the single simulations, as shown in Figure S5 (Supplementary Information).

Similarly to the simulations for younger infants (27), early exposure was overpredicted when applying software default parameters for the fasted state in infants with mean age of 10 months (28) and resulted in inaccurate predictions ($AAFE$ 1.442, Figure 7). In contrast, fasted conditions using the refined adult model (GTT 0.75 h) matched observed data well ($AAFE$ 1.201, Figure 7B). Following first order GE kinetics of the reference meal and caloric content of 170 kcal (the caloric needs of a 10-month-old infant), simulations employing default value for GTT = 1.89 h resulted in greater absorption delay than observed *in vivo*, as indicated by the $AAFE$ value of 1.87 (Figure 7C). The use of the adjusted GTT value for this study (Table II), although seeming to better predict the overall oral paracetamol performance compared to the default GTT values ($AAFE$ 1.274 vs $AAFE$ 1.87) led to overprediction of Cmax ($FD_{pred/obs}$ = 1.59). Under infant-formula-fed conditions, following zero order GE kinetics, default software settings (GTT 1.89 h) and adjusted GTT (1.47 h) underpredicted early exposure, however, employment of the adjusted GTT value showed slight improvement in the overall prediction compared to the default settings ($AAFE$ 1.40 vs $AAFE$ 1.695, Figure 7F and 7E, respectively). Population

simulations performed in 25 virtual subjects aged 3-36 months (28) indicated similar findings as the observations based on the single simulations with the mean population representative (Figure S7, Supplementary Information).

Parameter sensitivity analysis

PSA was performed for permeability and GTT under the three dosing conditions for the refined model for an adult population representative and a 4-month-old infant (9,27). Paracetamol PK showed sensitivity regarding the effective human permeability both in infant and adult population representatives, especially under fasted conditions (Figures S8 and S9, Supplementary Information). Decrease in paracetamol permeability negatively influenced the fraction of drug absorbed with up to 10 % compared to the baseline values (data not shown). Increase of GTT in adults and in infants resulted in lowered early exposure (Figure 8A), with prolonged Tmax values and Cmax decrease (Figure 9 and Figure S10, Supplementary Information). Furthermore, in infants, increased caloric content of the food translated into greater GTT values and led to more pronounced delay in paracetamol absorption under adjusted infant-formula-fed conditions when compared to extrapolation under adjusted fed conditions (Figure 8B and C and Figure 9 B and D). Overall, permeability and GTT changes demonstrated minor impact regarding total drug exposure. Additionally, reference solubility, bile salt solubilization ratio, dose volume, as well as the physiological parameters investigated demonstrated minor to no sensitivity in infants regardless of the dosing conditions applied, i.e. fasted, fed, or infant-formula-fed conditions (Table SI, Supplementary Information).

Discussion

Although PBPK modelling has been commonly used for the extrapolation from adults to paediatric populations, the usefulness of incorporating adult and/or infant-meal food effect data into PBPK modeling to extrapolate to infants has to the best of our knowledge not been reported yet. In this study, extrapolation to the infant paediatric subpopulation was performed based on the results of an exploratory clinical investigation of the paediatric paracetamol suspension in adults, which was designed to elucidate the effects of three different dosing conditions on drug performance, i.e., fasted, reference meal-fed and infant-formula-fed conditions (9). The applied PBPK modeling approach involved initial refinement of the adult oral absorption model for the different dosing conditions to match the *in vivo* observations reported by the Statelova *et al*. and these conditions were subsequently scaled to simulate paracetamol plasma concentration levels in infants observed after oral administration of paracetamol liquid formulations (27,28).

The discrepancy between predictions using default software values and predictions following adjustment of GTT values based on observed product performance highlighted the importance of model refinement that considered *in vivo* data collected under age-relevant dosing conditions using the commercially available paediatric formulation (Figure 4). Although PBPK modeling confidence with respect to oral drug absorption in adults has increased over the years and is considered to be reaching maturation for children (3,7,8), some aspects of GE and SI-transfer might not be accurately captured by a default approach regardless of age, i.e., discontinuous GE of liquid formulations and/or mixing processes between drug formulation and meal (61). In particular, the mismatch between the fasted state default prediction and observations for the suspension in adults could be explained by discontinuous GE-events resulting in a prolonged GE of the suspension as opposed to a single rapid GE event assumed for liquid formulations (9,19,62), i.e., Figure 2. The software platform enabled modeling of GE times for the administered drug as a function of different meal caloric contents, assuming homogeneous mixing of the drug and ingested meal. However, the default software assumptions of homogeneous mixing between drug formulation and the administered meals did not adequately reflect paracetamol GE patterns (63,64). Incomplete mixing of the formulation with the solid-liquid reference meal would lead to faster paracetamol emptying compared to the meal, as observed in the simulations (Figure 4C and 4D). On the other hand, paracetamol suspension mixes better with the liquid homogeneous infant formula, leading to paracetamol GE predominantly together with the infant formula bolus (9,63). It should be noted that, under both postprandial dosing conditions, independently of the meal texture, distinct paracetamol GE processes were not accurately reflected by the default ACAT™ model (9,63).

The present infant paracetamol PBPK model was discussed with focus on absorption parameters, as the paracetamol disposition and clearance parameters across ages employed in the model have been verified and discussed elaborately in previous works (7,32,33). In the present study, successful predictions were achieved for 4 month old infants (27) utilizing the refined model based on the *in vivo* performance of paracetamol suspension in adults under fasted conditions or infant-formula-fed conditions based on the recommended age-adjusted meal caloric content for the calculation of GTT in infants (Eq. 2 and Eq. 3), as shown in Figure 6B and 6F and summarized in Table III. Simulation of the administered dose in the population representative of the second available study [mean age of 10 months, (28)] led to most reasonable predictions using the refined model parameters for fasted conditions adjusted according to the study by Statelova *et al*. (Figure 7B and Table III). Similar observations resulted from population simulations for the adjusted dosing conditions (Figures S5 and S7, Supplementary Information), despite the simulation limitations based on the lack of variability included for GTT under both fed conditions. Although the prandial state in both infant studies was not reported, the adequacy of the predictions assuming infant-formula-fed state in a 4-month-old infant representative can be corroborated by the frequent feedings resulting in non-fasted conditions observed in young infants when compared to children and older age-ranges (3,65,66). In comparison, another age-dependent oral absorption modeling exercise employing default values for fasted and fed state in infants assuming a liquid feed and a semi-solid feed predicted slower absorption compared to the predictions in the present investigation (7,27). The delay in predicted absorption might be explained by the lack of meal size adjustment as a function of age and/or imperfect capturing of mixing events between formulation and meal. Lastly, within the current investigation, the extrapolation based on paracetamol GE kinetics after the ingestion of reference meal in adults (9) and the recommended age-adjusted meal calories for the estimation of GTT in infants resulted in overprediction of early exposure and rapid paracetamol absorption unlike the data observed in infants (27,28), thus appearing less suitable for the prediction of oral drug performance in infants.

