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Usefulness of the beagle model in the evaluation of paracetamol and ibuprofen exposure after oral administration to paediatric populations: An exploratory study

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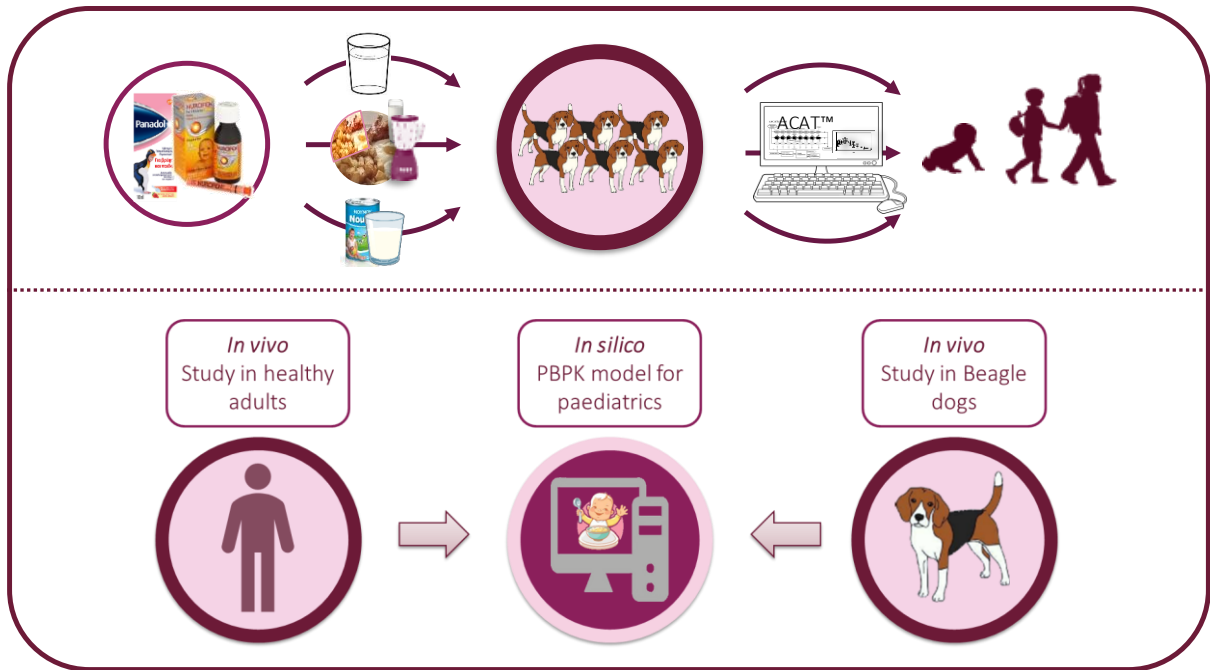
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1   Graphical abstract

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Abstract

The present study aimed to explore the usefulness of Beagle dogs in combination with physiologically based pharmacokinetic (PBPK) modelling in the evaluation of drug exposure after oral administration to paediatric populations at an early stage of pharmaceutical product development. An exploratory, single-dose, crossover bioavailability study in six beagles was performed. A paracetamol suspension and an ibuprofen suspension were co-administered in the fasted state conditions, under reference-meal fed state conditions, and under infant-formula fed state conditions. PBPK models developed with GastroPlus v9.7 were used to inform the extrapolation of beagle data to human infants and children. Beagle-based simulation outcomes were compared with published human-adult-based simulations. For paracetamol, fasted state conditions and reference-meal fed state conditions in beagles appeared to provide adequate information for the applied scaling approach. Fasted state and/or reference-meal fed state conditions in beagles appeared suitable to simulate the performance of ibuprofen suspension to paediatric populations. Contrary to human-adult-based translations, extrapolations based on beagle-data collected under infant-formula fed state conditions appeared less useful for informing simulations of plasma levels in paediatric populations. Beagle data collected under fasted and/or reference-meal fed state conditions appeared to be useful in the investigation of paediatric product performance of the two investigated highly permeable and highly soluble drugs in the upper small intestine. The suitability of the beagle as a pre-clinical model to understand paediatric drug product performance under different dosing conditions deserves further evaluation with broader spectrum of drugs and drug products and comparisons with paediatric *in vivo* data.

Keywords (max. 10)

Introduction

Although paediatric drug development and drug product evaluation has advanced over the recent years (1), age-appropriate tools and methodologies to predict formulation performance in paediatric populations are yet to be established. Doubtlessly paediatric drug product evaluation in the target population would be ideal to ensure safety and efficacy after administration to this vulnerable patient group, however, ethical and recruitment issues limit investigations in paediatrics. Therefore, data from bioavailability studies in adults are often extrapolated to the paediatric population of interest (1–3). In line, a recent draft guideline from the U.S. Food and Drug Administration (FDA) suggested that the sponsor should perform a food effect study in adults with the paediatric formulation and that "the sponsor can use foods and quantities of food that are commonly consumed with drugs in a particular pediatric population (e.g., formula for infants)" (4). Recent literature review highlighted the different food effect observations between studies performed in healthy adult volunteers in comparison to food effects observed in infants/young children who were administered the same drug (2,5). The discrepancy in the food effect outcomes could be attributed to several factors: age-dependent physiology differences, inconsistent protocols between adult and paediatric food effect studies, age-appropriate meal chosen for each study population (standard breakfast in adults and milk-based meal in paediatric populations) (2,5,6). A dedicated investigation of the factors affecting oral absorption of paediatric formulations in the presence of standard solid-liquid meal or infant milk-based feed in adult healthy volunteers revealed that food effects for infant formulations might not be adequately evaluated when applying common adult FE study protocols (5).

Furthermore, as paediatric product development usually commences during the clinical stages of adult drug product development, paediatric formulation development and the food effect evaluation are mainly guided by the knowledge gained throughout adult formulation investigations and the relevant applied protocols for adults (1,4,7,8). Within the recent FDA guidance, it is indicated that paediatric product development builds upon knowledge of the adult formulation performance, i.e., when the same to-be-marketed formulation that is approved for use in adults is approved for use in a pediatric population, a separate FE study is not necessary (4). However, before addressing this research question in the clinical setting in humans using the paediatric formulation, investigation of possible food effects with age-relevant meals and quantities at a preclinical level (e.g., in Beagle dogs) could de-risk paediatric formulation testing in clinic and reduce associated costs. Throughout adult drug product development, food effect evaluation in the preclinical stage is commonly supported by *in vitro* tools, pre-clinical animal models, and/or *in silico* tools (9,10) and has resulted in a confirmatory rather than exploratory nature of the clinical food effect studies for adult products (11–13). In paediatrics,

65 considering the limitations surrounding the establishment of validated age-appropriate *in vitro* and

66 *in silico* tools to be used as standalone methodologies for predicting product performance prior to

67 testing in human (7); adaptation of existing study protocols for animal models, as the beagle , could

68 offer additional insights to understand oral dosage behavior, especially regarding mechanical/physical

69 interactions between drug components/food components.

70 Based on the similarities between the canine and human adult gastrointestinal (GI) tract and the

71 relatively easy handling of the breed, preclinical bioavailability/food effect studies are often

72 performed in beagles (14–17). Despite several similarities, differences in GI anatomy and physiology

73 between humans and beagles may increase the complexity of directly translating preclinical outcomes

74 into human, e.g., basal gastric secretions, pH of fluids along segments of the GI tract, intraluminal bile

75 salt composition and levels, transit times, and intestinal permeability (17). A major difference between

76 beagles and human adults that might affect performance of ionizable, poorly soluble drugs is the lower

77 level of basal gastric secretions in beagles that could lead to elevated fasted gastric pH in the fasted

78 state and greater variability of intragastric pH compared to human adults (14,15,18,19). In an effort

79 to overcome this interspecies difference when investigating formulations for humans and to control

80 intragastric pH, oral administration of HCl/KCl solution prior to drug dosing has been demonstrated to

81 induce acidic environment in the canine stomach with an acceptable reproducibility (18).

82 In addition to the disparities between human and canine GI physiology that control drug absorption,

83 interspecies differences in disposition, metabolism, and elimination further complicated efforts for

84 direct results extrapolation from bioavailability/food effect studies from beagles to human adults (10).

85 Mechanistic approaches, such as physiologically based pharmacokinetic (PBPK) modeling have been

86 utilized to account for these differences and translate relevant biopharmaceutical information from

87 canine studies into the human adult model; furthermore, the preclinical model has been used to

88 identify sensitive parameters regarding oral drug/drug product performance in adults (20–23).

89 Furthermore, the dog model has been suggested to be potentially useful for paediatric formulation

90 testing based on its role in adult drug product development (7). To date, only few relevant studies

91 have been reported in the preclinical species and the evaluation of their usefulness has been limited

92 due to lack of paediatric clinical data to serve as confirmatory dataset (7). The elevated bile salt levels

93 in dogs have been mentioned as one factor that complicates results interpretation (7).

94 The aim of this study was to explore the usefulness of the beagle model in the evaluation of drug

95 exposure after oral administration to paediatric populations and compare it with the human adult

96 model. In line with the design of the human adult bioavailability data acquired under different dosing

97 conditions (5), the first objective was to design comparative bioavailability studies of two paediatric

98 drug products under different prandial and dosing conditions, i.e.,

99 • fasted state conditions

100 • fasted state conditions with gastric pH-lowering pretreatment

101 • reference-meal fed state conditions

102 • dosing conditions simulating the infant-formula fed state conditions.

103

104 The second objective was to propose a PBPK approach for modeling the collected data and investigate

105 if the conditions applied to beagles substantially affected extrapolation to paediatric populations. The

106 third objective was to compare the usefulness of the beagle data in evaluating drug exposure in

107 paediatric populations with the respective translation based on human adults (24,25).

108 Paracetamol (high solubility, weak acid, pka 9.5, BCS Class I) and ibuprofen (low solubility, weak acid,

109 pka 4.5, BCS Class II) (26–28) were selected as model drugs based on their luminal stability and high

110 intestinal permeability, as in the respective investigations in adults (5,24,25). Additionally, based on

111 their physicochemical properties, both drugs are expected to be highly soluble in the upper small

112 intestine. After confirming the lack of pharmaceutical and pharmacokinetic interaction (29,30), the

113 drugs were co-administered using the commercially available paediatric suspensions, i.e., variations

114 of dosing should impact primarily gastric emptying (paracetamol) or gastric emptying and, perhaps,

115 dissolution (ibuprofen).

116

Materials and methods

Materials

The paracetamol solution for intravenous (i.v.) administration was prepared in-house by dissolving paracetamol powder in saline for i.v. use with a final concentration of 10 mg/mL (Esco, European salt company GmbH & Co. KG, Germany). The ibuprofen solution for i.v. administration (5 mg/mL) was prepared in-house by dissolving ibuprofen in 50 mM Tris solution under addition of NaCl (876 mg/100 mL) to reach isotonicity, followed by pH adjustment to 7.6 with 1 M HCl. Both solutions were filtered through a 0.22 µm Millex®-GV PVDF filter. Nurofen® paediatric suspension with 100 mg ibuprofen/5 mL (Reckitt Benckiser UK Ltd., Berkshire, UK) and Panadol® paediatric suspension with 120 mg paracetamol/5mL (GlaxoSmithKline A.E.B.E., Middlesex, UK) were acquired from a local pharmacy in Athens, Greece.

Food products for the reference meal were supplied from a local supermarket. The reference meal consisted of two slices of toasted bread with butter, two strips of bacon fried in butter, two eggs fried in butter, French fries, and a glass of full-fat cow's milk; this resulted in 67 g of fat, 63 g of carbohydrates, 36 g of protein (60 % fat, 25 % carbohydrates, 15 % proteins) according to the recommended meal by regulators (4,31). The reference meal was prepared in a similar manner to a recently reported clinical study in adults (5) and was cooked in the evening prior to the relevant study day. On the study day, the meal was homogenized, and a portion of 100 g (200 kcal) was administered to each dog via gavage. Infant formula milk (Noulac® for infants) was used as in the clinical study (5). The infant formula was prepared according to instructions in the morning of the study day. The administered volume per dog was 150 mL (100 kcal) and it consisted of 43 % fats, 47 % carbohydrates, and 10 % proteins.

Acetonitrile and water (MilliQ®-System, Merck KGaA, Darmstadt, Germany) were of LC-MS/MS grade, trifluoroacetic acid (TFA, 99.5 % w/w) was of protein sequencing grade, HCl (1.0 M) and KCl were of analytical grade. All chemicals were supplied from Merck KGaA (Darmstadt, Germany).

Methods

Study in beagle dogs

The animal study described in this work was performed according to current relevant European directive on protection of animals used for scientific purposes (2010/63/EU) and Belgian low regulating Animal Welfare of test animals (https://www.lne.be/proefdierlabo). The study protocol

148 was approved by the institutional Ethics Committee of Janssen Pharmaceutica, Belgium (approval nr.
149 512).

150 Six healthy male beagles from the in-house colony (supplied from Marshall® colony, Marshall
151 BioResources, Lyon, France) aged between 1.7-4.0 years (mean 2.17) and weighing 7.9-13.3 kg
152 (mean 10.3) were included in this study. The dogs had unrestricted access to water throughout the
153 study. On study days, animals were placed and kept individually for four hours after dosing, after
154 which period the dogs were returned to their daily routine. On non-experimental days, the dogs
155 received their portion of canine dry pellets (LabDiet®, St. Louis, Mo, USA) once daily at noon.

156

157 *Experimental protocol*

158 This single-dose bioavailability study was performed on a crossover basis following a block design. Six
159 treatments were applied on separate occasions within the study and are listed in Table I. Drug doses
160 and meal quantities were scaled based on the mean body weight (BW) of the healthy adults in a
161 human relative bioavailability study performed with the same drug formulations under different
162 dosing conditions (≈76 kg) (4,24) and a typical BW of a beagle ≈13 kg (14,19,32). A single dose of
163 168 mg paracetamol and 140 mg of ibuprofen per dog were administered in all six study phases. In
164 Phases 1 and 2, paracetamol (168 mg per dog) or ibuprofen (140 mg per dog) were administered
165 intravenously on two separate study days. In the other four study phases the two drugs were co-
166 administered orally as their respective paediatric suspensions, i.e., 7 mL Panadol®
167 (168 mg paracetamol) and 7 mL Nurofen® (140 mg ibuprofen). Each study phase was separated by a
168 recovery/wash-out period of at least six days.

169 Intravenous drug administrations (Phases 1 and 2, Table I) were performed in the fasted state and
170 drugs were administered as single bolus injection into the cephalic vein. Oral administrations in the
171 fasted state were performed in Phases 3 and 4, as shown in Table . Several reports have indicated
172 elevated pH levels in the canine stomach and high variability in gastric pH values between
173 dogs (18,19,33,34). To evaluate the importance of this difference between beagles and humans, drug
174 exposure in the fasted state was evaluated with and without administration of 20 mL of 0.1 M HCl/KCl
175 oral solution with pH 1.6 prior to drug dosing (18).

176 Oral administrations in the fed state was performed in Phases 5 and 6. Based on BW-scaling, 100 g
177 portion of reference meal (4,24), i.e. 200 kcal, was administered to dogs "reference-meal fed state
178 conditions" (Phase 5). The scaling was based on a meal amount of 550 g and the above mentioned BW
179 for human adults and beagle dogs, resulting in 92 g of reference meal to be administered per dog – in

the present study the meal mass was rounded to 100 g to improve dosing feasibility in a preclinical setting. The meal was homogenized prior to dosing to enable administration via gavage. The two paediatric suspensions were co-administered within 10 minutes after ingestion of the homogenized meal (200 kcal). As for the reference meal, the volume of infant formula to be administered per dog was based on BW-scaling using the volume administered in the human relative BA study (4,24) and the above mentioned body weights. The resulting 138 mL of infant formula were rounded to improve drug performance feasibility. In the present dog PK study, 150 mL of infant formula (100 kcal) were administered to each dog to induce "infant-formula fed state conditions" within Phase 6. The two paediatric suspensions were dosed halfway through infant formula administration (Table I).

Table I Overview of dosing conditions applied in the canine study.

Phase	Time (hh: mm, a.m.)	Dosing conditions
Intravenous bolus – fasted state		
1	08:30	16.8 mL paracetamol solution (10 mg/mL, 168 mg of paracetamol)
2	08:30	28 mL ibuprofen solution (5 mg/mL, 140 mg ibuprofen).
Per os administration [a] – fasted state conditions		
3	08:30	7 mL Panadol® suspension [b] and 7 mL Nurofen® suspension [c], followed by tube rinse with 10 mL of tap water.
4	08:20 08:30	20 mL 0.1 M HCl/KCl-solution, pH 1.6 (pH-lowering pretreatment) 7 mL Panadol® suspension [b] and 7 mL Nurofen® suspension [c], followed by tube rinse with 10 mL of tap water.
Per os administration [a] – fed state conditions		
5	08:20 08:30	100 g homogenized reference meal (200 kcal) 7 mL Panadol® suspension [b] and 7 mL Nurofen® suspension [c], followed by tube rinse with 10 mL of tap water.
6	08:29 08:30 08:31	75 mL infant formula (50 kcal) 7 mL Panadol® suspension [b] and 7 mL Nurofen® suspension [c] 75 mL infant formula (50 kcal)

[a] dosing performed via gavage
[b] 7 mL of Panadol® suspension (24 mg paracetamol/mL) contain 168 mg paracetamol
[c] 7 mL of Nurofen® suspension (20 mg ibuprofen/mL) contain 140 mg ibuprofen

Each study day was initiated in the morning after a fasting period of at least 16 hours. The dogs were guided to their individual area designated for the duration of drug dosing. A pretreatment blood sample of 2 mL was collected through venipuncture of the jugular vein. Following drug dosing, blood

197 sampling was performed at pre-defined time intervals alternating between the left and right jugular

198 veins. Blood sampling after i.v. drug administration was performed at 10, 20, 30, 45 min and 1, 2, 3, 4,

199 6, 8, and 10 h post-dose. Within study phases employing oral route of drug administration, 2-mL blood

200 samples were collected 20, 40 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 h post-dose. On treatment days

201 requiring drugs administration under fasted state conditions, the dogs received the daily portion of

202 canine food pellets as usual around noon, i.e., 2.5 -3 h after drugs administration. In study phases 5

203 and 6, no further food was provided for the day. After collecting the 4-h-sample, animals could return

204 to their usual daily routine, while blood sampling continued occasionally until the end of the study

205 day.