In adults the usefulness of paracetamol as a GE marker to elucidate physiological events has been widely recognized under fasted state conditions (52,67), however, not after the high-calorie, high-fat meal recommended by regulatory agencies for the fed state (52,67). Within the present investigation of the fasted state in infants, when comparing the adjusted GTT value extrapolated from adults in the fasted state (GTT 0.75 h), the presence of thickening excipients in the paracetamol paediatric suspension could be the cause of delayed GE compared to GE of water in paediatrics, as in adults. As a note, reported GTT values in neonates who received 5 mL/kg non-caloric liquid and in infants who

495 received 20 mL/kg distilled water have been reported to be shorter, 0.17 h and 0.36 h, respectively,
496 (68,69), but a meta analysis across paediatric ages determined a GTT of 0.75 h for aqueous solutions
497 in the fasted state (55,70). Regarding the infant-formula-fed conditions, the adjusted paracetamol GTT
498 values cannot be compared with reported values from physiological studies in infants following infant
499 formula/milk feeds, because the GE kinetics in those studies are not always reported and/or different
500 infant formula types, caloric amounts, and formula compositions are used (3,71–74).

501

502 As PBPK modeling scaling of oral absorption processes to paediatrics relies on several assumptions
503 originating in knowledge gaps regarding physiological development and maturation in paediatrics (6),
504 parameters crucial for oral absorption and their impact on drug exposure in infants were investigated
505 using a one-factor-at-a-time PSA approach with primary focus on the prandial conditions. Drug
506 (formulation) related parameters and most physiological changes in infants appeared to be less
507 important for paracetamol exposure (Table SI) (45). As expected for paracetamol, prolonged GE
508 translated into absorption delay under fasted conditions (Figure 8 and Figure 9). Under fed conditions,
509 GE was investigated as a function of a range of caloric contents of an infant meal. Within the current
510 PBPK modeling exercise and extrapolation from adults to infants, recommended infant formula
511 volumes and caloric content thereof were used for the estimation of GTT in infants (Eq. 2 and 3) to
512 facilitate some standardization. PSA performed to understand the uncertainties underlying the caloric
513 content used in this study demonstrated delayed paracetamol absorption in infants for feeds with
514 greater caloric contents under infant-formula-fed conditions extrapolated from adults, with less
515 pronounced sensitivity within the range of 100-200 kcal feed (Figure 8 and Figure 9).

516

517 Paracetamol permeability was another sensitive parameter, the decrease of which led to drug
518 absorption delay and slight decrease in total exposure (Figures S6, S7, and S8). Interestingly, the PSA
519 under fed conditions induced with infant formula exhibited less sensitivity towards drug permeability
520 compared to the fasted conditions regarding Tmax. Drug permeability is generally considered to be an
521 age-dependent factor that reaches maturity the age of 2 years, with most of the conclusions originating
522 from investigations using dual sugar intestinal permeability tests (3). According to these studies (75–
523 79), increased permeability has been observed especially during the first days after birth, with
524 maturation (closure) of the junctions between epithelial cells ranging between the first days after birth
525 up to 15 months of age. Furthermore, age-dependent changes in permeability could be due to ongoing
526 morphological development of the shape and size of SI structures, i.e. villi and microvilli, leading to
527 surface-area-based decreased absorption capacity at young ages (7). While this parameter might bring

uncertainty into PBPK models for younger age-groups and should be carefully interpreted, population pharmacokinetic investigations suggested that age-related changes of paracetamol absorption rate were prominent in the early days after birth, i.e., neonates, who were not within the target group in the current investigation (7).

The present study for paracetamol highlighted the importance of informing the PBPK model during development with *in vivo* data employing age-relevant formulation and dosing conditions prior to extrapolation as opposed to using default settings to predict paracetamol oral absorption in infants (Figure 1). Along with PBPK modeling limitations highlighted and elaborately discussed elsewhere (3,7,8), specific limitations of the usefulness and applicability of the results from the present methodology include uncertainty regarding compounds whose bioavailability is affected by bile salt solubilization, ionizable compounds affected by intraluminal pH changes, drugs with permeability-limited absorption or transporter-substrates. In the present study, fasted conditions and/or infant-formula-fed conditions based on the study by Statelova *et al*. resulted in adequate predictions of paracetamol suspension performance in infants (27,28). In contrast, extrapolation following the reference meal appeared less useful to predict the observed plasma levels in infants (27,28). Coupling *in vivo* investigations of age-appropriate dosing conditions in adults with PBPK modeling and extrapolation to paediatrics provides a practical strategy for paediatric drug formulation testing with view to the complex interplay of formulation and age-appropriate meal characteristics.

Concluding remarks

Adult clinical data following paracetamol suspension administration under different dosing conditions was successfully extrapolated to infants using PBPK modeling. Reasonable simulations were achieved applying the refined model for fasted and/or fed state conditions employing a paracetamol meal-dependent GE based on infant formula. In contrast, default software parameters (GTT) and extrapolation to infants using paracetamol GE following the solid-liquid reference meal appeared less useful for predicting early exposure. The present investigation extended the utilization of PBPK modeling for simulating plasma concentration levels in infant populations in the context of its application within the biopharmaceutical investigations of age-appropriate fed conditions. Emphasis should be placed on age-dependent meal-drug-formulation interactions, as drug formulations for infants can be different than adults', i.e., suspensions, mini-tablets or multiparticulates and paediatric meals have commonly homogeneous texture unlike the reference meal. Our findings support the need of paediatric formulation investigations employing foods commonly used in the target paediatric subpopulation as recently introduced in regulatory guidelines (11). Furthermore, the present investigation indicated that caution should be exercised even when using bioavailability data of BCS Class I drugs with non-problematic absorption in adults to extrapolate to infants. Verification of the proposed methodology for infant formulation evaluation with broader spectrum of compounds with different physicochemical properties is required. Finally, availability of high-quality clinical data in infants is of paramount importance for evaluating the biopharmaceutics tools and methodologies and confirmation of their reliability.