206

207 *Sample handling and drug analysis*

208 Blood samples were collected into K_2-EDTA Vacutainer™ tubes (Becton, Dickinson U.K. Ltd., Berkshire,

209 UK) and were centrifuged for 10 minutes at $9,000\,g$ at 5° C to obtain plasma (Centrifuge 5430 R,

210 Eppendorf AG, Germany). Plasma was transferred into amber screw cap micro tubes of 2 mL (Thermo

211 Scientific™, Waltham, MA, USA), frozen and stored at -20°C. Analysis of paracetamol and ibuprofen

212 was performed according to the methods described in Statelova et al., 2020a (5) and the

213 Supplementary information, Part A.

214

215 *Data analysis*

216 Concentrations for samples under the low limit of quantification (LLOQ) were considered as zero.

217 Individual and mean plasma concentration-time profiles were evaluated using non-compartmental

218 pharmacokinetic (PK) analysis, i.e., area under the plasma concentration-time curve up to the last

219 sampling point 0-10 h (AUC_{0-10h}), AUC_{0-inf} (extrapolated to infinity based on the first order elimination

220 rate constant estimated from the last three sampling points), maximum concentration (C_{max}), and time

221 to reach C_{max} (T_{max}). Additionally, for the study arms investigating dosing after i.v. administration,

222 additionally, clearance (CL) and volume of distribution at steady state (Vss) were estimated for the

223 individual dog plasma concentration-time profiles and for the mean plasma concentration-time profile

224 (PKPlus™ tool within GastroPlus™ platform, Simulations Plus Inc.). Mean, standard deviation (SD), and

225 % relative standard deviation (RSD) values were calculated from the individual PK parameters.

PBPK modeling

227 *PBPK modeling workflow*

228 PBPK models were developed and refined for paracetamol and ibuprofen separately following an

229 identical modeling workflow using GastroPlus™ (Simulations Plus Inc., V9.7), Figure 1. As a first step,

230 the drug disposition model was developed based on i.v. data in beagles. As a second step, the oral

231 absorption ACAT™ model was refined for the different dosing conditions applied in the preclinical

232 study. In the third step, the different dosing conditions were extrapolated to the target paediatric

233 populations for each model drug based on previously developed and published paediatric PBPK

234 models (24,25). The paediatric simulations following oral dosing were evaluated using paediatric

235 clinical data in infants after oral paracetamol administration (35,36) and in mixed paediatric

236 populations (infants and children or children) after oral ibuprofen administration (37,38).
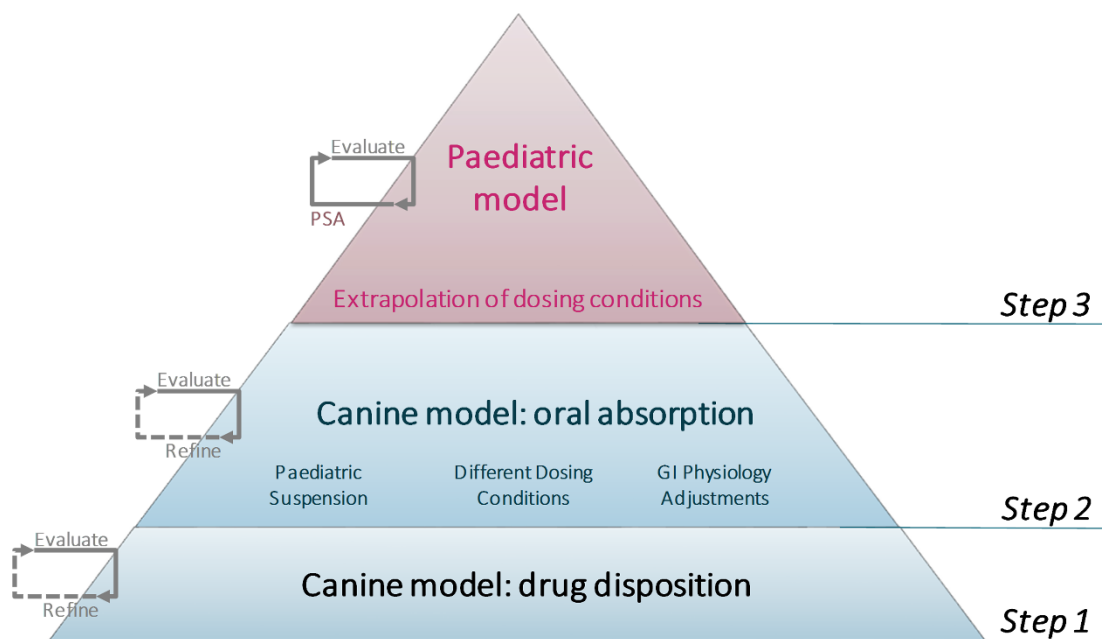
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238 **Figure 1** Modeling workflow for the extrapolation of bioavailability data in beagles to paediatrics.

239

240 *Step 1: Disposition model in beagles*

241 Drug-dependent and PK parameters used for model development of paracetamol and ibuprofen are

242 reported in Table B-SIII and Table B-SIV. One and two compartment models were tested for each drug

243 with the built-in PKPlus™ tool and evaluated using the Akaike Information Criterion (AIC), Bayesian

244 Information Criterion (BIC), and the adjusted coefficient of determination (adjusted R^2). The model

245 with the lower AIC and BIC and higher adjusted coefficient of determination (R^2) was selected to

246 describe disposition, if no substantial difference between the models was found based on the model

247 evaluation criteria, the simpler model was preferred. Fraction of drug unbound and blood to plasma

248 ratio were software-proposed parameters for the respective drug. The performance of the simulations

249 was evaluated against the *in vivo* data observed in dogs and used for model development based on

250 the Average (AFE), Absolute Average fold error (AAFE), and R^2.

251 *Step 2: Oral absorption model in beagles*

252 ACAT™ models were used to describe oral drug absorption processes along the canine GI tract. Default

253 software parameters were used for drug mean precipitation time, particle density, and mean particle

254 radius, while diffusion coefficients were estimated from the in-built ADMET predictor within

255 GastroPlus V9.7 (Simulations Plus Inc.), reported in Table B-SIII and Table B-SIV. Drug dissolution was

256 simulated with the Johnson model. For ibuprofen, the pH versus solubility profile from literature was

257 used to fit the pKa value and solubility factor. The lowest solubility value measured within the pH

258 range 1 - 7.4 was considered as reference drug solubility (the weak acid ibuprofen is assumed to be

259 predominantly present in nonionized form at pH 1, hence, intrinsic solubility) (25,26); the pH values

260 of the buffers used for solubility determination were used as input into the pH-solubility profile. To

261 estimate the bile salt-solubilization ratio, thermodynamic solubility in buffers containing defined bile

262 salt concentrations were integrated as in human adult modeling (25), i.e., Level III fasted state

263 simulated gastric fluid (FaSSGF), Level II fasted state simulated intestinal fluid (FaSSIF), and Level II fed

264 state simulated small intestinal fluid (FeSSIF-V2). The simulations utilized the estimated bile salt-

265 solubilization ratio and accounted for diffusion coefficient adjustment in presence of bile salts.

266 Effective human permeability (Peff) values from literature in adults were converted into Peff in dogs

267 by the in-built permeability converter within GastroPlus™ (23). The default GastroPlus® Opt LogD SA/V

268 6.1 model was utilized to calculate the absorption scale factors (ASF) used for regional

269 absorption/permeability in the virtual canine physiology. This model adjusts the regional Peff based

270 on the compound partitioning at the relevant GI pH, the ionized drug amount in the GI compartment,

271 and drug lipophilicity as well as the anatomical factors of the specific compartment; ASF can have

272 direct impact on the simulated drug permeation and absorption. The default ASF model employed in

273 this study has been applied for modeling and simulation for dog models using the GastroPlus™

274 platform (23,39–42). This method of estimating ASF has been previously linked to overestimation of

275 the colonic drug absorption of lipophilic compounds due to their high logP; this potential artifact might

276 be of lesser concern for the two model drugs paracetamol and ibuprofen, as they are rapidly and

277 completely absorbed in the upper SI within the simulations.

278 Fasted state conditions with and without pH-lowering pretreatment were simulated using the "Beagle

279 fasted physiology" ACAT™ settings, while "Beagle fed physiology" ACAT™ settings were employed to

simulate the fed state conditions induced with the homogenized reference meal or infant formula. Relevant parameters were adjusted according to the *in vivo* observations in dogs, i.e., (i) gastric transit time (GTT) adjustment was needed to describe drug performance under fasted state conditions assuming first order gastric emptying (GE) kinetics or (ii) GTT adjustment and GE kinetics change to zero order GE to capture performance under fed state conditions induced with the homogenized reference meal and infant formula. Within the software platform, GTT for a first order GE process (fasted state conditions) represented the mean GTT described as the GE half-time ($t_{1/2}$) divided by ln2, while the GTT for a zero order GE process (fed state conditions following a homogeneous liquid) described the total GTT.

Step 3: Extrapolation to paediatrics using PBPK modeling

The paediatric models for paracetamol (24) and for ibuprofen **Error! Reference source not found.**(25) were used as basis for the present PBPK modeling exercise. The model parameters utilized for paracetamol and ibuprofen are reported in the Supplementary Information, Table B-SVI and Table B-SVIII, respectively. For simulating fasted state conditions, GTT found to best describe drug/drug product performance were inherited directly from the adjusted Beagle ACAT™ physiology. Extrapolation of drug exposure under fed state conditions was based on the typical meal types for the target age/age range, i.e., infant formula for paediatric subjects younger than 2.5 years or homogenized reference meal utilized for all age groups. To account for the age-dependent caloric content of meals and enable scaling from the beagle study to different paediatric age groups, a calorie-based normalization was performed according to Equation 1:

$$Drug\ GTT_{paediatrics} = \frac{Meal\ caloric\ content\ _{paediatrics} \times Drug\ GTT_{Beagle,meal}}{Meal\ caloric\ content\ _{Beagle}}$$

Equation 1,

where $Drug\ GTT_{paediatrics}\ (h)$: the calculated GTT value to be employed within the paediatric simulation

$Meal\ caloric\ content\ _{paediatrics}\ (kcal)$: the recommended meal calories for the age

$Drug\ GTT_{Beagle,\ meal}\ (h)$: the drug GTT employed in the refined canine oral absorption model for the meal utilized, i.e. homogenized reference meal or infant formula

$Meal\ caloric\ content\ _{adults}\ (kcal)$: the meal calories of the meal employed in beagles, i.e. 200 kcal for the reference meal or 100 kcal for the infant formula.

310   Single simulations were performed for the mean population representatives of the available clinical

311   datasets in paediatric populations.

312   For paracetamol simulations model, a 4-month-old infant (male, 4 kg) receiving a 19.6 mg/kg dose was

313   simulated (herein, population representative), based on the mean demographic parameters from the

314   infant study including five infants (36), i.e., mean age 4 months (range 2-6 months), mean BW 4 kg

315   (2.6-6 kg). Furthermore, based on the older paediatric age group reported by Walson et al. (35), a 10-

316   month-old, 10-kg male infant virtual physiology was generated (population representative),

317   representing the mean demographic parameters reported in the paediatric study (n=12 subjects,

318   mean age 10 months, mean BW 8.6 kg). Simulations for the 10-month-old population representative

319   utilized the reported mean dose administered in this study, 12.14 mg/kg (24). For the age of 4 months

320   and 10 months, meal caloric contents of 140 kcal and 170 kcal were assumed, respectively (1,24); the

321   caloric content of the meals was based on recommended meals/portions for the specific age (1). The

322   fasted state conditions, reference-meal fed state conditions using relevant ACAT™ information from

323   the homogenized reference meal in dogs, and infant-formula fed state conditions using the relevant

324   ACAT™ information from the infant formula-fed state conditions in dogs were simulated for each

325   population representative. The simulations were compared to the mean plasma data observed in the

326   respective populations (35,36). The simulated conditions and adjusted parameters are reported in

327   Table II.

328   For the ibuprofen model, simulations for a mixed age group [infants and children, 0.3 - 12 years (38)]

329   and a children group [2 - 11 years (37)] who received 10 mg/kg ibuprofen were performed using a

330   bracketing approach (25) according to the reported age ranges in each clinical dataset. Single

331   simulations were performed for a 1-year-old (male, 10 kg), 6-year-old (male, 23 kg), and 12-year-old

332   (male, 48.5 kg); the resulting simulated mean profiles were compared to the mean profile reported in

333   the clinical dataset (38). Single simulations were performed for a 2-year-old (male, 13 kg), a 6-year-

334   old (male, 23 kg), and a 11-year-old (43.6 kg); the resulting mean simulated profiles were compared

335   to the mean reported plasma concentrations in the clinical dataset (37). Fasted state and fed state

336   ibuprofen performance adjusted according to drug performance following the homogenized

337   reference meal in beagles were simulated for all paediatric age representatives, while infant-formula

338   fed state conditions were considered for the 1- and 2-year-olds. The age-adjusted caloric contents of

339   the meals were 170 kcal, 200 kcal, 260 kcal, and 340 kcal for a 1-year-old, 2-year-old, 6-year-old, and

340   11/12-year-old, respectively. Mean simulated profiles were calculated for the (i) fasted state

341   conditions, (ii) reference-meal fed state performance, and (iii) mixed fed state conditions using infant-

342   formula-fed state performance for subjects < 2.5 years and reference-meal fed state conditions for

343   the children population representatives. The simulated conditions and simulation parameters are

344   reported in Table III.

345

346   *Model performance evaluation*

347   To compare drug exposure, the Fold Difference of simulated vs. observed (*FDpred/obs*) parameters

348   were employed for AUC, C_{max}, and T_{max} values. Individual and/or mean simulated plasma

349   concentration-time profiles were compared using the average fold error (*AFE*) and the absolute

350   average fold error *(AAFE)* according to Eq. 2 and Eq. 3, respectively.

351
$$AFE = 10^{\left(\frac{1}{n}\Sigma \log \left(\frac{PREDi}{OBSi}\right)\right)}$$
Equation 2

352
$$AAFE = 10^{\left(\frac{1}{n}\Sigma \left|\log \left(\frac{PREDi}{OBSi}\right)\right|\right)}$$
Equation 3

353   where n denotes the number of observed sampling points, PREDi and OBSi denote the simulated and

354   observed plasma concentration, respectively, at the sampling time point i.

355   To evaluate simulations in a mixed population or children populations following oral dosing of

356   ibuprofen, the mean simulated profiles and PK parameters were calculated, i.e. *FD_pred/obs*, *AFE*, and

357   *AAFE*. *AFE* values indicated the trend for underestimation (*AFE* < 1) or overestimation (*AFE* > 1) of

358   the observed plasma concentrations, while *AAFE* values close to unity indicated precision of the

359   simulations. Simulations were considered adequate when *FD_pred/obs* and *AFE* values were within two-

360   fold and *AAFE* values were below two (43), while simulations were considered successful when

361   *FD_pred/obs* and *AFE* fell between 0.66 - 1.5 and *AAFE* below 1.5 (44).

362

363   *Parameter sensitivity analysis*

364   One-factor-at-a-time sensitivity analysis was performed to explore the impact of different parameters

365   within the canine model. Drug/drug product-related parameters explored were drug reference

366   solubility, particle radius, diffusion coefficient, precipitation time, and permeability. Beagle

367   physiology-relevant parameters investigated included fluid volumes and intraluminal pH in the

368   different GI compartments, GTT, small intestinal transit time, intestinal radius, length, and surface

369   area. Lastly, clinical study uncertainties were tested regarding the volume of fluid co-administered

370   with the formulation. The parameters investigated for paracetamol are reported in Table B-SV and for

371   ibuprofen in Table B-SVI. The PSA of the paediatric models has previously been performed and

372   discussed for paracetamol (24) and ibuprofen (25).

Results

Paracetamol

Disposition model in beagles

A two-compartment model was found to adequately describe paracetamol performance following i.v. bolus administration at a dose of 168 mg per dog. The results of the compartmental PK analysis for the i.v. administration performed on individual basis and for the mean profile are reported in Table B-SI. Simulations performed using the developed disposition model, adequately simulated the mean observed profile, as indicated by AFE 1.060, AAFE 1.104, and R^2 0.996 and shown in Figure B-S1. For paracetamol the mean clearance normalized for body weight observed 1.29 ± 0.10 L/h/kg was higher than in human adult subjects (0.23 - 0.335 L/h/kg), (24) or paediatrics (0.55 - 0.29 L/h/kg) (45). The clearance in dogs observed in the present investigation was in line with previous investigations in beagles reported in the literature (46,47). Furthermore, the estimated fu,p and B/P-ratios for dog were similar to the ones in human, i.e., 0.82 and 1.09, respectively (Table B-SVII).

Oral absorption model in beagles

Oral drug absorption was simulated using the default fasted state conditions for the ACAT™ Beagle physiology. GTT values were adjusted to match the observed paracetamol absorption when drug was dosed following a pH-lowering pretreatment or when no pretreatment was applied. First order GE kinetics and a GTT of 0.5 h were found to adequately describe both dosing conditions (Figure 2A and FigureB-S2), with AAFE 1.349 and 1.195 for the fasted state conditions with and without HCl/KCl pretreatment, respectively. To simulate the two fed state conditions, the ACAT™ physiology was changed to "Beagle fed physiology", zero order GE and GTT 1.5 h were employed to match the observed paracetamol performance, Figure 2B and 3C.
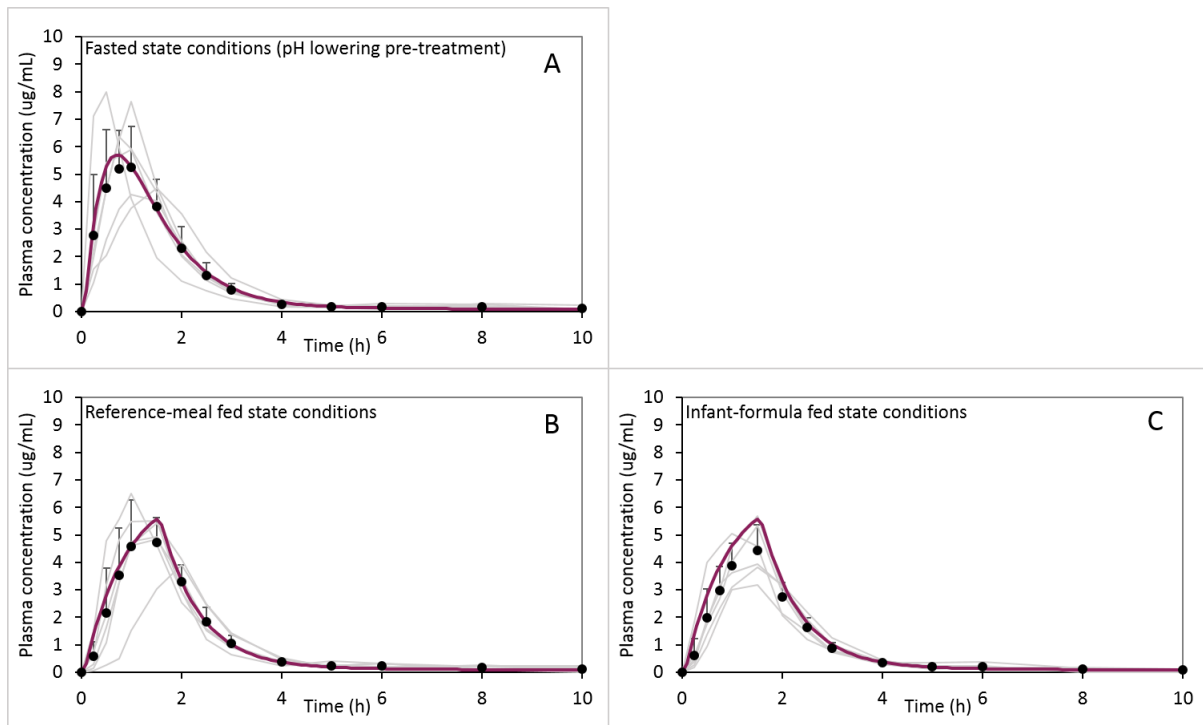
Figure 2 Observed and simulated paracetamol plasma concentration-time profiles following oral administration of 168 mg paracetamol dose (7 mL Panadol® suspension) on a crossover basis to 6 beagles applying pH-lowering pretreatment under fasted state conditions (A), under reference-meal fed state conditions (B), and infant-formula fed state conditions (C). Purple bold lines represent simulated profiles, grey lines the individual observed profiles; symbols and error bars denote observed mean concentrations and standard deviations.

Extrapolation to infants using PBPK modeling

A published paracetamol PBPK model was utilized for simulations of paediatric subjects; within the published PBPK model a full-body PBPK model was utilized to scale age-dependent drug disposition and enzyme-based clearance was employed to describe age-dependent clearance (24). The usefulness of the paracetamol bioavailability data obtained under different dosing conditions in beagles was evaluated using two datasets in infants who were administered paracetamol liquid formulations (35,36). To allow for the extrapolation of the fed state dosing conditions investigated in beagles to different paediatric ages, stomach transit times observed in dogs for the relevant meals were scaled on caloric basis to paediatric population representatives employing age-relevant caloric quantities for the population representatives (Eq. 1), (24).

417 **Table II** Adjusted paracetamol gastric transit time (GTT) values employing zero order gastric emptying
418 kinetics in beagles and in infants based on meal caloric content.

Meal	Beagle dog		Infants			
	2-year-old, 10 kg body weight [a]		4-month-old, 4 kg body weight [b]		10-month-old, 9 kg body weight [c]	
	Caloric content (kcal)	GTT (h)	Caloric content (kcal)	GTT (h)	Caloric content (kcal)	GTT (h)
Reference meal	200	1.5	140	1.05	170	1.28
Infant formula	100	1.5	140	2.1	170	2.55

419 [a] mean age and body weight of male beagles (n=6)
420 [b] mean infant population representative (36)
421 [c] mean infant population representative (35)

422

423 The use of fasted state GTT based on observations in beagles led to successful simulation of the 4-
424 month-old population representative compared to observed mean data of five 2-6 month-old infants
425 (36) and of the 10-month-old population representative compared to mean data observed in 12 3-36-
426 month-old infants/young children (35) (Figure 3A and Figure 3B). These observations were reflected
427 by the model evaluation metrics, Figure 4. The extrapolation based on beagle data acquired following
428 the homogenized reference meal, resulted in a reasonable simulation of a 4-month-old population
429 representative compared to the mean observed data in Hopkins et al. (AAFE 1.178), but led to a slight
430 underestimation of mean plasma concentrations at early times in the 10-month-old population
431 representative compared to the mean observed profile in the study by Walson et al. (AAFE 1.327)
432 (Figure 3C and Figure 3D), respectively. Simulations based on paracetamol performance in beagles
433 under infant-formula fed state conditions resulted in delayed paracetamol absorption, compared with
434 the *in vivo* observations in infants (Figure 3E and Figure 3F). Although AAFE values and FD of AUC and
435 C_{max} were within two-fold from the mean observations Figure 4C, T_{max} fold differences indicate the
436 estimation mismatch, Figure 4A.

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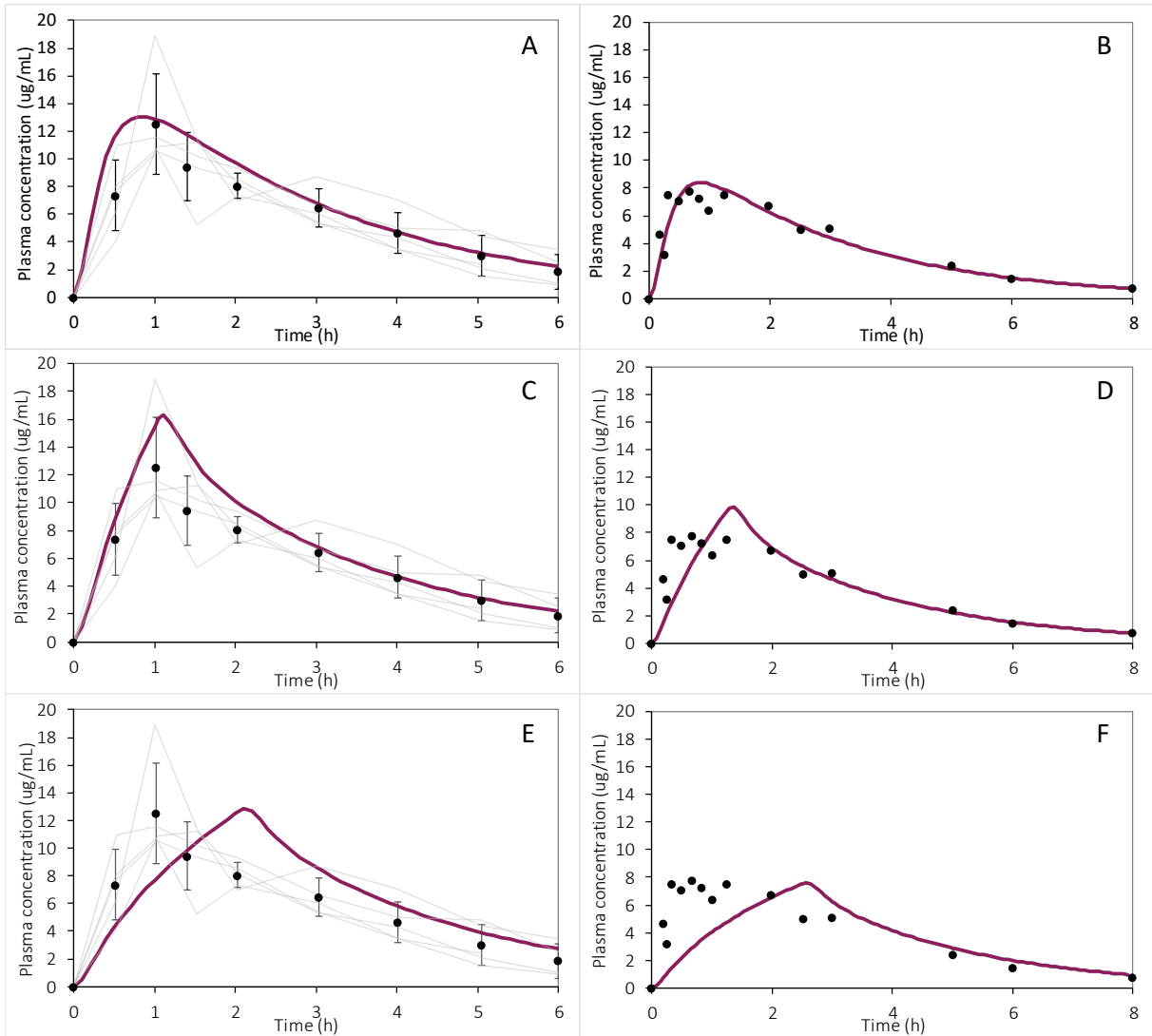
Figure 3 Simulated paracetamol plasma concentration-time profiles (purple lines) based on formulation performance in beagles for a 4-month-old infant following the administration of 19.6 mg/kg dose (A, C, E) and in a 10-month-old infant following a dose of 12.14 mg/kg (B, D, F). Fasted state conditions (A, B), reference-meal fed state conditions (C, D), and infant-formula fed state conditions (E, F). Grey lines denote individual observed plasma concentration-time profiles, symbols and error bars denote mean plasma concentrations and standard deviation in 4-month-old infants (Hopkins *et al*., 1990 (36); A, C, E); symbols denote mean plasma concentrations in 10-month-old infants (Walson *et al*., 2013 (35); B, D, F).
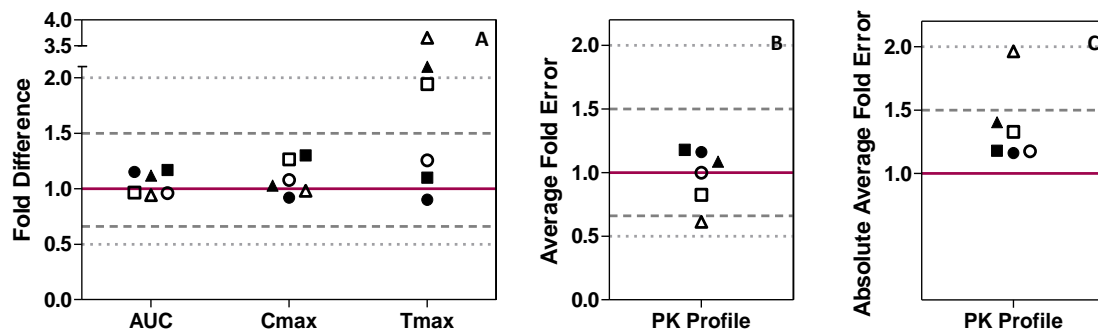
449
450 **Figure 4** Fold Difference (simulated/observed) for AUC_{0-8h}, C_{max}, and T_{max} (A), Average Fold Error (B), and
451 Absolute Average Fold Error (C) for the mean profile from the study dataset from infants 2-6 months with
452 mean age 4 months (closed symbols, Hopkins *et al*. 1990 (36)) and infants 3-36 months with mean age
453 10 months (open symbols, Walson *et al*. 2013 (35)) under fasted state conditions (circles), reference-meal
454 fed state conditions (squares), and infant-formula fed state conditions (triangles). The solid line represents
455 the line of unity, grey dashed lines 0.66-1.5 range indicating successful simulations, and grey dotted lines
456 the 0.5-2 range indicating adequate simulation.

457

Parameter sensitivity analysis

459 Parameters related to drug/drug product properties, physiology, and dosing conditions were
460 investigated for the paracetamol dog PBPK model (Table B-SV). Paracetamol absorption rates were
461 decreased by prolonged gastric transit times, indicated by the pronounced C_{max} decrease and T_{max}
462 prolongation under all dosing conditions (Figure B-S5 and Figure B-S7). Increase in liver first pass
463 metabolism resulted in lower total and peak exposure, as presented in Figure B-S6 and Figure B-S8. In
464 contrast to the human PBPK models for adults and infants (24), the canine PBPK model was not greatly
465 affected by the effective permeability value employed, with lower absorption observed firstly at 10-
466 fold lower permeability, i.e., canine Peff 1.0 cm/s $\times10^{-4}$. The canine PBPK model was overall robust
467 and changes in the rest of the parameters tested (Table B-SV) exhibited no impact on exposure.

Ibuprofen

Disposition model in beagles

A one-compartment model was found to adequately describe performance of ibuprofen following i.v. bolus administration at a dose of 140 mg per dog. As for paracetamol, compartmental PK analysis of i.v. ibuprofen performance was performed on individual basis and for the mean profile, as shown in Table B-SII. The developed disposition model was applied and adequately simulated the mean observed profile in beagles, as indicated by AFE 1.060, AAFE 1.104, and R^2 0.983 and shown in Figure B-S3. For the weak acid ibuprofen, the mean clearance normalized for BW observed 0.048 ± 0.005 L/h/kg was within the range of clearance reported in human adult (0.036 - 0.054 L/h/kg) and paediatrics subjects (0.060 - 0.083 L/h/kg) (25). Furthermore, the estimated $f_{u,p}$ for dog was in line with the high binding to plasma proteins in human plasma (human $f_{u,p}$ was 0.0155), while the estimated B/P-ratio for dog (Table B-SIV) was two-fold lower than the human B/P-ratio, i.e., 1.55.

Oral absorption model in beagles

As for paracetamol, the fasted ACAT™ model settings were adjusted to match ibuprofen performance under fasted state conditions with or without pH-lowering pretreatment. Both performances were successfully simulated using the same GTT of 0.25 h for each dosing condition, i.e., AAFE 1.172 and 1.099 for fasted state simulations with and without the pretreatment, respectively, Figure 5A and Figure B-S4. To simulate both fed state conditions, the ACAT™ physiology was changed to "Beagle fed physiology" and GTT was adjusted to 1.1 h along with employing zero order GE kinetics, Figure 5B and Figure 5C. Simulations of fed conditions following the administration of 200 kcal homogenized reference meal and 100 kcal infant formula, were adequately described by the same ACAT™ model settings with AAFE 1.144 and 1.088, respectively.
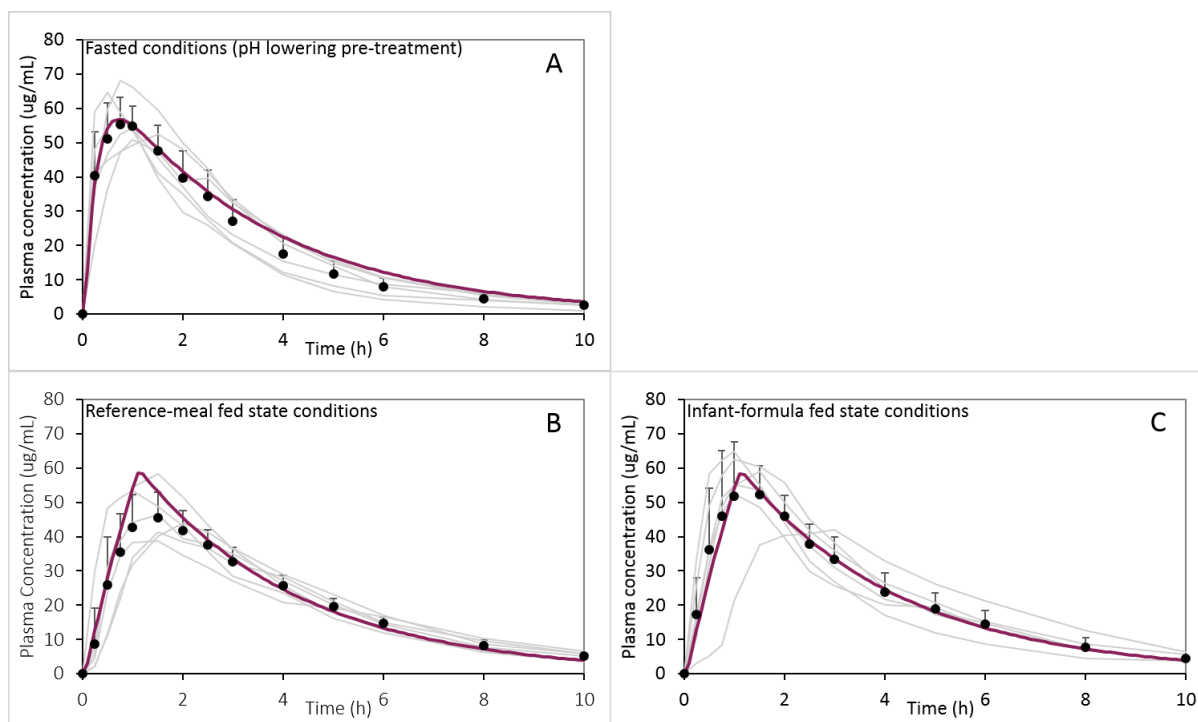
Figure 5 Observed and simulated ibuprofen plasma concentration-time profiles following oral administration of 140 mg ibuprofen dose (7 mL Nurofen® suspension) on a crossover basis to 6 beagles applying pH-lowering pretreatment under fasted state conditions (A), under reference-meal fed-state conditions (B), and under infant-formula fed state conditions (C). Purple bold lines represent simulated profiles, grey lines the individual observed profiles; symbols and error bars denote observed mean concentrations and standard deviations.

Extrapolation to paediatrics using PBPK modeling

The usefulness of the bioavailability data following ibuprofen administration under different dosing conditions in beagles was evaluated using the two most relevant, published datasets in paediatric populations, i.e., a mixed infant/children population 0.3 - 12 years (38) and a children population 2 - 11 years (37), who received ibuprofen liquid formulations (37,38). To simulate fasted state formulation performance, the adjusted GTT in fasted beagles was directly inherited into the paediatric fasted state simulations. The extrapolation of the fed dosing conditions investigated in beagles to different paediatric ages was performed as for paracetamol, whereby GTT observed in dogs for the relevant meals were scaled on caloric basis to the paediatric population representative employing age-relevant caloric quantities for the population representatives (Equation 1), Table III. Mean simulated profiles in the paediatric groups were calculated based on the individual simulations of the population representatives according to the study age ranges and were compared to the mean clinical data. Mean simulated profiles were calculated for three different dosing scenarios: (i) fasted state conditions, (ii) fed state conditions using GTT scaling for all population representatives based on the homogenized reference meal, and (iii) age-dependent fed state conditions employing infant formula for population representatives < 2.5 h and homogenized reference meal for population representatives > 2.5 yr.

22

517 **Table III** Adjusted ibuprofen gastric transit time (GTT) values employing zero order emptying kinetics
518 for beagles and paediatric population representatives based on meal caloric content.