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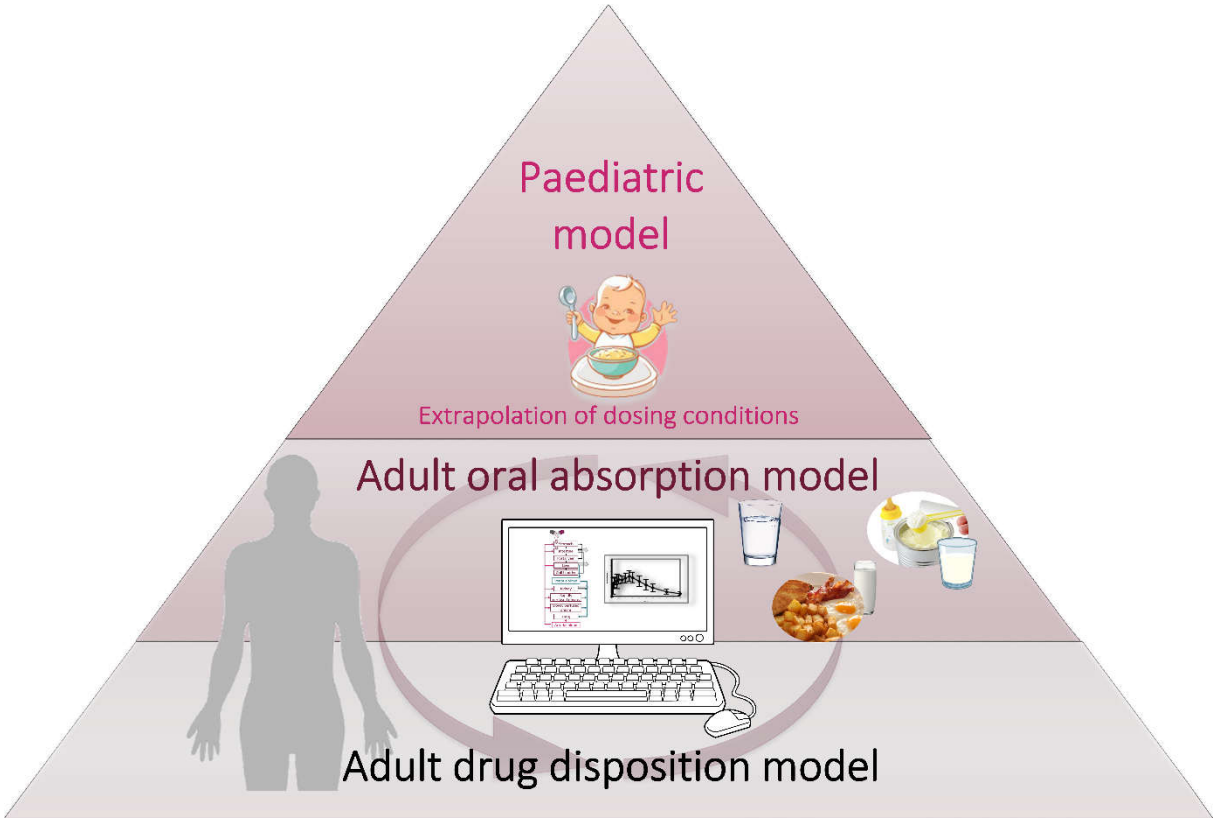
Graphical abstract



List of figures

Figure 8 Simulated plasma concentration-time profile (continuous line) in infant population representative under fasted conditions with variation of the GTT between 0.1-1.5 h (A), under fed conditions with different caloric intake (70-200 kcal) and adjusted GTT based on the paracetamol reference-meal dependent gastric emptying (B), and under infant fed conditions with different caloric intake (70-200 kcal) and adjusted GTT extrapolated based on the paracetamol infant-formula-dependent gastric emptying (C). The color gradient represents increasing GTT values and caloric content of the meals from dark to light grey. Observed mean data and standard deviation are presented as symbols and error bars (27).

Figure 9 Parameter sensitivity analysis for Cmax and Tmax results in a population representative infant (4 months old, (27)) as a function of GTT under adjusted fasted conditions (A and C), or caloric content (GTT) influence under adjusted reference-meal-conditions (continuous lines B and D), or caloric content influence under adjusted infant-formula-fed conditions (discontinuous lines B and D). Values used within the paediatric simulations employing adjusted MGTT values are shown as open circles.

Figure 1

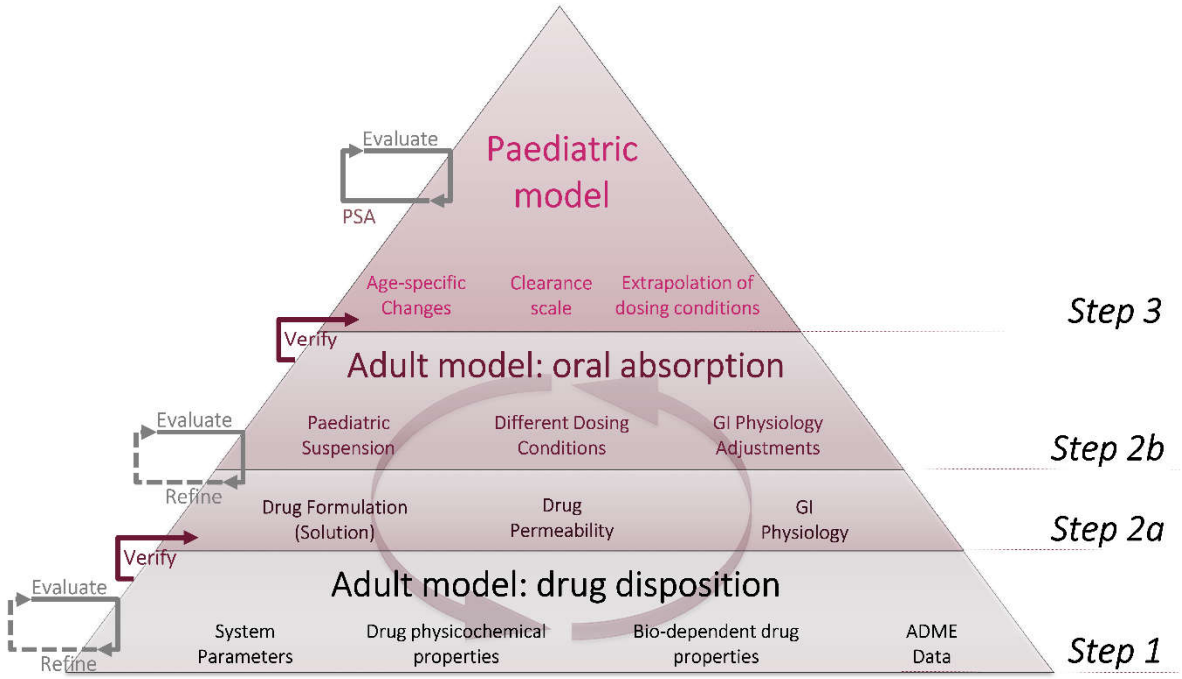

Evaluate
PSA
Paediatric model
Age-specific Changes
Clearance scale
Extrapolation of dosing conditions
Step 3
Verify
Adult model: oral absorption
Evaluate
Refine
Paediatric Suspension
Different Dosing Conditions
GI Physiology Adjustments
Step 2b
Drug Formulation (Solution)
Drug Permeability
GI Physiology
Step 2a
Verify
Adult model: drug disposition
Evaluate
Refine
System Parameters
Drug physicochemical properties
Bio-dependent drug properties
ADME Data
Step 1