Meal	Beagle dog		Infant		Infant/Child		Child			
	2-year-old, 10 kg body weight [a]		12-month-old, 9.5 kg body weight [b]		2-year-old, 12.9 kg body weight [c]		6-year-old, 23 kg body weight [b, c]		11 [c]-12 [b] year-old, 43.6/48.6 kg body weight [d]	
	Caloric content (kcal)	GTT (h)	Caloric content (kcal)	GTT (h)	Caloric content (kcal)	GTT (h)	Caloric content (kcal)	GTT (h)	Caloric content (kcal)	GTT (h)
Reference meal	200	1.1	170	0.94	200	1.1	260	1.43	340	1.87
Infant formula	100	1.1	170	1.87	200	2.2	-	-	-	-

519 [a] mean age and body weight of the male beagles (n=6)
520 [b] population representative (38)
521 [c] population representative (37)
522 [d] the recommended average daily energy needs for the 11- and 12-year-old population
523 representatives were the same, resulting in the same caloric content per meal and adjusted GTT value
524 for these population representatives
525

526 The resulting individual and mean simulated plasma concentration-time profiles based on the two
527 study datasets are presented in Figure 6 and the respective model evaluation criteria are presented
528 in Figure 7. Using the adjusted GTT from the canine absorption model (0.25 h) to simulate fasted state
529 conditions in the target paediatric populations, reasonable simulations were achieved for the mean
530 plasma concentration data for the mixed paediatric population Figure 6A, AAFE 1.135 (38). On the
531 other hand, simulations for the children age group (37) appeared to overestimate mean observed
532 plasma concentrations at early times and underestimate T_{max} (Figure 6B and Figure 7A), leading to an
533 overall simulation inaccuracy (AAFE 1.350). Simulations using the ibuprofen reference-meal fed state
534 performance in beagles and following zero order GE resulted in an overall ibuprofen absorption delay
535 unlike *in vivo* mean observations in the mixed population, Figure 6C. For the children population, the
536 application of adjusted reference-meal fed state conditions appeared to improve model estimations
537 (Figure 6D, AAFE 1.203) compared to fasted state calculations (Figure 7A and C). Simulations with
538 adjusted infant-formula fed state conditions based on ibuprofen performance in beagles for
539 population representatives younger than 2.5 years in the paediatric population are shown in
540 Figure 6E and F. Resulting mean simulations for the mixed population underestimated the observed
541 mean early plasma levels and could not capture the overall ibuprofen performance *in vivo* with
542 AAFE 1.504, (Figure 7A and C). Consideration of the infant formula-fed state conditions for the children
543 dataset (37), as for mixed populations, resulted in delayed estimated absorption (Figure 6F), unlike
544 observation from the mean profile in the clinical dataset, thereby leading to some simulation
545 inaccuracies (Figure 7A and C). Lastly, despite the trends observed for the simulations vs. the mean

546 observed profiles, it is important to note that simulations for all three dosing conditions fell within the
547 observed variability of the study in the majority of cases (Figure 6).

548

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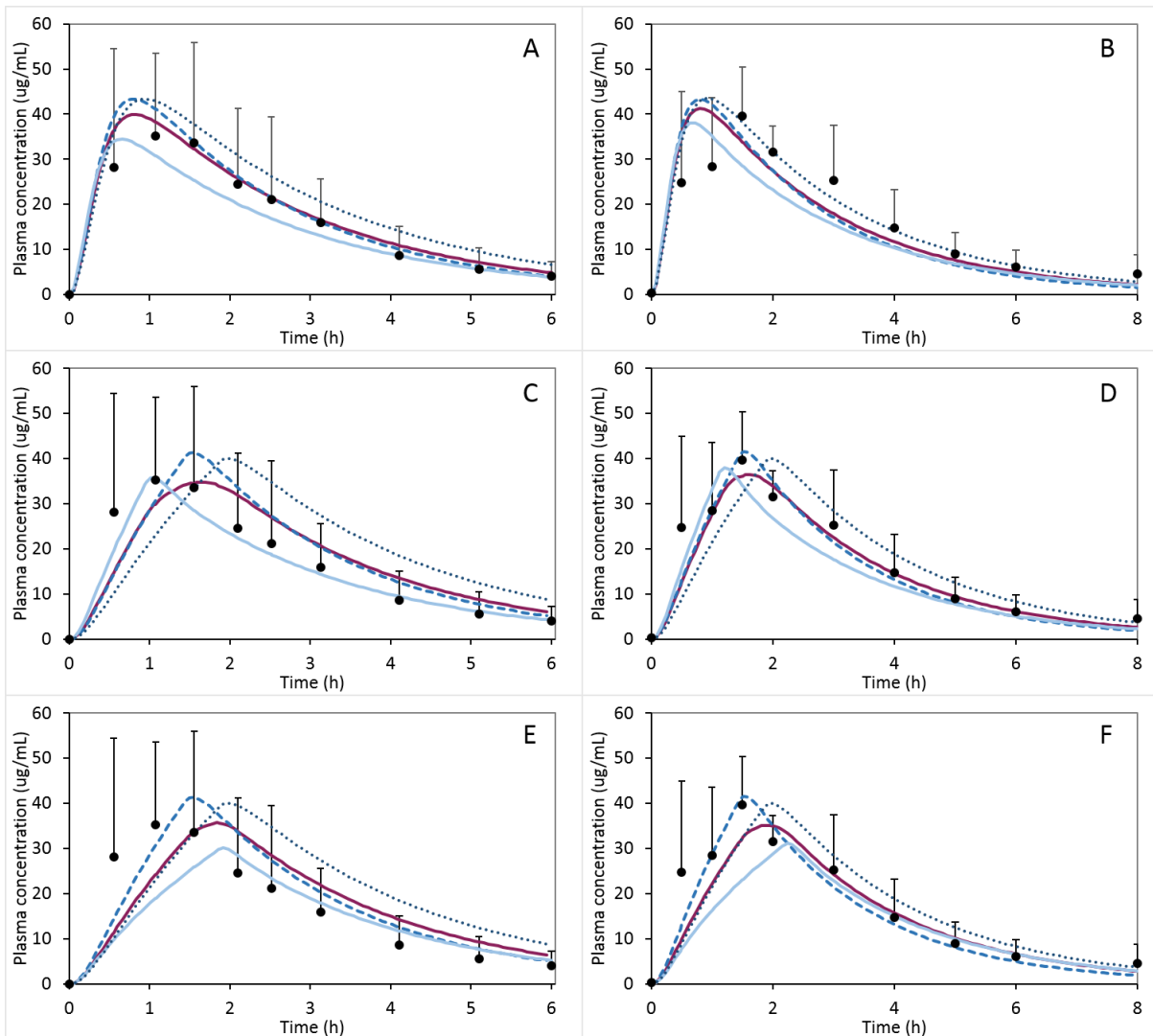
550 **Figure 6** Simulated ibuprofen plasma concentration-time profiles (lines) following oral administration of
551 ibuprofen under different dosing conditions based on drug formulation performance in beagles. Thin light
552 blue continuous line (–) 1- year-old population representative (A, C, E) or 2-year-old child (B, D, F), dashed
553 line (- -) 6-year-old child, dotted line (⋯) 12-year-old child (A, C, E) or 11-year-old child (B, D, F), bold purple
554 continuous lines (–) mean profiles for the three age groups. Fasted state conditions (A, B); reference-meal
555 fed state conditions (C, D); reference-meal fed state conditions (> 2.5 years) and infant-formula fed state
556 conditions (< 2.5 years) (E, F). Symbols and error bars denote mean observed plasma levels and standard
557 deviations (Brown *et al.*, 1992 (38); A, C, E) and (Walson *et al.*, 1989 (37); B, D, F).
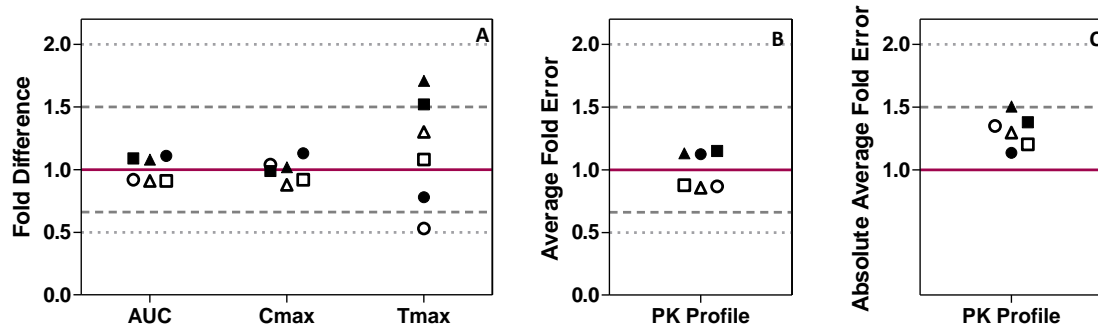
Figure 7 Fold Difference (simulated/observed) for AUC_{0-8h}, C_{max}, and T_{max} (A), Average Fold Error (B), and Absolute Average Fold Error (C) for the mean profile from the mixed population group (closed symbols, Brown *et al*. 1992 (38)) and children population (open symbols, Walson *et al*. 1989 (37)) under fasted state conditions (circles), reference-meal fed state conditions (squares), and reference-meal fed state conditions (> 2.5 years) and infant-formula fed state conditions (< 2.5 years) (triangles). The solid line represents the line of unity, grey dashed lines 0.66-1.5 range indicating successful simulations, and grey dotted lines the 0.5-2 range indicating adequate simulations.

Parameter sensitivity analysis

Parameters related to drug/drug formulation properties, physiology, and dosing conditions were investigated for the ibuprofen dog PBPK model (Table B-SVI). Greatest impact on drug absorption was observed regarding gastric transit times prolongation resulting in lower peak exposure and longer times to reach C_{max}, Figure B-S9 and Figure B-S11. Increase in liver first pass metabolism resulted in lower plasma levels and total exposure and peak exposure (Figure B-S10 and Figure B-S12). As for paracetamol, only after 10-fold permeability decrease ibuprofen drug absorption was retarded. Additionally, pH lowering in the duodenum resulted in slightly prolonged T_{max} and lowered C_{max} values, while gastric and jejunal pH had little to no impact on ibuprofen performance in beagles.

Comparison of the usefulness of canine and human adult bioavailability data of paracetamol and ibuprofen for exposure extrapolation to paediatrics

Using GastroPlus V9.7, the beagle based PBPK model led to successful simulations of paracetamol performance in infants under fasted state conditions; likewise, extrapolation of fasted state data in human adults resulted in successful simulations of paracetamol performance in infants (Figure 8A and B). It might be worth noting that under fasted state conditions, a slightly greater absorption delay was estimated based on human adult data (Figure 8A and B). Simulations based on human adult data suggested that infant-formula fed state conditions were suitable to simulate mean paracetamol exposure in infants, while the same dosing conditions applied in beagles led to less

adequate simulations, (Figure 8E and F and Figure B-S13). Exposure extrapolation based on reference-meal fed state conditions (solid-liquid) in adults was less useful for capturing performance in infants based on the mean observed profiles, as indicated by the difference in simulated vs observed T_{max}. In contrast, when extrapolations were based on the beagle model, the reference-meal fed state conditions appeared to be closer to the average PK profiles and the observed T_{max} (Figure 8C and D and Figure B-S13). Furthermore, GE order appeared different in the canine and human adult simulations of the reference-meal fed state conditions, zero vs. first order GE process, respectively (Figure 8C and D).
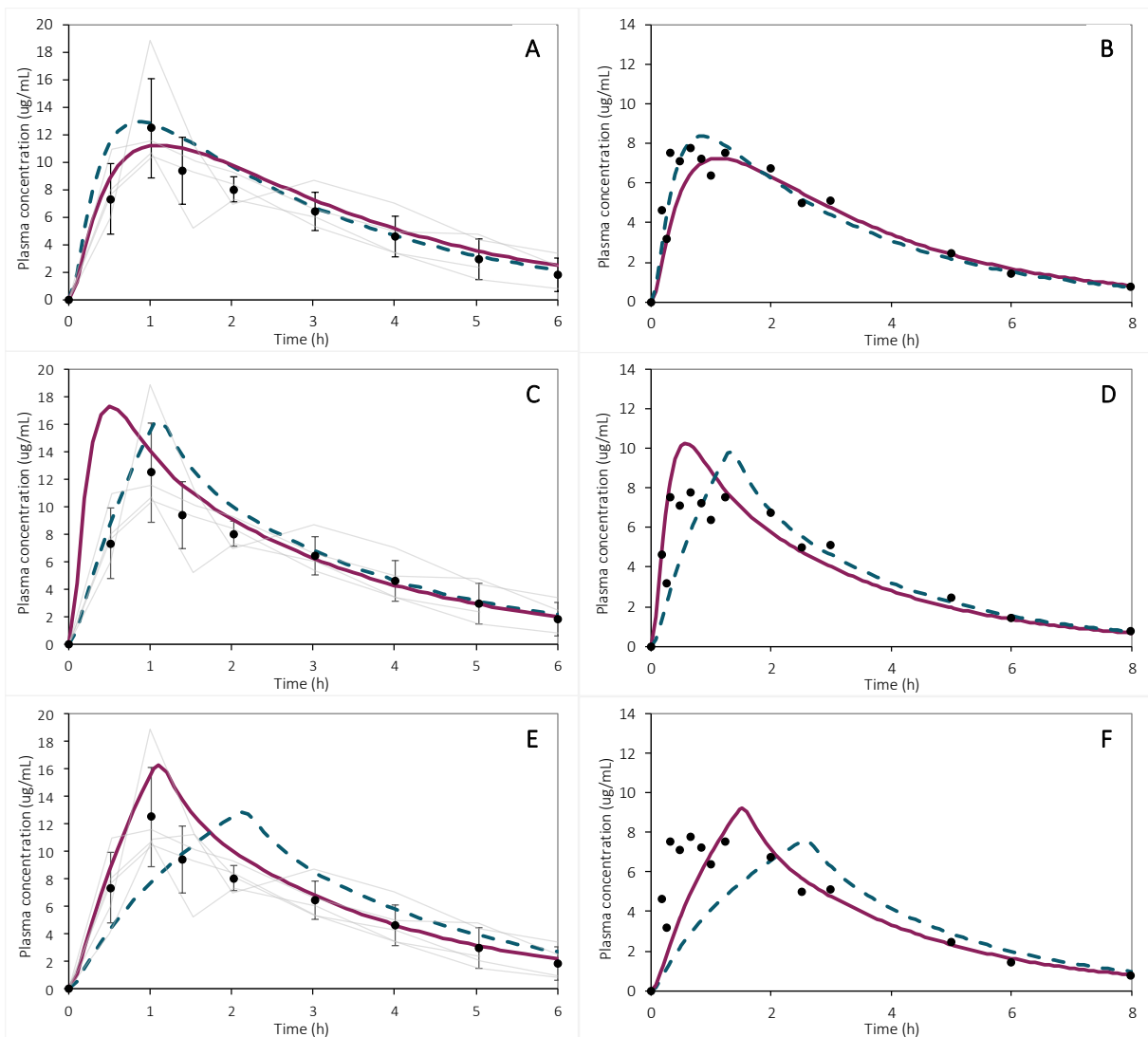
Figure 8 Comparison of simulated plasma paracetamol levels in a population representative using PBPK models based on human adult bioavailability data (purple continuous lines, (24)) vs. Beagle bioavailability data (blue dashed lines, present study) under (A, B) fasted state conditions, (C, D) reference-meal fed state conditions, and (E, F) infant-formula fed state conditions. Left panel (Hopkins et al. 1990 (36); A, C, E) with a population representative of 4 months (study age range 2-6 months), individual observed plasma concentration-time profiles depicted as grey lines, observed mean concentrations and standard deviations depicted with black symbols and error bars; right panel (Walson et al., 2013 (35); B, D, E) with a population

602 representative of 10 months (study age range 3-36 months), observed mean concentrations depicted with
603 black symbols.

604 Ibuprofen simulations based on product performance under fasted state conditions in adults led to
605 successful simulations for the paediatric studies (37,38); adequate simulations were achieved using
606 the beagle-based model under fasted state conditions (Figure 9A and B and Figure B-S14). As for
607 paracetamol, simulated ibuprofen GE (MGTT) under fasted state conditions appeared somewhat
608 faster in canine data as opposed to human adults (Figure 9A and B).

609
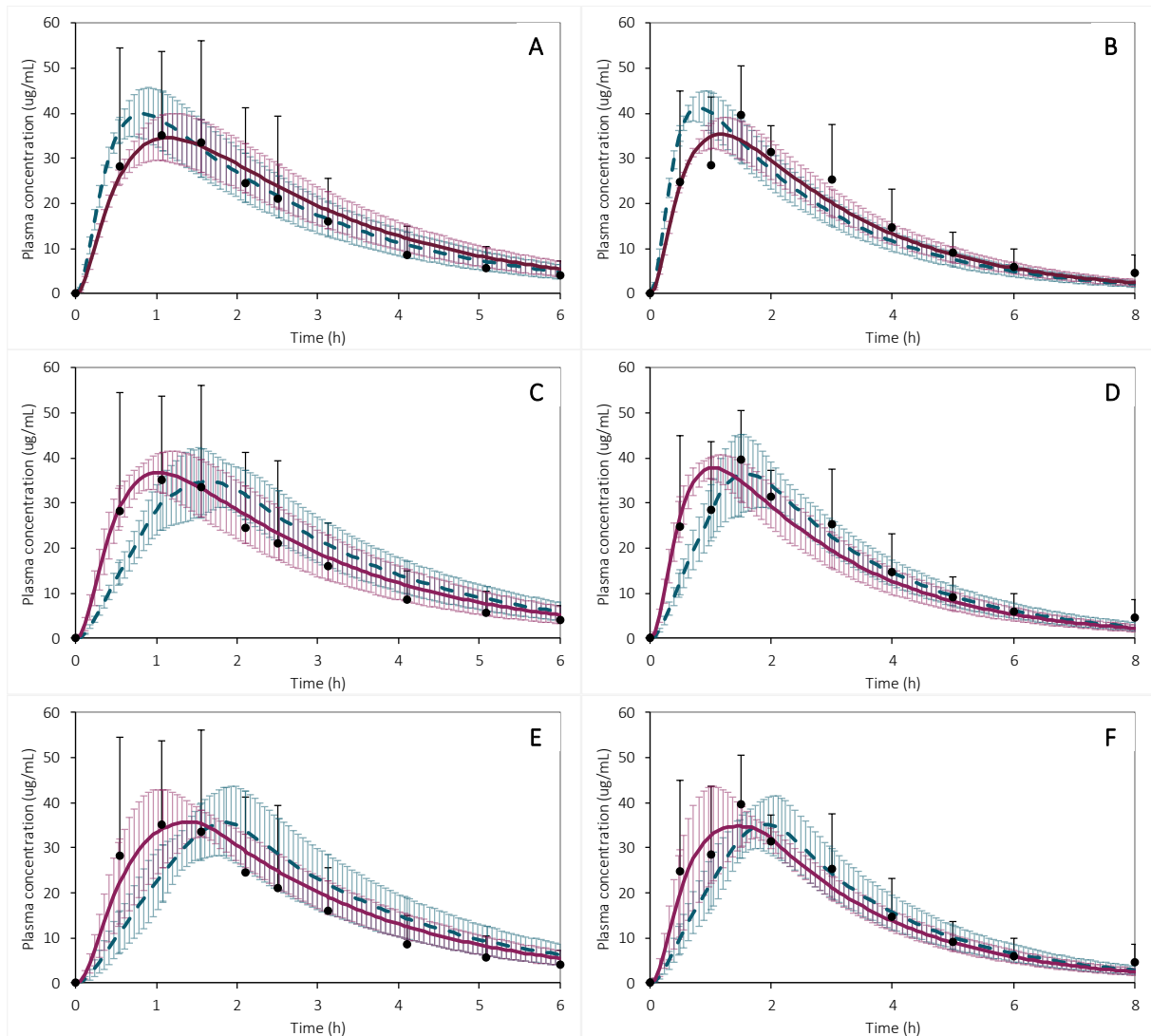
610 **Figure 9** Comparison of mean simulated plasma ibuprofen levels in mixed paediatric populations using
611 PBPK models based on human adult bioavailability data (purple continuous lines and error bars, (25)) vs.
612 beagle bioavailability data (blue dashed lines and blue error bars, present study) under (A, B) fasted state
613 conditions, (C, D) reference-meal fed state conditions, and (E, F) reference-meal fed state conditions for
614 children and infant formula-fed state conditions for subjects < 2.5 years. Left panel (Brown et al., 1992 (38);
615 A, C, E) with study age range 0.3-12 years and right panel (Walson et al., 1989 (37); B, D, F) with study age
616 range 2-12 years, observed mean concentrations and standard deviations depicted with black symbols and
617 error bars.

618 Extrapolations based on the human adult model under reference-meal fed state conditions (solid-
619 liquid) and infant-formula fed state conditions successfully matched data observed in paediatric
620 groups (Figure 9C-F and Figure B-S14). In contrast, exposure simulations of the canine model based
621 on the reference-meal fed state conditions, but not infant-formula fed state conditions, were able to
622 capture the mean *in vivo* performance observed in paediatric patients (Figure 9C-F and Figure B-S14).
623 As for paracetamol, under the reference-meal fed state conditions, differences in ibuprofen GE
624 kinetics could be observed in human adults versus canine data, i.e., first versus zero order apparent
625 GE processes. Overall, as the simulations under different dosing conditions appeared to be matching
626 observed *in vivo* concentrations and to be within the study variability, therefore, comparisons
627 regarding the usefulness of human vs. dog as basis for oral model translation to paediatric populations
628 should be interpreted as indication of a trend, rather than generalizing finding.

629

Discussion

631 In the present study we collected paracetamol and ibuprofen plasma data in beagles, after
632 administering paediatric formulations under various dosing conditions, in order to evaluate their
633 usefulness in informing the plasma profile in simulations of systemic exposure in paediatric
634 populations. Simulations outcomes were then compared to the outcomes of simulations based on
635 published plasma data collected after administering the same formulations under various dosing
636 conditions to human adults.

637 .

638 Within the preclinical investigation under fasted state conditions, paracetamol and ibuprofen
639 exhibited rapid absorption (Figure 2 and Figure 5). In the paracetamol canine PBPK model, the
640 adjusted GTT value fell within physiologically observed GE half-life and Tmax reports (47–50). In this
641 context, it should be noted that the adjusted GTT value represents the formulation's gastric transit
642 time, rather than a general GE time for a clear fluid. Under fasted conditions, the application of HCl/KCl
643 pretreatment appeared not to affect paracetamol performance or GE substantially in the present
644 study; no effects were expected based on paracetamol high solubility and the drugs ionization
645 properties. Although ibuprofen is a weak acid and elevated pH levels in the fasted canine stomach
646 (33,34) could potentially impact drug performance, no considerable changes were observed with
647 respect to the ACAT™ physiology parameters employed to adequately describe drug behavior *in vivo*.
648 In the present investigation, the utilization of the HCl/KCl pre-treatment indicated no noticeable
649 impact on the two highly permeable drugs. Impact of acidifying pretreatment is expected to be more

650 pronounced for weakly basic lipophilic compounds, which rely on dissolution in the acidic stomach

651 environment to allow for supersaturation and absorption upon gastro-intestinal transfer or for

652 modified-release formulations utilizing functional pH-sensitive polymers (18,33,51).

653

654 Interestingly, in the present study, despite the two-fold difference in caloric content between the

655 homogenized reference meal (200 kcal) and the infant formula (100 kcal), the two meals resulted in

656 similar paracetamol/ibuprofen absorption delay and could be described by the same GE rate order

657 and GTT for the two meals. Gastric emptying of drug product and chyme under post-prandial

658 conditions is a complex process that is governed by multiple factors such as meal energy content,

659 volume, solid vs. liquid texture, viscosity, density, particle size of gastric contents, and osmolarity, to

660 name some (e.g., 52). Multiple investigations have showcased that increase of meal amounts and

661 calorific content results in a delay of gastric emptying in comparison to meals with fewer calories in

662 dogs (53–56). In comparison to humans where osmoreceptors providing the feedback leading to

663 gastric emptying delay are located in the duodenum, in dogs these receptors were found in the

664 jejunum and not in the first duodenal part (57,58). In the present beagle study, additional factors

665 might contribute to the overall GE of the two meals, such factors could be meal viscosity or volume

666 (59–63) , while osmolarity appeared to play a minor role in comparison to the presence of nutrients.

667 It should be noted that volume effects on GE were more evident at volumes higher than the ones

668 utilized in the present study, i.e., 150-1200 mL (63).

669 In humans, caloric dependence of gastric emptying rate has been demonstrated in several

670 investigations (64–71), with differences in volumes appearing to be a minor contributor (72).

671 Additionally, interplay between volume and calorific density has been proposed to describe the fed

672 state GE half-life in adults, for volumes > 100 mL (73). In addition to the linear relationship assumed

673 in the present study to enable caloric-based translation, other scaling approaches for estimating meal-

674 dependent GE have been reported for adults (74), advanced *in vitro* models (52), meal types (75), and

675 breastfeeding in newborns/infants (76); the predictive capabilities of these approaches for different

676 meal types and populations are yet to be confirmed with further clinical evidence. Meal compositions,

677 calories, textures, and volumes evolve with age progression of the specific paediatric populations, e.g.,

678 liquid milk-based feeds for infants, semi-solid meals for infants and toddlers, solid small meals for pre-

679 school children and school children, and increased adult-like portions for adolescents (1); based on

680 this, greatest potential differences between paediatric populations and adults would be expected for

681 the youngest – newborns and infants. Although caloric regulation of GE has been established in adults,

682 the postprandial GE behavior and main contributors in paediatric subjects are less studied. An

683 investigation in 10 premature infants who received different amounts of calories at the same formula

684 volume (22 mL/kg BW) revealed that significant inhibition of GE was related to increasing caloric

685 density over the entire test duration, leading to the conclusion that GE regulation by caloric density in

686 premature infants was qualitatively similar to that in adults (77). Furthermore, in pre-term newborns,

687 duodenal activity following feeding increased for newborns receiving infant formula infusion, while

688 duodenal activity for diluted infant formula resembled duodenal activity following water (78).

689 It should be considered that although the reference meal utilized in the beagle study was

690 homogenized, while the reference meal in the clinical study was a solid-liquid meal chewed by the

691 volunteers (5), the different texture might not have been a key contributor. In a recent study

692 conducted in human healthy adults, the reference meal was homogenized to facilitate meal

693 administration via nasogastric tube and no pronounced differences were reported due to meal

694 homogenization in comparison to a chewed meal (79).

695 Based on the present *in vivo-in silico* investigation of paracetamol suspension performance in beagles

696 and subsequent translation to infants, fasted state dosing conditions in beagles appeared useful to

697 inform modeling for infant formulations. Beagle data collected after the homogenized reference meal

698 were also useful for simulating the mean plasma concentrations of the younger population

699 (2-6 months (36)), but not for simulating the mean plasma concentrations of the older population

700 (3-36 months (35)). In contrast, beagle data collected under infant-formula fed state conditions were

701 less useful for extrapolating to infants, indicated by the mismatch between the simulated and

702 observed T_{max}. For ibuprofen, fasted state performance in beagles appeared useful for simulating

703 clinical datasets in a mixed paediatric population (38), while adjusted fed state conditions using the

704 homogenized reference meal appeared to lead to adequately describe mean concentrations in the

705 children dataset (37). For both drugs, fasted state and reference-meal fed state bioavailability data in

706 beagles led to adequate representation of the mean observed PK profiles in the target paediatric

707 populations in most cases; while infant-formula fed state conditions overestimated the oral

708 absorption delay observed in vivo in paediatrics, in line with the misalignment of the simulated vs

709 observed Tmax for this dosing condition. In contrast, extrapolation based on fasted state and infant-

710 formula fed bioavailability data in human adults appeared to capture well the mean observed data in

711 most paediatric/infant datasets. Despite these trends for the human-based and dog-based

712 extrapolation processes, the conclusions should be interpreted with caution, as the adequacy of the

713 simulations was based on a small number of paediatric patients. Furthermore, as the datasets were

714 collected from patient populations, the impact of the disease on drug intraluminal performance

715 cannot be excluded. The presented extrapolation methodology and different dosing conditions

716 deserve further, more comprehensive evaluation utilizing compounds with various biopharmaceutics

717 properties and formulation principles to allow for a clear conclusion on their usefulness within drug

718 product design and development.

719 For both model drugs, performed PSA for the beagle model indicated limited sensitivity to changes in

720 drug and/or physiology-related parameters, including the uncertainty pertaining to utilizing the in-

721 built 3-fold higher permeability for dogs vs. humans. Compared to humans, passive permeability in

722 dogs has been demonstrated to be higher, especially for drugs/compounds which are predominantly

723 absorbed via the paracellular route (17,80–82); the high permeability utilized in the canine models for

724 paracetamol and ibuprofen led to lack of preclinical model sensitivity regarding this parameter.

725 Conversely, the transcellular absorption pathway is expected to be similar for humans and dogs, which

726 is in line with the reported similar effective permeability in both species for two highly permeable

727 drugs (81). In addition to the passive permeability routes, anatomical differences between dogs and

728 humans can have an impact on the regional effective permeability of the drug (17). Employing human-

729 like Peff in the paracetamol and ibuprofen canine models had no impact on the canine simulations

730 and the estimated GE parameters under the applied dosing conditions; this indicated lack of

731 confounding effect of the employed Peff in the preclinical model for the estimated GE parameters for

732 the different dosing conditions. A further uncertainty pertaining to the regional

733 absorption/permeability in the virtual canine physiology could be the utilization of the Opt LogD SA/V

734 6.1 model to estimate ASF. The method selection for scaling of regional permeability in the virtual

735 ACAT™ physiologies should be decided with caution for the specific model drugs and adjusted if

736 further scientific evidence is available for specific cases (22,82). In the paediatric models (24,25),

737 sensitivity towards reduced permeability model was demonstrated for lower permeability resulting in

738 delayed absorption under fasted state conditions. The potentially higher permeability in dogs (and

739 lack of model sensitivity to permeability changes) could pose a challenge for translating or identifying

740 the rate-limiting absorption process in the preclinical species vs. humans. Careful consideration of

741 compound permeability and its adequate representation *in silico* is imperative in extrapolations to

742 paediatric populations; specifically in cases where permeability is a confirmed critical bioavailability

743 attribute, as intestinal barrier maturation occurs in infant age groups and permeability has been

744 identified as a parameter of uncertainty for paediatric extrapolations into young age groups (1,24,25).

745 Although beagles have been and still are used in preclinical formulation investigations for adults, their

746 usefulness for evaluation of product performance in paediatric patients has not been explored. Based

747 on the similarities of the human adult and canine GI tract, the beagle food effect model has proven its

748 utility in several cases in literature for predicting food effects in human adults (23,32,83,84). On the

749 other hand, studies have shown that food effects on total exposure and C_{max} in human adults were

750 not accurately captured by investigations performed in dogs (83,85). For celecoxib the increase in drug

751 exposure was 3-5-fold following food, while human adult food effect studies indicated only 11 %

752 increase in the fed state (85). In this context, within the present investigation, limited exposure effects

753 were expected for the two model drugs, however, differences were observed between human and

754 beagle regarding the observed absorption characteristics that could be linked to mechanical/physical

755 interaction of drug with the chyme, i.e., mixing and sieving events leading to different gastric transit

756 times. As paracetamol and ibuprofen are highly soluble and permeable in the upper SI, the observed

757 changes in drug performance among the different dosing conditions would be expected to be less

758 sensitive to differences in intraluminal fluid composition under the applied dosing conditions; gastric

759 transit time alterations as a function of prandial state can be a potential factor to explain the observed

760 *in vivo* performance. Differences in drug transit behavior was reflected in the simulations and affected

761 simulations adequacy for the paediatric populations studied, even for a simple drug product such as

762 an aqueous suspension. Implications for more complex formulations (e.g., enabling formulations) and

763 poorly water-soluble compounds with more challenging physicochemical properties deserve further

764 evaluation, building upon the understanding gained from the present investigation.

Concluding remarks

Despite the differences observed between the beagle-based and human adult-based model predictions, the beagle appears suitable for investigating paediatric products of the two studied highly soluble, highly permeable drugs in the upper small intestine at early drug product development stages, when applying fasted and/or reference-meal fed state conditions for paracetamol and ibuprofen. During later stages, human-based simulations for the two model drugs appeared superior in most of the cases, especially regarding the usefulness of infant-formula fed state conditions. It should be noted that the majority of simulations performed were within the error of the observed clinical data in the paediatric studies and were within the targeted accuracy. A deeper understanding of the differences regarding meal calorie- and texture-dependent drug GE between human adults and beagles could improve pre-clinical protocols applied at present to investigate food effects. To overcome some of the interspecies difference, the present work demonstrated an approach utilizing PBPK modeling for paediatric formulation evaluation at an early development stage for two highly soluble and highly permeable in the upper small intestine model drugs. Nevertheless, verification of the proposed methodologies for infant formulation evaluation with broader spectrum of compounds with different physicochemical properties as well as different formulation principles is required. Lastly, availability of high-quality clinical data in infants is of paramount importance for evaluating the biopharmaceutics tools and methodologies and confirmation of their reliability.

References

1. Guimarães M, Statelova M, Holm R, Reppas C, Symilllides M, Vertzoni M, et al. Biopharmaceutical considerations in paediatrics with a view to the evaluation of orally administered drug products - a PEARRL review. J Pharm Pharmacol. 2019;71(4):603–42.

2. Batchelor H. Influence of food on paediatric gastrointestinal drug absorption following oral administration: a review. Children. 2015;2(2):244–71.

3. Elder DP, Holm R, Kuentz M. Medicines for Pediatric Patients—Biopharmaceutical, Developmental, and Regulatory Considerations. J Pharm Sci. 2017;106(4):950–60.

4. CDER, Center for Drug Evaluation and Research Food F and DA. Assessing the Effects of Food on Drugs in INDs and NDAs - Clinical pharmacology considerations Guidance for industry. FDA Guid. 2022;(June). Available from: https://www.fda.gov/media/121313/download

5. Statelova M, Goumas K, Fotaki N, Holm R, Symillides M, Reppas C, et al. On the Design of Food Effect Studies in Adults for Extrapolating Oral Drug Absorption Data to Infants : an Exploratory Study Highlighting the Importance of Infant Food. AAPS J. 2020;22(6):1–11.

6. Wollmer E, Ungell A-L, Nicolas J-M, Klein S. Review of paediatric gastrointestinal physiology relevant to the absorption of orally administered medicines. Adv Drug Deliv Rev. 2022;181:114084.

7. Batchelor H, Kaukonen AM, Klein S, Davit B, Ju R, Ternik R, et al. Food effects in paediatric medicines development for products co-administered with food. Int J Pharm. 2018 Feb;536(2):530–5.

8. U.S. Department of Health and Human Services Food and Drug Administration. Leveraging Existing Clinical Data for Extrapolation to Pediatric Uses of Medical Devices. 2016 p. 1–37.

9. Pentafragka C, Symillides M, McAllister M, Dressman J, Vertzoni M, Reppas C. The impact of food intake on the luminal environment and performance of oral drug products with a view to *in vitro* and *in silico* simulations: a PEARRL review. J Pharm Pharmacol. 2018 Sep;

10. Van den Bergh A, Van Hemelryck S, Bevernage J, Van Peer A, Brewster M, Mackie C, et al. Preclinical Bioavailability Strategy for Decisions on Clinical Drug Formulation Development: An In Depth Analysis. Mol Pharm. 2018 Jul;15(7):2633–45.

11. Selen A, Dickinson PA, Müllertz A, Crison JR, Mistry HB, Cruañes MT, et al. The Biopharmaceutics Risk Assessment Roadmap for Optimizing Clinical Drug Product Performance. J Pharm Sci. 2014;103(11):3377–97.

12. Tistaert C, Heimbach T, Xia B, Parrott N, Samant TS, Kesisoglou F. Food Effect Projections via Physiologically Based Pharmacokinetic Modeling: Predictive Case Studies. J Pharm Sci. 2019 Jan 1;108(1):592–602.

13. Pepin XJH, Huckle JE, Alluri R V, Basu S, Dodd S, Parrott N, et al. Understanding Mechanisms of Food Effect and Developing Reliable PBPK Models Using a Middle-out Approach. AAPS J. 2021;23(1):12.

14. Koziolek M, Grimm M, Bollmann T, Schäfer KJ, Blattner SM, Lotz R, et al. Characterization of the GI transit conditions in Beagle dogs with a telemetric motility capsule. Eur J Pharm Biopharm. 2019;136(August 2018):221–30.

15. Sjögren E, Abrahamsson B, Augustijns P, Becker D, Bolger MB, Brewster M, et al. In vivo methods for drug absorption - Comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. Vol. 57, European Journal of Pharmaceutical Sciences. 2014. 99–151 p.

16. Martinez MN, Papich MG. Factors influencing the gastric residence of dosage forms in dogs. J Pharm Sci. 2009;98(3):844–60.

17. Martinez MN, Mochel JP, Neuhoff S, Pade D. Comparison of Canine and Human Physiological Factors: Understanding Interspecies Differences that Impact Drug Pharmacokinetics. AAPS J. 2021;23(3).

18. Polentarutti B, Albery T, Dressman J, Abrahamsson B. Modification of gastric pH in the fasted dog. J Pharm Pharmacol. 2010;62(4):462–9.