Figure 2

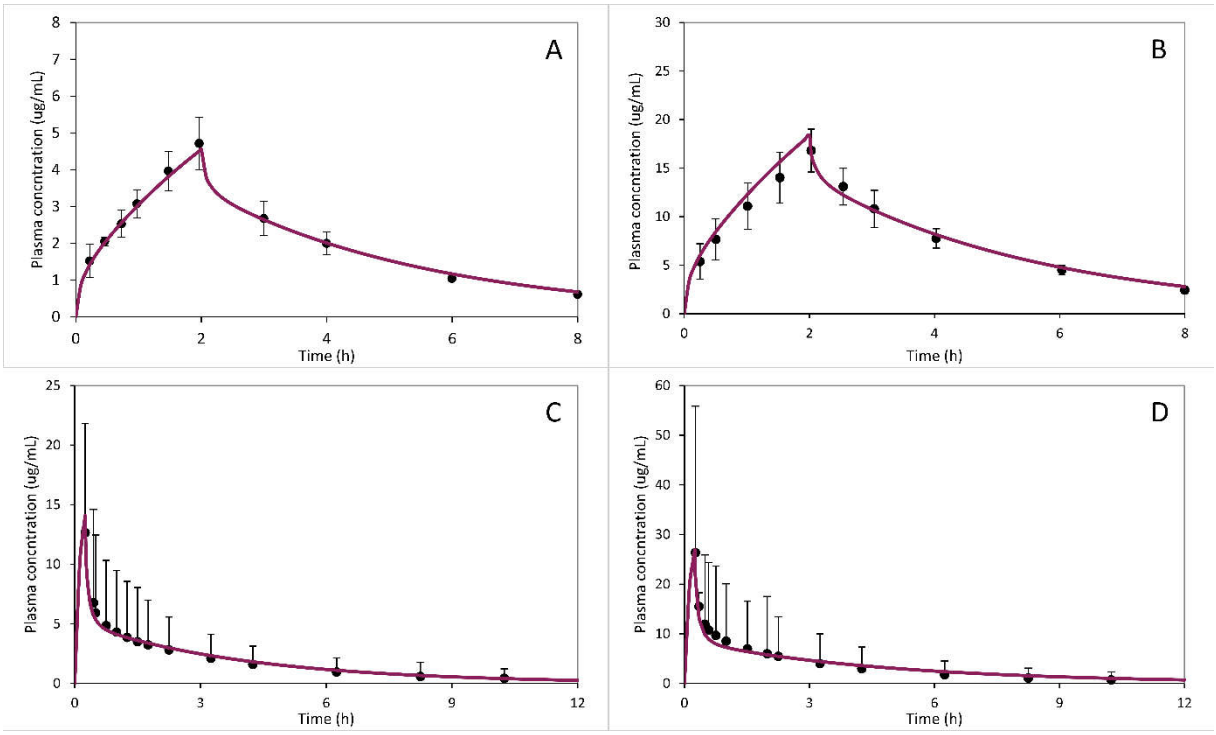

A
Plasma concntration (ug/mL)
Time (h)
B
Plasma concntration (ug/mL)
Time (h)
C
Plasma concntration (ug/mL)
Time (h)
D
Plasma concntration (ug/mL)
Time (h)

Figure 3

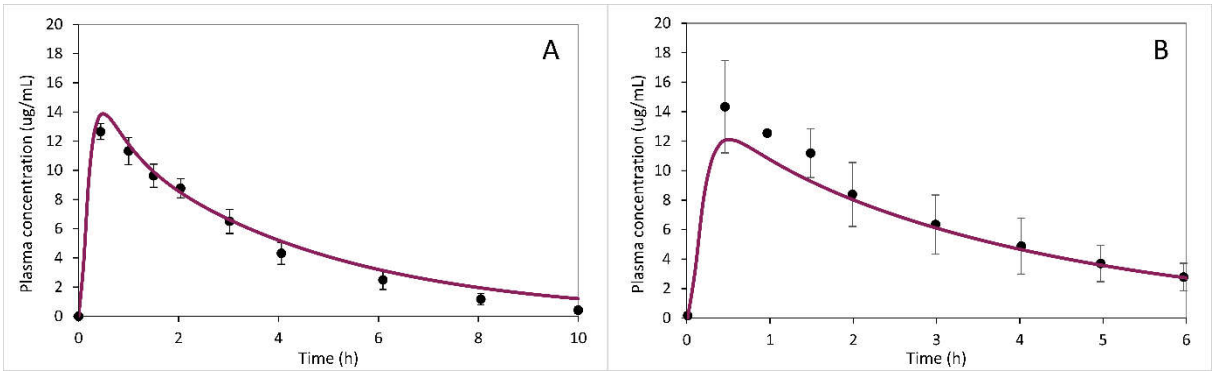

A
Plasma concentration (ug/mL)
Time (h)
B
Plasma concentration (ug/mL)
Time (h)

Figure 4

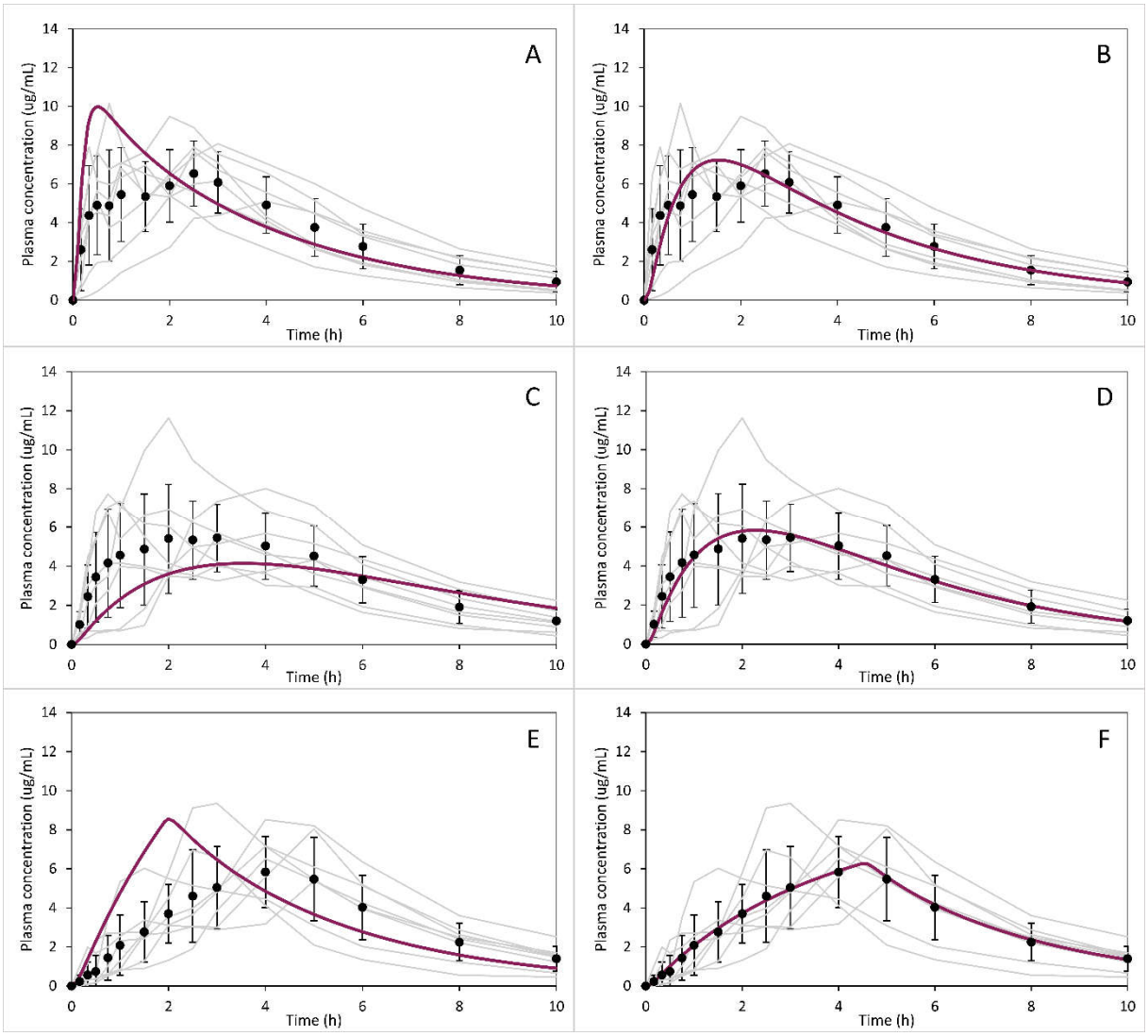


Figure 5

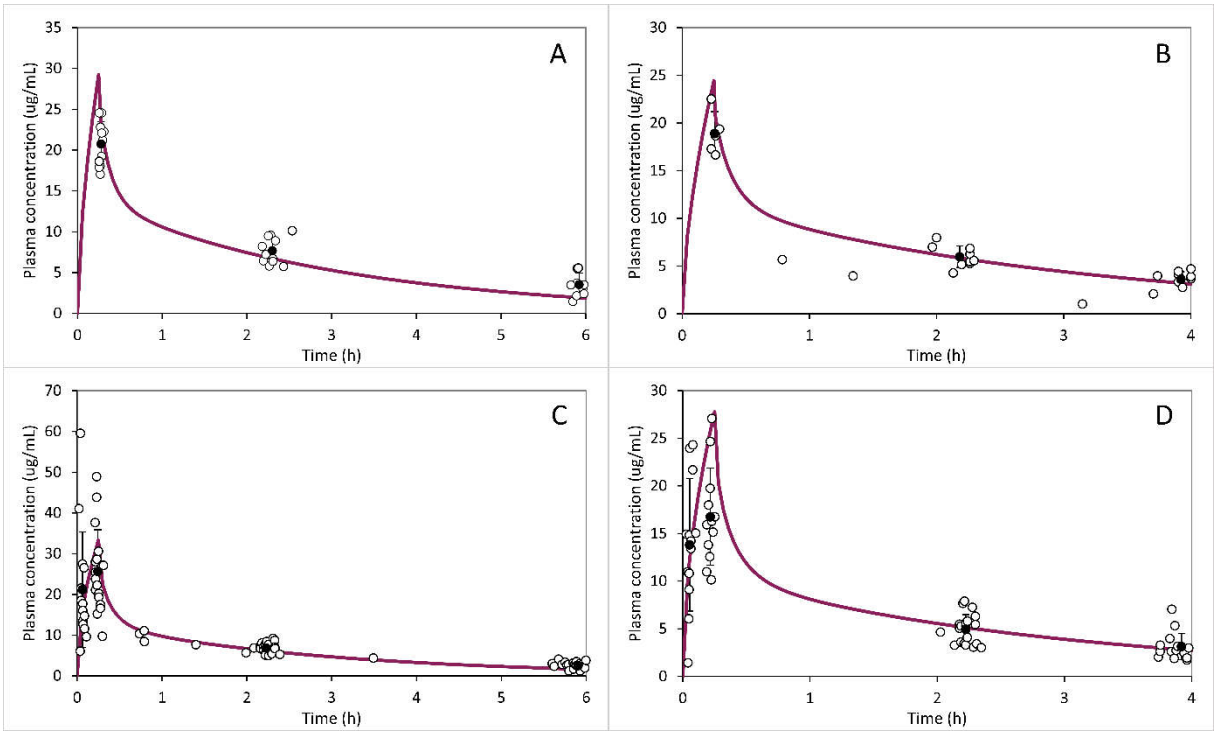


Figure 6