19. Sagawa K, Li F, Liese R, Sutton SC. Fed and fasted gastric pH and gastric residence time in conscious beagle dogs. J Pharm Sci. 2009;98(7):2494–500.

20. Heikkinen AT, Fowler S, Gray L, Li J, Peng Y, Yadava P, et al. In vitro to in vivo extrapolation and physiologically based modeling of cytochrome P450 mediated metabolism in beagle dog gut wall and liver. Mol Pharm. 2013;10(4):1388–99.

21. Lin Z, Li M, Gehring R, Riviere JE. Development and application of a multiroute physiologically based pharmacokinetic model for oxytetracycline in dogs and humans. J Pharm Sci. 2015;104(1):233–43.

22. Kesisoglou F, Balakrishnan A, Manser K. Utility of PBPK Absorption Modeling to Guide Modi fi ed Release Formulation Development of Gaboxadol , a Highly Soluble Compound With Region-Dependent Absorption. J Pharm Sci. 2016;105(2):722–8.

23. Li X, Yang Y, Zhang Y, Wu C, Jiang Q, Wang W, et al. Justification of Biowaiver and Dissolution Rate Specifications for Piroxicam Immediate Release Products Based on Physiologically Based Pharmacokinetic Modeling: An In-Depth Analysis. Mol Pharm. 2019;16(9):3780–90.

24. Statelova M, Holm R, Fotaki N, Reppas C, Vertzoni M. 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. AAPS J. 2020;22(126):1–17.

25. Statelova M, Holm R, Fotaki N, Reppas C, Vertzoni M. Factors affecting successful extrapolation of ibuprofen exposure from adults to paediatric populations after oral administration of a paediatric aqueous suspension. AAPS J. 2020;In press.

26. Potthast H, Dressman JB, Junginger HE, Midha KK, Oeser H, Shah VP, et al. Biowaiver monographs for immediate release solid oral dosage forms: Ibuprofen. J Pharm Sci. 2005;94(10):2121–31.

27. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: Transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res. 2005;22(1):11–23.

28. European Medicines Agency E. Ibuprofen oral use immediate release formulations 200 - 800 mg product-specific bioequivalence guidance. 2018. p. 1–4.

29. Atkinson HC, Stanescu I, Frampton C, Salem II, Beasley CPH, Robson R. Pharmacokinetics and bioavailability of a fixed-dose combination of ibuprofen and paracetamol after intravenous and oral administration. Clin Drug Investig. 2015;35(10):625–32.

30. Wright CE, Antal EJ, Gillespie WR, Albert KS. Ibuprofen and acetaminophen kinetics when

taken concurrently. Clin Pharmacol Ther. 1983;34(5):707–10.

31.   European Medicines Agency (EMA). Guideline on the investigation of drug interactions. Guid Doc [Internet]. 2012;44(June):1–59. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions-revision-1_en.pdf

32.   Wu Y, Loper A, Landis E, Hettrick L, Novak L, Lynn K, et al. The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: A Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. Int J Pharm. 2004;285(1–2):135–46.

33.   Zane P, Guo Z, Macgerorge D, Vicat P, Ollier C. Use of the pentagastrin dog model to explore the food effects on formulations in early drug development. Eur J Pharm Sci. 2014;57(1):207–13.

34.   Akimoto M, Nagahata N, Furuya a, Fukushima K, Higuchi S, Suwa T. Gastric pH profilles of beagle dogs and their use as an alternative to human testing. Eur J Pharm Biopharm. 2000;49(2):99–102.

35.   Walson PD, Halvorsen M, Edge J, Casavant MJ, Kelley MT. Pharmacokinetic Comparison of Acetaminophen Elixir Versus Suppositories in Vaccinated Infants (Aged 3 to 36 Months): A Single-Dose, Open-Label, Randomized, Parallel-Group Design. Clin Ther. 2013;35(2):135–40.

36.   Hopkins CS, Underhill S, Booker PD. Pharmacokinetics of paracetamol after cardiac surgery. Arch Dis Child. 1990;65(9):971–6.

37.   Walson PD, Galletta G, Pharm D, Braden NT, Alexander L, Columbus BS. Ibuprofen, acetaminophen, and placebo treatment of febrile children. Clin Pharmacol Ther. 1989;46(1):9–17.

38.   Brown RD, Wilson JT, Kearns GL, Eichler VF, Johnson VA, Bertrand KM. Single-dose pharmacokinetics of ibuprofen and acetaminophen in febrile children. J Clin Pharmacol. 1992;32:232–41.

39.   Gobeau N, Stringer R, De Buck S, Tuntland T, Faller B. Evaluation of the GastroPlus™ Advanced Compartmental and Transit (ACAT) Model in Early Discovery. Pharm Res. 2016;33(9):2126–39.

40.   Xia B, Heimbach T, Lin TH, Li S, Zhang H, Sheng J, et al. Utility of physiologically based modeling and preclinical in vitro/in vivo data to mitigate positive food effect in a BCS class 2 compound. AAPS PharmSciTech. 2013;14(3):1255–66.

41.   Mudie DM, Stewart AM, Rosales JA, Adam MS, Morgen MM, Vodak DT. In vitro-in silico tools for streamlined development of acalabrutinib amorphous solid dispersion tablets. Pharmaceutics. 2021;13(12).

42.   Parrott N, Lukacova V, Fraczkiewicz G, Bolger MB. Predicting pharmacokinetics of drugs using physiologically based modeling - Application to food effects. AAPS J. 2009;11(1):45–53.

43.   Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, Macintyre F, et al. The Prediction of Human Pharmacokinetic Parameters from Preclinical and In Vitro Metabolism Data. J Pharmacol Exp Ther. 1997 Oct;283(1):46 LP – 58.

44.   Mahmood I, Ahmad T, Mansoor N, Sharib SM. Prediction of Clearance in Neonates and Infants (≤ 3 Months of Age) for Drugs That Are Glucuronidated: A Comparative Study Between Allometric Scaling and Physiologically Based Pharmacokinetic Modeling. J Clin

Pharmacol. 2017;57(4):476–83.

45. Zuppa A, Hammer G, Barrett J, Kenney B, Kassir N, Moukassi S, et al. Safety and Population Pharmacokinetic Analysis of Intravenous Acetaminophen in Neonates, Infants, Children, and Adolescents With Pain or Fever. J Pediatr Pharmacol Ther. 2011;16(4):246–61.

46. Neirinckx E, Vervaet C, De Boever S, Remon JP, Gommeren K, Daminet S, et al. Species comparison of oral bioavailability, first-pass metabolism and pharmacokinetics of acetaminophen. Res Vet Sci. 2010;89(1):113–9.

47. Sjödin L, Visser S, Al-Saffar A. Using pharmacokinetic modeling to determine the effect of drug and food on gastrointestinal transit in dogs. J Pharmacol Toxicol Methods. 2011;64(1):42–52.

48. Koyanagi T, Yamaura Y, Yano K, Kim S, Yamazaki H. Age-related pharmacokinetic changes of acetaminophen, antipyrine, diazepam, diphenhydramine, and ofloxacin in male cynomolgus monkeys and beagle dogs. Xenobiotica. 2014;44(10):893–901.

49. Sagara K, Mizuta H, Ohshiko M, Shibata M, Haga K. Relationship Between the Phasic Period of Interdigestive Migrating Contraction and the Systemic Bioavailability of Acetaminophen in Dogs. Vol. 12, Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists. 1995. p. 594–8.

50. Madsen M, Enomoto H, Messenger K, Papich MG. Effects of housing environment on oral absorption of acetaminophen in healthy Beagles. Vol. 83. 2022.

51. Fancher RM, Zhang H, Sleczka B, Derbin G, Rockar R, Marathe P. Development of a canine model to enable the preclinical assessment of pH-dependent absorption of test compounds. J Pharm Sci. 2011 Jul;100(7):2979–88.

52. Smeets-Peeters M, Watson T, Minekus M, Havenaar R. A review of the physiology of the canine digestive tract related to the development of in vitro systems. Nutr Res Rev. 1998 Jun;11(1):45–69.

53. Mizuta H, Kawazoe Y, Haga K, Ogawa K. Effects of meals on gastric emptying and small intestinal transit times of a suspension in the beagle dog assessed using acetaminophen and salicylazosulfapyridine as markers. Chem Pharm Bull. 1990;38(8):2224–7.

54. Tanaka T, Mizumoto A, Haga N, Itoh Z. A new method to measure gastric emptying in conscious dogs: A validity study and effects of EM523 and L-NNA. Am J Physiol - Gastrointest Liver Physiol. 1997;272(4 35-4).

55. Wyse CA, Preston T, Love S, Morrison DJ, Cooper JM, Yam PS. Use of the 13C-octanoic acid breath test for assessment of solid-phase gastric emptying in dogs. Am J Vet Res. 2001;62(12):1939–44.

56. Meyer JH, Elashoff JD, Domeck M, Levy A, Jehn D, Hlinka M, et al. Control of canine gastric emptying of fat by lipolytic products. Am J Physiol - Gastrointest Liver Physiol. 1994;266(6 29-6).

57. Cooke AR, Clark ED. Effect of First Part of Duodenum on Gastric Emptying in Dogs: Response to Acid, Fat, Glucose, and Neural Blockade. Gastroenterology [Internet]. 1976;70(4):550–5. Available from: http://dx.doi.org/10.1016/S0016-5085(76)80494-5

58. Cooke AR. Localization of Receptors Inhibiting Gastric Emptying in the Gut. Gastroenterology. 1977;72(5):875–80.

59. Gupta PK, Robinson JR. Effect of volume and viscosity of coadministered fluid on

gastrointestinal distribution of small particles. Int J Pharm. 1995;125(2):185–93.

60.   Gupta PK, Robinson JR. Gastric emptying of liquids in the fasted dog. Int J Pharm. 1988;43(1–2):45–52.

61.   Ehrlein H -J, Pröve J. Effect of Viscosity of Test Meals on Gastric Emptying in Dogs. Q J Exp Physiol. 1982;67(3):419–25.

62.   Reppas C, Dressman JB. Viscosity modulates blood glucose response to nutrient solutions in dogs. Diabetes Res Clin Pract. 1992;17(2):81–8.

63.   Lin HC, Elashoff JD, Gu Y, Meyer JH. Effect of meal volume on gastric emptying. Neurogastroenterol Motil. 1992;4(3):157–63.

64.   Collins PJ, Horowitz M, Cook DJ, Harding PE, Shearman DJ. Gastric emptying in normal subjects - a reproducible technique using a single scintillation camera and computer system. Gut. 1983;24(12):1117–25.

65.   Kong F, Singh RP. Disintegration of solid foods in human stomach. J Food Sci. 2008;73(5).

66.   Shafer RB, Levine AS, Marlette JM, Morley JE. Do calories, osmolatity, or calcium determine gastric emptying? Am J Physiol - Regul Integr Comp Physiol. 1985;17(4):479–83.

67.   Halawi H, Camilleri M, Acosta A, Vazquez-Roque M, Oduyebo I, Burton D, et al. Relationship of gastric emptying or accommodation with satiation, satiety, and postprandial symptoms in health. Am J Physiol - Gastrointest Liver Physiol. 2017;313(5):G442–7.

68.   Guiastrennec B, Sonne DP, Hansen M, Bagger JI, Lund A, Rehfeld JF, et al. Mechanism-Based Modeling of Gastric Emptying Rate and Gallbladder Emptying in Response to Caloric Intake. CPT pharmacometrics Syst Pharmacol. 2016 Dec;5(12):692–700.

69.   Calbet JAL, MacLean DA. Role of caloric content on gastric emptying in humans. J Physiol. 1997;498(2):553–9.

70.   Okabe T, Terashima H, Sakamoto A. A comparison of gastric emptying of soluble solid meals and clear fluids matched for volume and energy content: a pilot crossover study. Anaesthesia. 2017;72(11):1344–50.

71.   Okabe T, Terashima H, Sakamoto A. Determinants of liquid gastric emptying: comparisons between milk and isocalorically adjusted clear fluids. Br J Anaesth. 2015;114(1):77–82.

72.   Noakes TD, Rehrer NJ, Maughan RJ. The importance of volume in regulating gastric emptying. Med Sci Sports Exerc. 1991 Mar;23(3):307–13.

73.   Hunt JN, Stubbs DF. The volume and energy content of meals as determinants of gastric emptying. J Physiol. 1975;245(1):209–25.

74.   Winter F, Schick P, Weitschies W. Bridging the Gap between Food Effects under Clinical Trial Conditions and Real Life: Modeling Delayed Gastric Emptying of Drug Substances and Gastric Content Volume Based on Meal Characteristics. Mol Pharm. 2023 Feb 6;20(2):1039–49.

75.   Johnson TN, Bonner JJ, Tucker GT, Turner DB, Jamei M. Development and application of a physiologically-based model of paediatric oral drug absorption. Eur J Pharm Sci. 2018;

76.   Yeung CHT, Fong S, Malik PR V, Edginton AN. Quantifying breast milk intake by term and preterm infants for input into paediatric physiologically based pharmacokinetic models. Matern Child Nutr. 2020 Apr;16(2):e12938.

77.   Siegel M, Lebenthal E, Krantz B. Effect of caloric density on gastric emptying in premature

infants. J Pediatr. 1984;104(1):118–22.

78.   Baker JH, Berseth CL. Duodenal motor responses in preterm infants fed formula with varying concentrations and rates of infusion. Pediatr Res. 1997 Nov;42(5):618–22.

79.   Pentafragka C, Vertzoni M, Symillides M, Goumas K, Reppas C. Disposition of two highly permeable drugs in the upper gastrointestinal lumen of healthy adults after a standard high-calorie, high-fat meal. Eur J Pharm Sci. 2020;149(January).

80.   He YL, Murby S, Warhurst G, Gifford L, Walker D, Ayrton J, et al. Species differences in size discrimination in the paracellular pathway reflected by oral bioavailability of poly(ethylene glycol) and D-peptides. J Pharm Sci. 1998;87(5):626–33.

81.   Dahlgren D, Roos C, Johansson P, Lundqvist A, Tannergren C, Abrahamsson B, et al. Regional Intestinal Permeability in Dogs: Biopharmaceutical Aspects for Development of Oral Modified-Release Dosage Forms. Mol Pharm. 2016 Sep 6;13(9):3022–33.

82.   Eckernäs E, Tannergren C. Physiologically Based Biopharmaceutics Modeling of Regional and Colon Absorption in Dogs. Mol Pharm. 2021;18(4):1699–710.

83.   Lentz KA, Quitko M, Morgan DG, Jr. JEG, Gleason C, Marathe PH. Development and validation of a preclinical food effect model. J Pharm Sci. 2007;96(2):459–72.

84.   Cook CS, Hauswald CL, Grahn AY, Kowalski K, Karim A, Koch R, et al. Suitability of the dog as an animal model for evaluating theophylline absorption and food effects from different formulations. Int J Pharm. 1990;60(2):125–32.

85.   Paulson SK, Vaughn MB, Jessen SM, Lawal Y, Gresk CJ, Yan B, et al. Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. J Pharmacol Exp Ther. 2001;297(2):638–45.

Usefulness of the beagle model in the evaluation of paracetamol and ibuprofen exposure after oral administration to paediatric populations: An exploratory study

Supplementary Information

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Part A: Experimental (drug determination in plasma samples)

Paracetamol and ibuprofen were analyzed separately using a different sample preparation procedure for each drug. Protein precipitation with subsequent centrifugation, and dilution were applied as sample treatment procedure according to previously described methods (1–3). The diluted supernatant was analysed via ultra-high-pressure liquid chromatography (UPLC) employing an Acquity UPLC System (Waters Corporation, Milford, MA USA).

Chromatographic conditions

The Acquity UPLC system utilized in this study consisted of a binary solvent manager, sample manager with integral column temperature control, coupled with a photodiode array (PDA) detector. System control, data acquisition and processing were performed using the Empower 3® chromatography data software (Waters Corporation, Milford, MA USA). Sample separation was achieved on an Acquity UPLC™ BEH C_{18} column (2.1 mm x 50 mm, 1.7 μm, 130Å) equipped with an ACQUITY UPLC™ BEH C_{18} VanGuard pre-column (2.1 mm X 5 mm, 130Å, 1.7 μm). Gradient elution was performed using 0.1 % trifluoracetic acid in water (*v/v*) as Solvent A and ACN as Solvent B, shown in Table A-S1 Optimal separation was achieved with a constant column temperature of 55° C at a flow rate of 0.4 mL/min for paracetamol and 0.6 mL/min for ibuprofen.

Table A-SI Gradient elution steps applied in the UPLC method

Elution	Solvent A	Solvent B	Time (min)
Isocratic	90	10	0-0.8
Linear gradient	0	100	0.8-2.8
Isocratic	0	100	2.8-3.1
Linear gradient	90	10	3.1-3.2
Isocratic	90	10	3.2-4.0

Paracetamol analysis

The paracetamol bioassay involved precipitation of 100 μL plasma sample with 200 μL 10% aqueous dilution of TFA (v/v), followed by vortex-mixing over one minute and centrifugation over 10 minutes at 10° C and 12 000 *g* (Centrifuge 5430 R, Eppendorf AG, Germany). After the collection of 150 μL clear supernatant and dilution with 150 μL water, 8 μL were injected into the UPLC system. The detection wavelength was 242 nm. Calibration curves were linear between the ranges 0.01 – 6 μg/mL, R2 > 0.9991 with a lower limit of quantification of 60 ng/mL in plasma. Quantification of the samples

51 analyzed was performed using standards diluted in a 90:10 Solvent A:Solvent B mixture; each standard

52 was injected 3 times, and quality control standards were injected after every 10th injection performed

53 (percent error <9.8%).

54 *Ibuprofen analysis*

55 The ibuprofen bioassay involved precipitation of 100 µL plasma sample acidified with 10 µL 5%

56 aqueous dilution of TFA (v/v) by addition of 190 µL ice-cold ACN, followed by vortex-mixing over one

57 minute and centrifugation over 10 minutes at 10° C and 12 000 *g*. After the collection of 150 µL clear

58 supernatant and dilution with 150 µL diluent mixture of 60:40 Solvent B:Solvent A (v/v), 8 µL were

59 injected into the UPLC system. Ibuprofen was detected at a wavelength of 220 nm. Calibration curves

60 were linear between 0.01 – 10 µg/mL, R2 > 0.9991 with a lower limit of quantification 60 ng/mL in

61 plasma. Quantification of the samples analyzed was performed using standards diluted in a 90:10

62 Solvent A:Solvent B mixture, where each standard was injected 3 times, and quality control standards

63 were injected after every 10th injection performed (percent error < 11.8%).

64

Part B: PBPK modeling and simulations

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132 **Table B-SI** Two-compartment-model parameters for paracetamol performance following intravenous
133 administration of 168 mg of paracetamol to six healthy male beagles. Pharmacokinetic analysis was
134 performed using the PKPlus™ tool within the GastroPlus™ platform.

Parameter	Mean Profile	Mean values ± SD
Clearance, CL (L/h)	12.66	13.13 ± 0.99
Volume of distribution in the central compartment, V_c (L/kg)	1.03	1.01 ± 0.09
Elimination half-life, $t_{1/2}$ (h)	7.95	5.26 ± 2.24
k_{12} h^{-1}	0.214	0.185 ± 0.062
K_{21} h^{-1}	0.104	0.175 ± 0.060
Volume of distribution in the second compartment, V_2 (L/kg)	2.115	1.29 ± 0.88

135

136

137 **Table B-SII** One-compartment-model parameters for ibuprofen performance following intravenous
138 administration of 140 mg of ibuprofen to six healthy male beagles. Pharmacokinetic analysis was
139 performed using the PKPlus™ tool within the GastroPlus™ platform.

Parameter	Mean Profile	Mean values ± SD
Clearance, CL (L/h)	0.478	0.479 ± 0.049
Volume of distribution in the central compartment, V_c (L/kg)	0.146	0.148 ± 0.022
Elimination half-life, $t_{1/2}$ (h)	2.19	2.20 ± 0.29

140

141 **Table B-SIII** Input parameters in the canine PBPK model for paracetamol.

Parameter		Source
Physicochemical properties		
Molecular weight (g/mol)	151.2	
Compound type	Monoprotic weak acid	(4–6)
pKa	9.45 (acidic)	
logP	0.51	
Reference solubility in water (mg/mL)	14	(4)
Absorption		
Model	ACAT	GastroPlus™
Effective permeability, human (cm/s $\times 10^4$)	3.897	Calculated based on references (7–9)
Effective permeability, dog (cm/s $\times 10^4$)	10.39	Scaled from human GastroPlus™ (10)
Dissolution model	Johnson	GastroPlus™
Drug particle radius (μm)	25	
Particle density (g/mL)	1.2	Default GastroPlus™
Mean precipitation time (s)	900 [a]	
Diffusion coefficient (cm^2/s $\times 10^5$)	1.109	ADMET Predictor within GastroPlus™ (10,11)
Absorption scale factor (ASF) estimation	Opt LogD Model SA/V 6.1	GastroPlus™ (10)
Compartmental PK model (based on i.v. dosing)		
Fraction unbound (dogs), fu [b]	0.664	ADMET Predictor within GastroPlus™ (10,11)
Blood-plasma ratio (dogs)	1.01	
Clearance, CL (L/h)	12.66	
Volume of distribution in the central compartment, V_c (L/kg)	1.03	2-compartment-model fit to the mean i.v. profile using the PKPlus™ tool within GastroPlus™
Elimination half-life, $t_{1/2}$ (h)	7.95	
k_{12} h^{-1}	0.214	
K_{21} h^{-1}	0.104	
Volume of distribution in the second compartment, V_2 (L/kg)	2.115	

142 [a] default value was used for precipitation time due to drug high solubility and lack of model

143 sensitivity for this parameter; [b] adjusted fu,p option used within simulations; no bile salt

144 solubilization was assumed for paracetamol due to its high aqueous solubility.

145 **Table B-SIV** Input parameters in the canine PBPK model for ibuprofen.

Parameter		Source
Physicochemical properties		
Molecular weight (g/mol)	206.3	(12)
pKa	4.42 (acidic)	(13)
Compound type	Monoprotic acid	
clogP [a]	3.65	Predicted GastroPlus™
Reference solubility (mg/mL)	0.038	
Aqueous solubility in mg/mL (pH)	0.038 (1.0)	(14)
	0.043 (3.0)	
	0.084 (4.5)	
	0.685 (5.5)	
	3.37 (6.8)	
	3.44 (7.4)	
Absorption		
Model	ACAT™	GastroPlus™ (15)
Effective permeability, human (cm/s ×10^4)	6.6	Calculated (8,16)
Effective permeability, dog (cm/s ×10^4)	18.05	Scaled from human GastroPlus™ (10)
Solubility in biorelevant media (mg/mL) Level III FaSSGF Level II FaSSIF Level II FeSSIF-V2	0.048 1.953 2.290	In house data
Bile salt-solubilization ratio	$1.4×10^3$	Estimated in GastroPlus™
Dissolution model	Johnson	GastroPlus™ (15)
Particle radius (μm)	25	Default GastroPlus™
Particle density (g/mL)	1.2	
Mean precipitation time (s) [b]	900	
Diffusion coefficient (cm^2/s × 10^5)	0.939	ADMET Predictor within GastroPlus™ (10,11)
Absorption scale factor (ASF) estimation	Opt LogD Model SA/V 6.1	GastroPlus™ (10)
Compartmental PK model (based on i.v. dosing)		
Fraction unbound, fu [c]	0.0586	ADMET Predictor within GastroPlus™ (10,11)
Blood-plasma ratio	0.7	
Clearance, CL (L/h)	0.478	1-compartment-model fit to the mean i.v. profile using the PKPlus™ tool within GastroPlus™
Volume of distribution in the central compartment, Vc (L/kg)	0.146	
Elimination half-life, $t_{1/2}$ (h)	2.19	

146 [a] calculated/predicted logP (octanol/water) by GastroPlus™, experimental logP range 3.23-4.13

147 (13,17–19); [b] default value was used for precipitation time due to drug high solubility at intestinal pH

148 and lack of model sensitivity for this parameter (drug is a weak acid, no precipitation occurs during

149 transfer from stomach to duodenum); [c] adjusted fu,p option used within simulations

150

151 **Table B-SV** One-factor-at-a-time parameter sensitivity analysis performed for the paracetamol beagle
152 model (10 kg male beagle physiology): parameters, baseline values (value applied in the model) and
153 ranges for the parameters tested.

Parameter	Baseline	Lower range limit	Upper range limit
All prandial/dosing conditions			
Effective permeability (cm/s $\times 10^4$)	10.39	1.039	35.0
Diffusion coefficient (cm^2/s $\times 10^5$)	1.109	0.111	1.109
Particle size (μm)	25	2.5	100
Precipitation time (s)	900	90	9000
Dose volume (mL)	24	2	35
Small intestinal length (cm)	150	75	300
Small intestinal radius (cm)	0.5	0.25	1.0
Small intestinal transit time (h)	1.82	0.91	3.64
Fraction of small intestinal fluid in fasted state (%)	40	20	80
Duodenal pH	6.2	0.5	8.0
Jejunal pH	6.2	0.5	8.0
Liver first pass effect (%)	12	6	25
Fasted state conditions			
Gastric pH	1.6	0.5	8.0
Gastric volume (mL)	51	25.5	102
Gastric transit time (h)	0.5	0.1	1.0
Reference-meal/Infant-formula fed state conditions			
Gastric pH	5.0	0.5	8
Gastric volume (mL)	1000	500	2000
Gastric transit time (h), Zero order gastric emptying	1.5	0.75	3.0

154

155

156   **Table B-SVI** One-factor-at-a-time parameter sensitivity analysis performed for the ibuprofen beagle
157   model (10 kg male beagle physiology): parameters, baseline values (value applied in the model) and
158   ranges for the parameters tested.

Parameter	Baseline	Lower range limit	Upper range limit
All prandial/dosing conditions			
Effective permeability (cm/s $\times 10^4$)	18.05	1.0	36.1
Bile salt solubilization ratio	1.4×10^3	1.0×10^3	2.8×10^3
Diffusion coefficient (cm^2/s $\times 10^5$)	0.939	0.47	1.0
Reference solubility (mg/mL)	0.038	0.0038	0.076
Particle size (µm)	25	2.5	100
Precipitation time (s)	900	90	9000
Dose volume (mL)	24	2	35
Small intestinal length (cm)	150	75	300
Small intestinal radius (cm)	0.5	0.25	1.0
Small intestinal transit time (h)	1.82	0.91	3.64
Fraction of small intestinal fluid in fasted state (%)	40	20	80
Duodenal pH	6.2	0.5	8.0
Jejunal pH	6.2	0.5	8.0
Liver first pass effect (%)	22	10	35
Fasted state conditions			
Gastric pH	1.6	0.5	8.0
Gastric volume (mL)	51	25.5	102
Gastric transit time (h)	0.25	0.1	1.0
Reference-meal/Infant-formula fed state conditions			
Gastric pH	5.0	0.5	8
Gastric volume (mL)	1000	500	2000
Gastric transit time (h), Zero order gastric emptying	1.1	0.1	2.2

159

160   **Table B-SVII** Input parameters used to build the PBPK model for paracetamol

Parameter			Source
Physicochemical properties			
Molecular weight (g/mol)		151.2	(4–6)
Compound type		Monoprotic weak acid	(4–6)
pKa		9.45 (acidic)	(4–6)
logP [a]		0.51	(4–6)
Reference solubility in water (mg/mL)		14	(4)
Absorption			
Model		ACAT	GastroPlus™
Effective permeability, human (cm/s ×10^4)		3.897	Calculated based on (7–9)
Dissolution model		Johnson	GastroPlus™
Drug particle radius (µm)		25	Default GastroPlus™
Absorption scale factor (ASF) estimation		Opt LogD Model SA/V 6.1	GastroPlus™ (10)
Distribution			
Fraction unbound, fu		0.82	(11)
Blood-plasma ratio		1.09	(20)
Predicted Vss (L/kg) [b]		0.86	Predicted using the Lukacova, Rodgers and Rowland method (21–23)
Clearance			
In vivo clearance (L/h)		19.7	(24)
Enzyme kinetics			
	Km (µM)	Vmax (pmol/min/mg microsomal protein)	
CYP1A2 [c]	220	30.78	(25)
CYP2C9 [c]	660	8.42	(25)
CYP2C19 [c]	2000	25.53	(25)
CYP2D6 [c]	440	5.62	(25)
CYP2E1 [c]	4020	76.97	(25)
CYP3A4 [c]	130	57.16	(25)
UGT1A1 [d]	5500	6102.67	(26)
UGT1A9 [d]	9200	10208.11	(26)
UGT2B15 [d]	23000	34045.84	(26)
SULT1A1 [e]	2400	1374.06	(27)
SULT1A3 [e]	1500	202.89	(27)
SULT1E1 [e]	1900	146.22	(27)
SULT2A1 [e]	3700	828.35	(27)

161   [a] to achieve the benchmark Vss values observed *in vivo*, initially logP value of 1.2 was used for the
162   calculation of the tissue partitioning coefficients (Kp) (6); measured logP value 0.51 was used thought
163   simulations; [b] Predicted volume of distribution at steady state (Vss); [c] Cytochrome P450 (CYP)
164   isoenzyme, [d] UDP-glucuronosyltransferase (UGT) isoenzyme, and [e] cytosolic sulfotransferases (SULT)
165   isoenzyme contributing to paracetamol metabolism

166

167

168   **Table B-SVIII** Input parameters used to build the human PBPK model for ibuprofen

Parameter		Source
Physicochemical properties		
Molecular weight (g/mol)	206.3	(12)
pKa	4.42 (acidic)	(13)
Compound type	Monoprotic weak acid	
clogP*	3.65	Predicted GastroPlus™
Reference solubility (mg/mL)	0.038	(14)
Aqueous solubility in mg/mL (pH)	0.038 (1.0)	(14)
	0.043 (3.0)	
	0.084 (4.5)	
	0.685 (5.5)	
	3.37 (6.8)	
	3.44 (7.4)	
Absorption		
Model	ACAT™	
Effective permeability, human (cm/s ×10^4)	6.6	Calculated based on (8,16)
Solubility in biorelevant media (mg/mL) Level III FaSSGF Level II FaSSIF Level II FeSSIF-V2	0.048 1.953 2.290	In house data
Dissolution model	Johnson	GastroPlus™, (15)
Particle size, radius (μm)	25	Default GastroPlus™
Absorption scale factor (ASF) estimation	Opt LogD Model SA/V 6.1	GastroPlus™ (10)
Distribution		
Fraction unbound, fu	0.0155	(28)
Blood-plasma ratio	1.55	(29)
Vss (L/kg) [a]	0.11	Predicted using the Lukacova, Rodgers and Rowland method (21,23)
Clearance		
Clearance (L/h)	3.81	Adjusted based on healthy adults Pavliv *et al.* (30)

169   *calculated/predicted logP (octanol/water) by GastroPlus™, experimental logP range 3.23-4.13

170   (13,17–19)

171

172 Clearance scaling

173 The hepatic intrinsic clearance ($CL_{int,u,H}$) parameter was incorporated as whole organ clearance in the

174 model and was calculated according to the well-stirred clearance model (6,10,31), i.e. Eq. S1.

175
$$CL_{int,u,H} = \frac{Q_{H,B} \times CL_H}{Fu_p \times (Q_{H,B} - CL_H/B:P)}$$
Eq. S1

176 where $Q_{H,B}$ is the hepatic blood flow (L/h), fu is the fraction of drug unbound in plasma, CL_H is the

177 hepatic clearance observed *in vivo* (L/h) and B:P denotes the blood to plasma concentration ratio of

178 the drug (Table B-SVII).

179

180 Although the two ibuprofen enantiomers exhibit quantitatively different metabolic contributions of

181 the different CYP and UGT isoenzymes, the proportions of (*S*)-ibuprofen metabolized by the different

182 isoenzymes was considered in the present modeling exercise of the racemic ibuprofen. This

183 simplification was adopted to facilitate clearance scaling to paediatrics and because the (R)-

184 enantiomer undergoes extensive systemic inversion to the S-enantiomer (17,32). Pre-systemic

185 inversion of (R)- to (S)- ibuprofen has been considered negligible in literature (32), as studies

186 investigating intravenous and oral (R)-ibuprofen administration in adults revealed no pharmacokinetic

187 differences between the two administration routes (33–35). Based on urinary recovery data of

188 ibuprofen (metabolites) in adults (36,37), fraction metabolized (fm) values for CYP2C9, CYP2C8,

189 UGT1A9, and UGT2B7 were estimated and reported in Table S-BVIII.

190 **Table S-BIX** Fraction metabolized (fm) and enzyme maturation factors employed in the age-dependent
191 clearance estimations for ibuprofen.

Metabolizing enzyme	Fraction metabolized (fm)		Maturation factor (MF) [a]		
	Adult/Paediatric	Source	Infant (12 - 24 months)	Child (6-year-old)	Source
CYP2C9	0.8460	(36,37)	0.90	1.00	(38)
CYP2C8	0.0059	(36,37)	0.99	1.00	(38)
UGT1A9	0.1502	(36,37)	1.25	1.16	(39)
UGT2B7	0.0038	(36,37)	0.87	2.16	(39)

192 [a] Maturation factor calculated from enzyme abundance/activity in paediatric microsomes *vs.* adults

193

194 Furthermore, maturation processes in paediatrics were considered for age-dependent ibuprofen

195 clearance estimations, based on their crucial role for capturing drug clearance at young ages, i.e.

196 younger children and infants (38,40). The approach applied in the present study is commonly

197 implemented in PBPK modeling routines (38,40,41). Clearance scaling was performed based on an

198 allometric scaling factor of 0.75 (ASF) and a maturation factor (MF_{age}) for the involved metabolizing

199 enzymes as shown in Eq. S2 and Eq. S3, respectively.

13

$$CL_{paediatrics} = CL_{adult} \times \left(\frac{BW_{paediatrics}}{BW_{adult}}\right)^{0.75} \times MF_{age} \qquad \text{Eq. S2}$$

200

201 where $CL_{paediatrics}$ is the clearance in the paediatric population representative (L/h), CL_{adult} is the

202 clearance in adults (L/h), $BW_{paediatrics}$ and BW_{adult} are the body weights of the paediatric and adult

203 representatives, respectively, and the MF_{age} is the maturation factor for the specific age (Eq. S3) .

$$MF_{age} = a \times MF_{CYP2C9} + b \times MF_{CYP2C8} + c \times MF_{UGT1A9} + d \times MF_{UGT2B7} \qquad \text{Eq. S3}$$

204

205 where a, b, c, and d are the fm(CYP2C9), fm(CYP2C8), fm(UGT1A9), and fm(UGT2B7), respectively;

206 MF_{CYP2C9}, MF_{CYP2C8}, $MF_{UGT1A9,}$ and MF_{UGT2B7} denote the relative isoenzyme activity at the relevant

207 paediatric age *vs.* the adult activity for CYP2C9, CYP2C8, UGT1A9, and UGT2B7, respectively. Values

208 employed in the present study are reported in Table S-BVIII. MPPGL ontogeny was not taken into
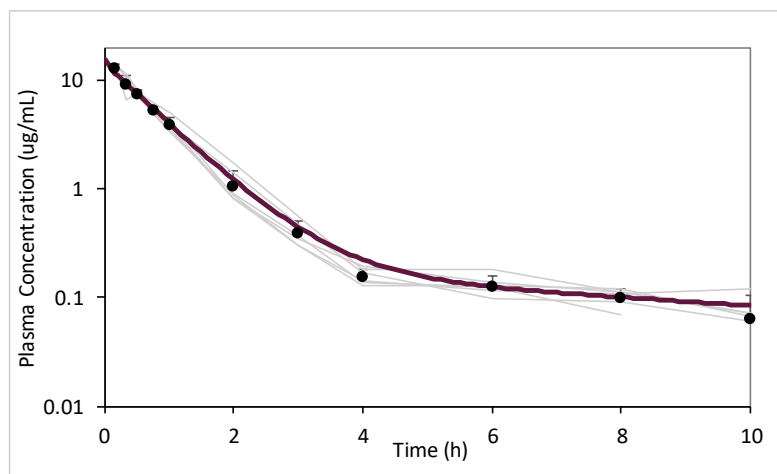
209 consideration in the present model