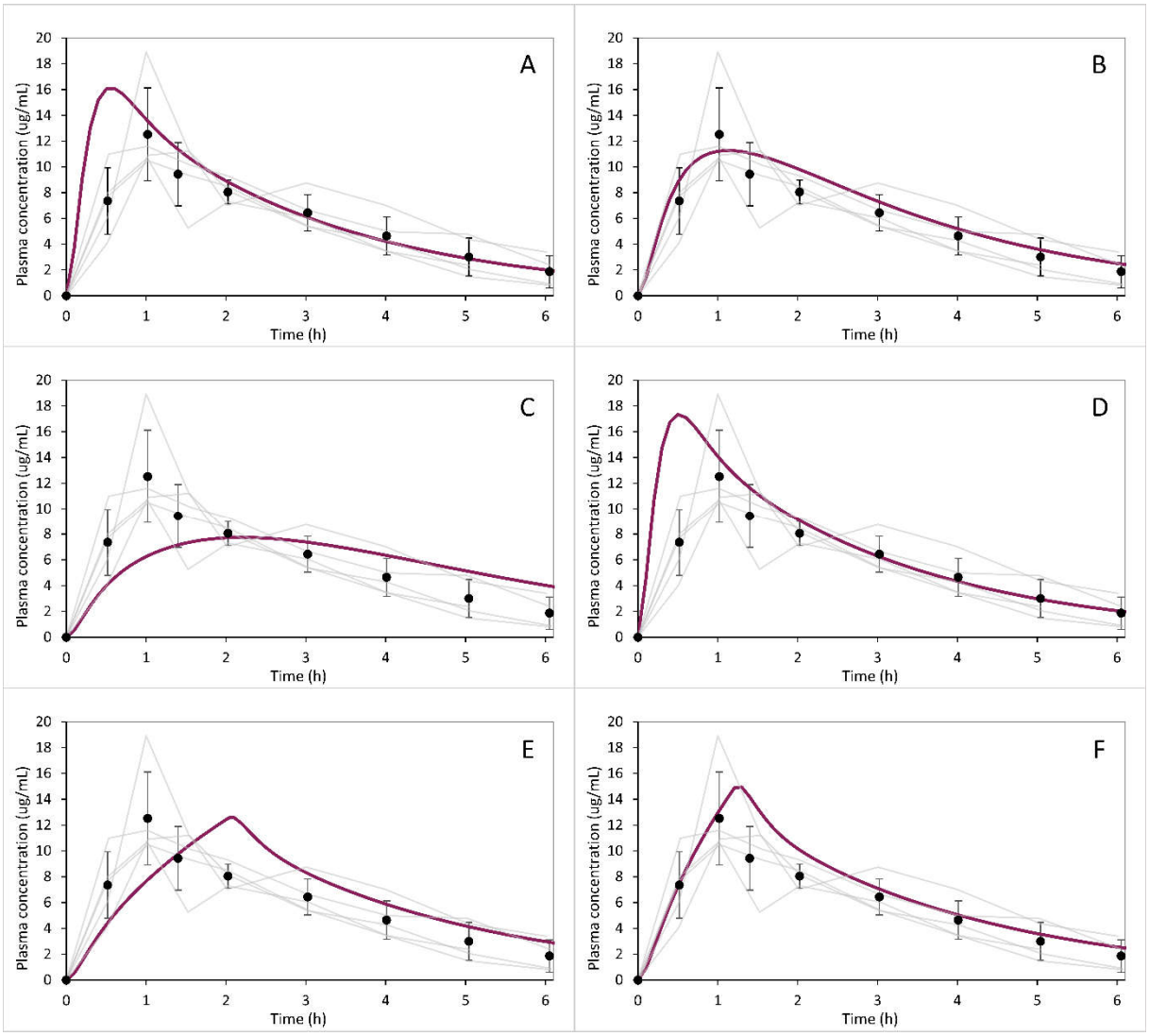

A
B
C
D
E
F
Plasma concentration (ug/mL)
Time (h)
0
2
4
6
8
10
12
14
16
18
20
1
3
5

Figure 7

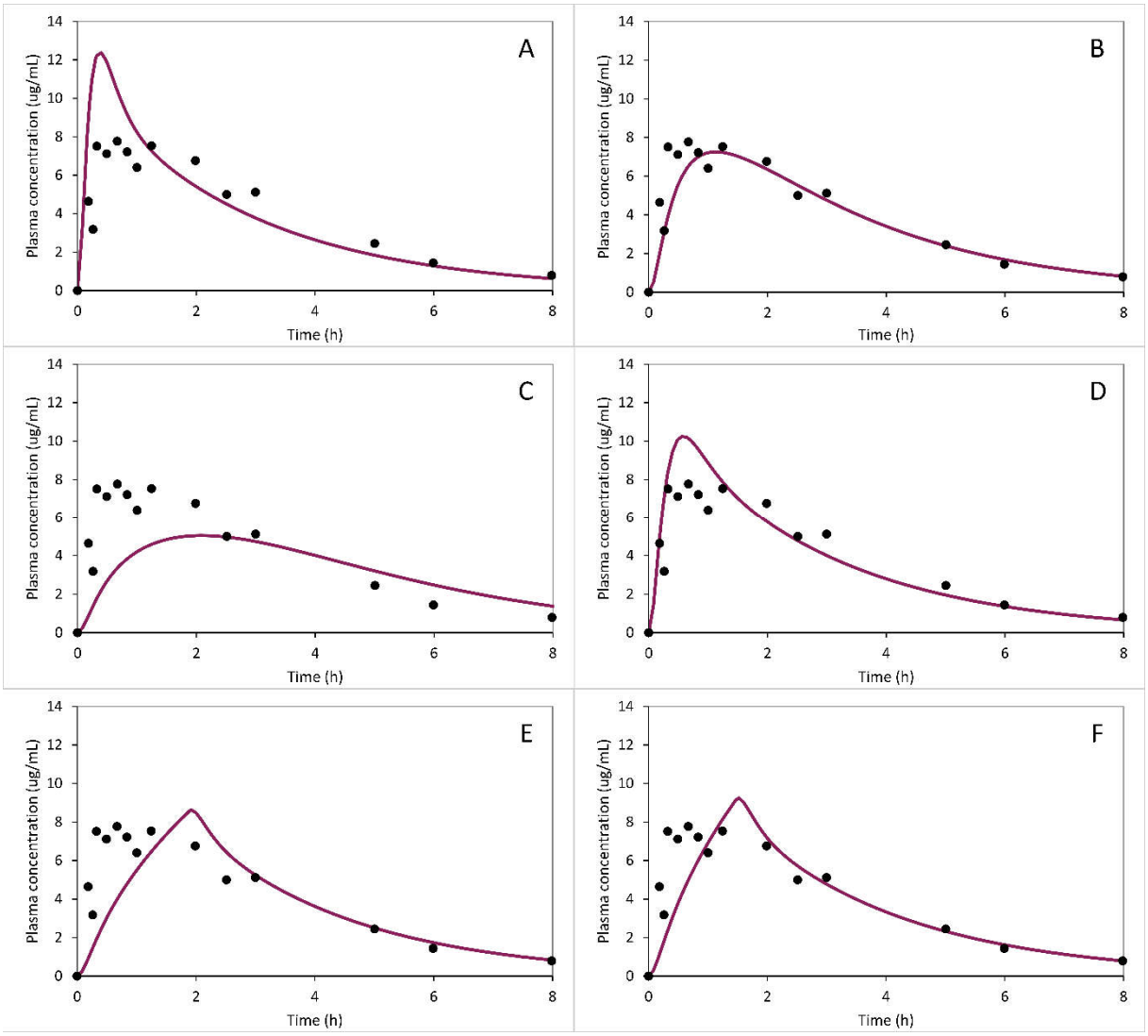


Figure 8

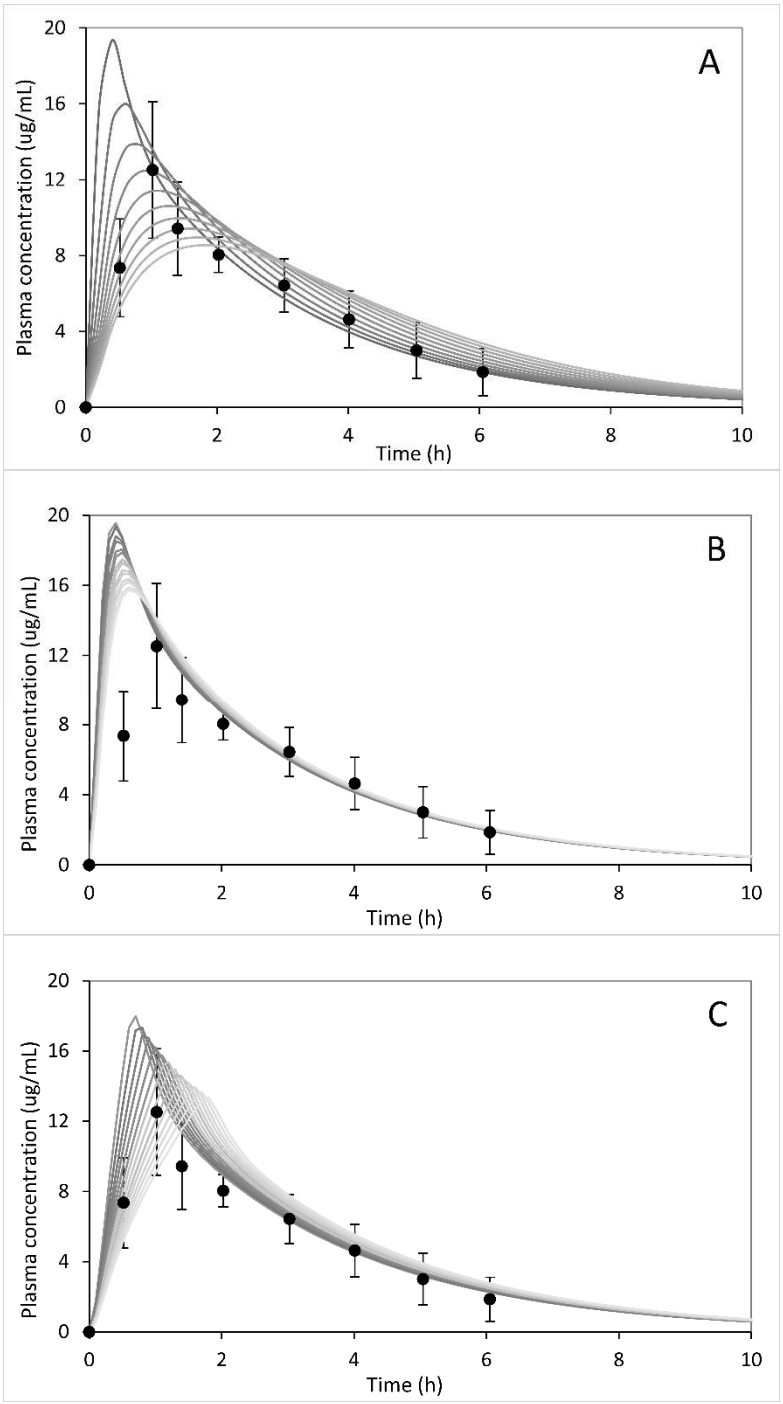


Figure 9

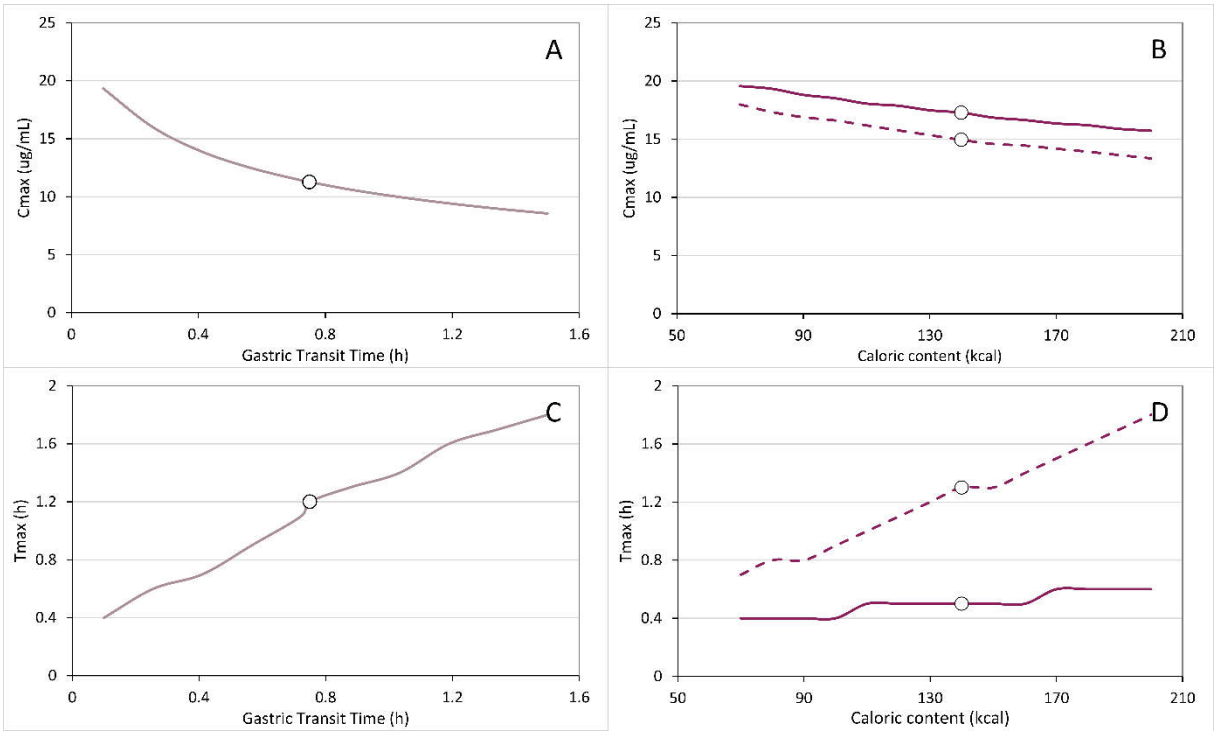

A
Cmax (ug/mL)
Gastric Transit Time (h)
B
Cmax (ug/mL)
Caloric content (kcal)
C
Tmax (h)
Gastric Transit Time (h)
D
Tmax (h)
Caloric content (kcal)

Table I Input parameters used to build the PBPK model for paracetamol

Parameter			Source
Physicochemical properties			
Molecular weight (g/mol)		151.9	(31–33)
Compound type		Monoprotic weak acid	(31–33)
pKa		9.45 (acidic)	(31–33)
logP [a]		0.51	(31–33)
Reference solubility in water (mg/mL)		14	(31)
Absorption			
Model		ACAT	GastroPlus™
Effective permeability, human (cm/s ×10^4)		3.897	Calculated based on (7,34,35)
Dissolution model		Johnson	GastroPlus™
Drug particle radius (µm)		25	Default GastroPlus™
Distribution			
Fraction unbound, fu		0.82	(46)
Blood-plasma ratio		1.09	(47)
Predicted Vss (L/kg) [b]		0.86	Predicted using the Lukacova, Rodgers and Rowland method (6,38,39)
Clearance			
In vivo clearance (L/h)		19.7	(16)
Enzyme kinetics			
	Km (µM)	Vmax (pmol/min/mg microsomal protein)	
CYP1A2 [c]	220	30.78	(40)
CYP2C9 [c]	660	8.42	(40)
CYP2C19 [c]	2000	25.53	(40)
CYP2D6 [c]	440	5.62	(40)
CYP2E1 [c]	4020	76.97	(40)
CYP3A4 [c]	130	57.16	(40)
UGT1A1 [d]	5500	6102.67	(41)
UGT1A9 [d]	9200	10208.11	(41)
UGT2B15 [d]	23000	34045.84	(41)
SULT1A1 [e]	2400	1374.06	(42)
SULT1A3 [e]	1500	202.89	(42)
SULT1E1 [e]	1900	146.22	(42)
SULT2A1 [e]	3700	828.35	(42)

[a] to achieve the benchmark Vss values observed *in vivo*, initially logP value of 1.2 was used for the calculation of the tissue partitioning coefficients (Kp) (36); measured logP value 0.51 was used thought simulations; [b] Predicted volume of distribution at steady state (Vss); [c] Cytochrome P450 (CYP) isoenzyme, [d] UDP-glucuronosyltransferase (UGT) isoenzyme, and [e] cytosolic sulfotransferases (SULT) isoenzyme contributing to paracetamol metabolism

Table II Paracetamol meal-dependent gastric emptying (GE) based on the gastric transit time (GTT) values employed in the refined adult model for the reference meal and the infant formula used for inducing fed and infant-formula-fed conditions (9) and adjusted GTT values for paracetamol gastric emptying in infants according to recommended meal calories for age (4 and 10 months).

Meal and Paracetamol GE kinetics	Adult		Paracetamol GE (meal-dependent, expressed as kcal/min)	Infants			
	28-years-old male, 78 kg body weight [a]			4-month-old, 4 kg body weight [b]		10-month-old, 8.6 kg body weight [c]	
	Caloric content (kcal)	GTT (h)		Caloric content (kcal)	GTT (h)	Caloric content (kcal)	GTT (h)
Reference meal (Solid-liquid) 1st order GE	990	1.5	11	140	0.21	170	0.26
Infant formula (Liquid homogeneous) Zero order GE	520	4.5	1.93	140	1.21	170	1.47

[a] mean adult population representative of the study by Statelova *et al*. (9)

[b] mean infant population representative of the study by Hopkins *et al*. (27)

[c] mean infant population representative of the study by Walson *et al*. (28)

Table III Observed and predicted pharmacokinetic parameters in studies performed in infants (27,28). Simulations in infants were extrapolated based on the refined adult model for different dosing conditions as described in Statelova *et al.* (9).

Study	Parameter	Observed	Simulated fasted conditions [a]			Simulated fed conditions (solid-liquid meal) [a]			Simulated infant-formula-fed condition (liquid homogenous meal) [a]		
			Predicted	$FD_{pred/obs}$ [b]	AFE [c] / $AAFE$ [d]	Predicted	$FD_{pred/obs}$ [b]	AFE [c] / $AAFE$ [d]	Predicted	$FD_{pred/obs}$ [b]	AFE [c] / $AAFE$ [d]
Hopkins *et al.* n= 5 subjects 3 male/2 female Dose 19.6 mg/kg	AUC_{0-t} [e] (ug/mL·h)	35.93	40.49	1.127	1.129/ 1.187	43.78	1.219	1.053/ 1.201	41.30	1.149	1.212/ 1.215
	AUC_{0-inf} [f] (ug/mL·h)	40.21	47.22	1.172		49.24	1.225		49.06	1.220	
	Cmax [g] (ug/mL)	12.52	11.27	0.900		17.33	1.384		14.94	1.193	
	Tmax [h] (h)	1.0	1.1	1.1		0.5	0.5		1.3	1.3	
	AUC_{0-Tmax} [i] (ug/mL·h)	6.88	7.48	1.087		13.51	1.963		6.83	0.992	
Walson *et al.* n= 13 subjects 7 male/5 female Dose 12.1 mg/kg	AUC_{0-t} [e] (ug/mL·h)	30.13	28.60	0.949	0.948/ 1.201	29.15	0.967	1.107/ 1.274	30.92	1.026	0.847/ 1.401
	AUC_{0-inf} [f] (ug/mL·h)	32.76	30.89	0.943		30.99	0.946		28.72	0.876	
	Cmax [g] (ug/mL)	7.77	7.26	0.934		10.24	1.318		9.25	1.190	
	Tmax [h] (h)	0.70	1.12	1.60		0.56	0.80		1.52	2.17	
	AUC_{0-Tmax} [i] (ug/mL·h)	3.62	2.65	0.733		5.05	1.396		1.78	0.492	

[a] Conditions simulated based on the refined adult model for different dosing condition as described in Statelova *et al.* (9)

[b] $FD_{pred/obs}$: Fold difference predicted/observed

[c] AFE average fold error

[d] $AAFE$ absolute average fold error

[e] Area under the plasma concentration-time curve from 0h until the last observed time point (t) AUC_{0-t} (ug/mL·h)

[f] Area under the plasma concentration-time curve from 0h to infinity AUC_{0-inf} (ug/mL·h)

[g] Maximum plasma concentration Cmax (ug/mL)

[h] Time to reach Cmax (h)

[i] Area under the plasma concentration-time curve from 0h until the mean Tmax observed in the simulated study AUC_{0-Tmax} (ug/mL·h)