Figure B-S1 Observed and simulated plasma concentration-time profiles for paracetamol following the i.v. administration of 168 mg of paracetamol to 6 beagles. Mean observed plasma concentrations and standard deviations are depicted with filled circles and error bars, individual profiles with grey lines; simulated mean profile with a purple bold line.
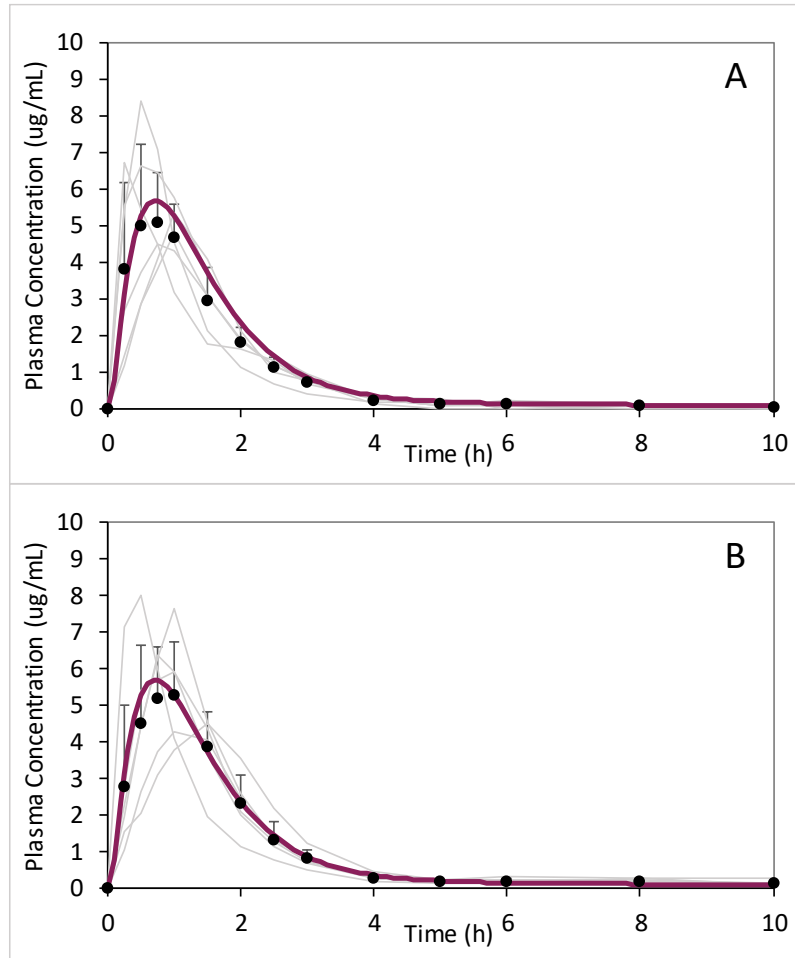
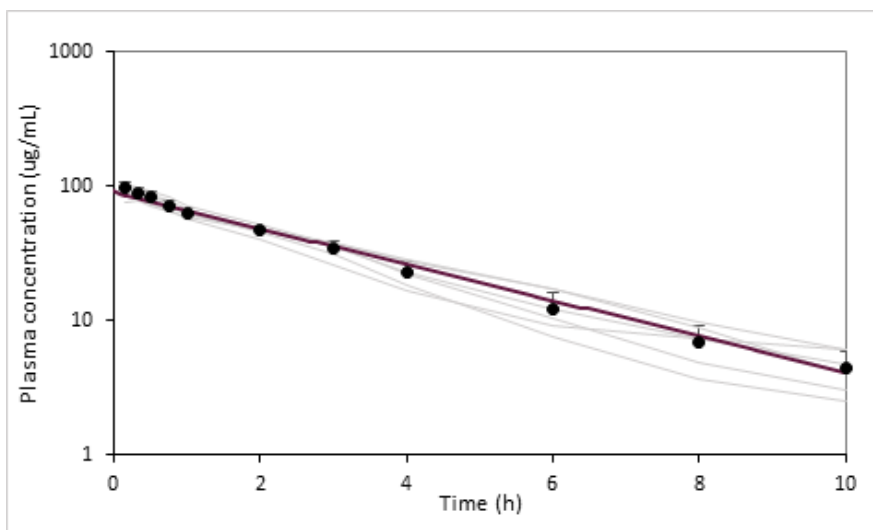**Figure B-S2** Observed and simulated paracetamol plasma concentrations in beagles (n=6) following oral administration under fasted state conditions without (A) and with (B) gastric pH-lowering pretreatment. For both simulations gastric transit time of 0.5 h was employed. Simulations without pretreatment (A) resulted in AFE / AAFE of 1.307 / 1.349, while simulations of drug performance with pretreatment resulted in AFE / AAFE of 0.960 / 1.195. Mean observed plasma concentrations and standard deviations are depicted with filled circles and error bars, individual profiles are presented with grey lines, simulated mean profile is presented with a purple bold line.

Figure B-S3 Observed and simulated plasma concentration-time profiles for ibuprofen following i.v. administration of 140 mg of ibuprofen to 6 beagles. Mean observed plasma concentrations and standard deviations are depicted with filled circles and error bars; individual profiles grey lines; simulated mean profile with a purple bold line.
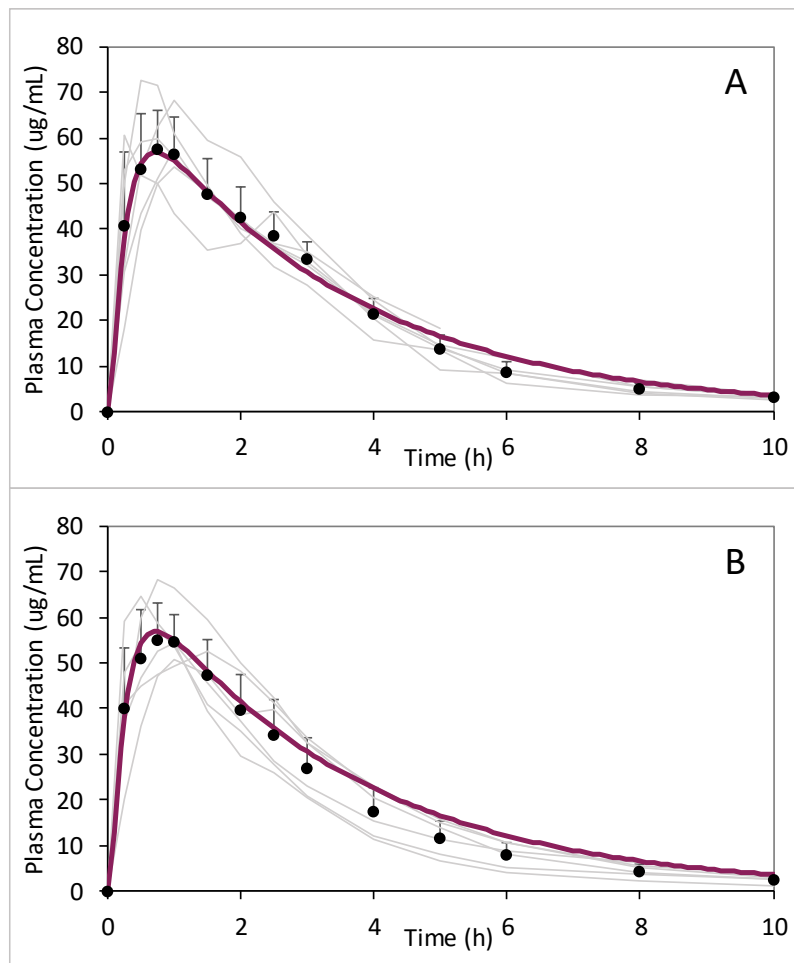
Figure B-S4 Observed and simulated ibuprofen plasma concentrations in beagles (n=6) following oral administration under fasted state conditions without (A) and with (B) gastric pH-lowering pretreatment. For both simulations gastric transit time of 0.25 h was employed. Simulations without pretreatment (A) resulted in AFE / AAFE of 1.049 / 1.099, while simulations of drug performance with pretreatment resulted in AFE / AAFE of 1.162 / 1.172. Mean observed plasma concentrations and standard deviations are depicted with filled circles and error bars, individual profiles are presented with grey lines, simulated mean profile is presented with a purple bold line.
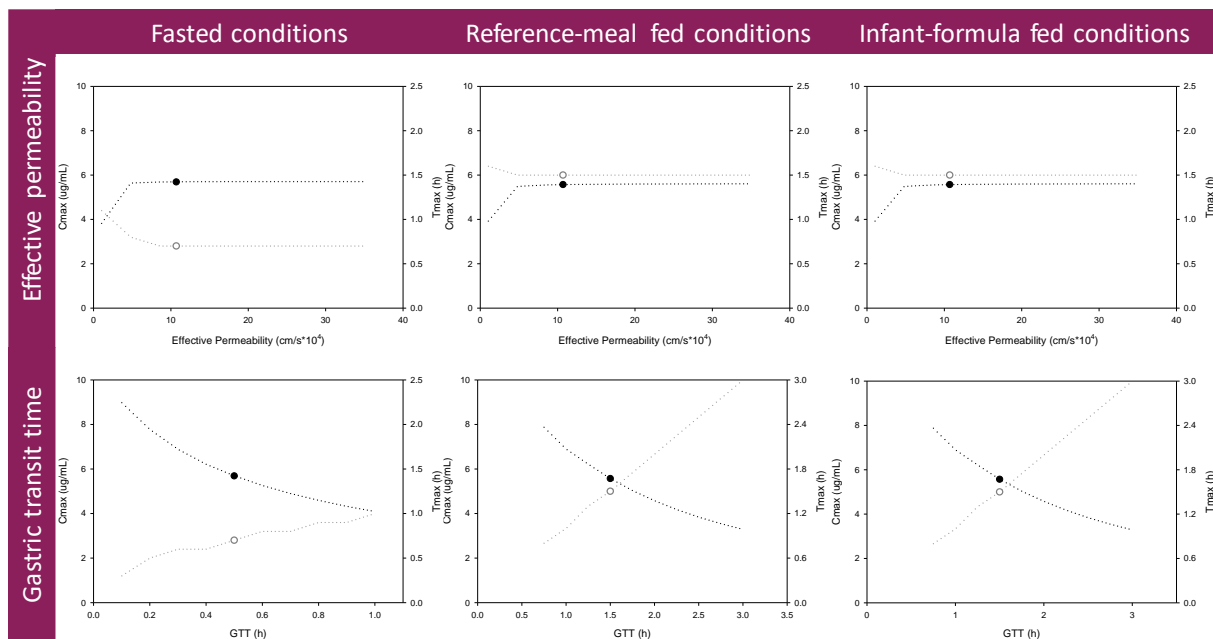
Figure B-S5 Paracetamol parameter sensitivity analysis performed for effective permeability (upper panel) and gastric transit times (lower panel) in a beagle (male, 10 kg) under fasted state conditions, reference-meal fed state conditions, and infant-formula fed state conditions. Black lines depict C_{max} values (left Y-axis) and grey lines depict T_{max} values (right Y-axis). Symbols denote the parameter value (baseline) employed in the performed beagle simulations.
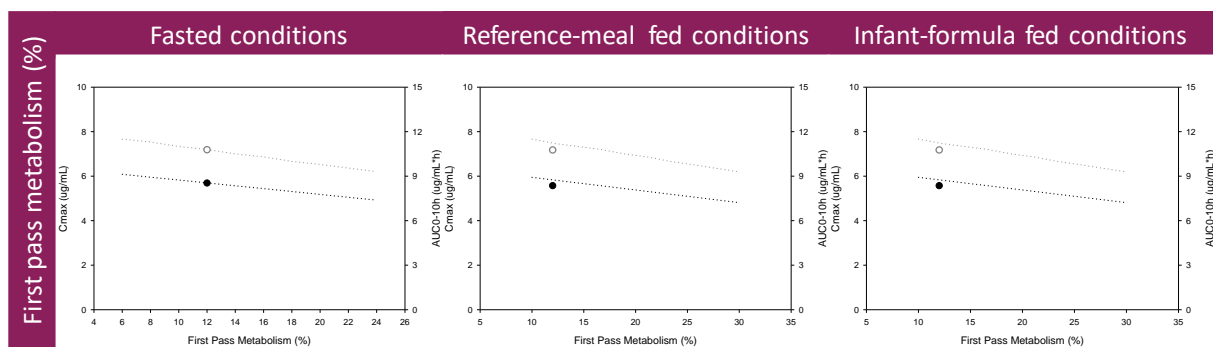

Figure B-S6 Paracetamol parameter sensitivity analysis performed for first pass metabolism changes between 6 - 24 % in a beagle (male, 10 kg) under fasted state conditions, reference-meal fed state conditions, and infant-formula fed state conditions. Black lines depict C_{max} values (left Y-axis) and grey lines depict AUC_{0-10h} values (right Y-axis). Symbols denote the parameter value (baseline) employed in the performed beagle simulations.
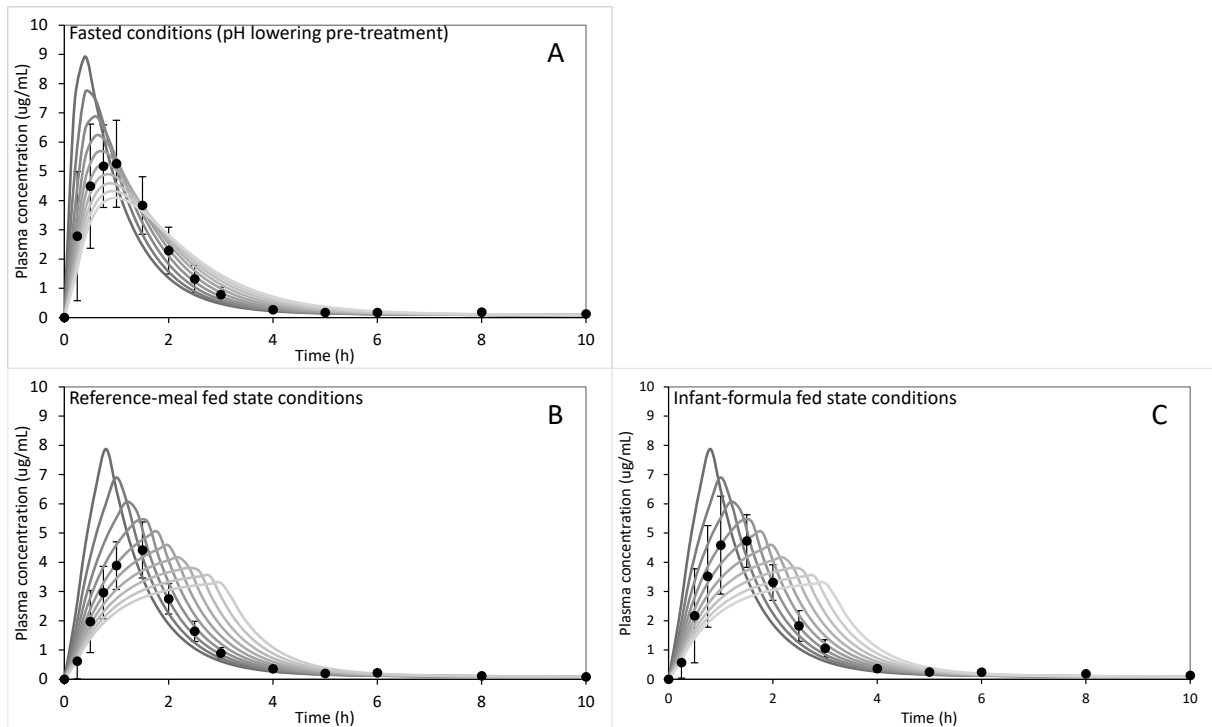
Figure B-S7 Paracetamol simulations investigating sensitivity to gastric transit time (GTT) changes in beagles under the different dosing condition: fasted state conditions: range 0.1 h to 1 h (A), reference-meal fed state conditions: range 0.75 h to 3 h (B), and infant-formula fed state conditions: range 0.75 h to 3 h (C). Grey lines denote simulated plasma concentration-time profiles, with dark to light color gradient denoting rapid to slow GTT values (10 steps), symbols and error bars denote mean and standard deviation observed in the canine study (n=6 Beagles).
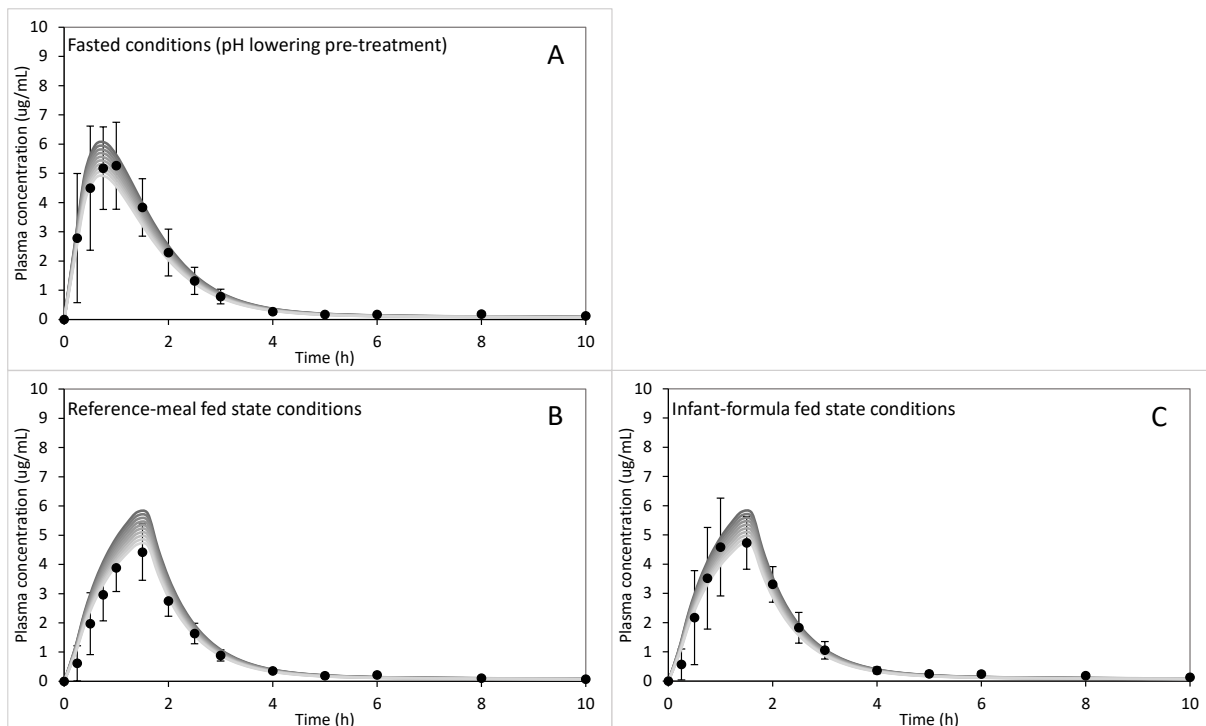
Figure B-S8 Paracetamol simulations investigating sensitivity to liver first pass metabolism (%) changes 6-24 % under fasted state conditions (A), reference-meal fed state conditions (B), and infant-formula fed state conditions (C). Grey lines denote simulations, with dark to light color gradient denoting low to high first pass metabolism values (10 steps), symbols and error bars denote mean and standard deviations observed in the *in vivo* study in beagles.
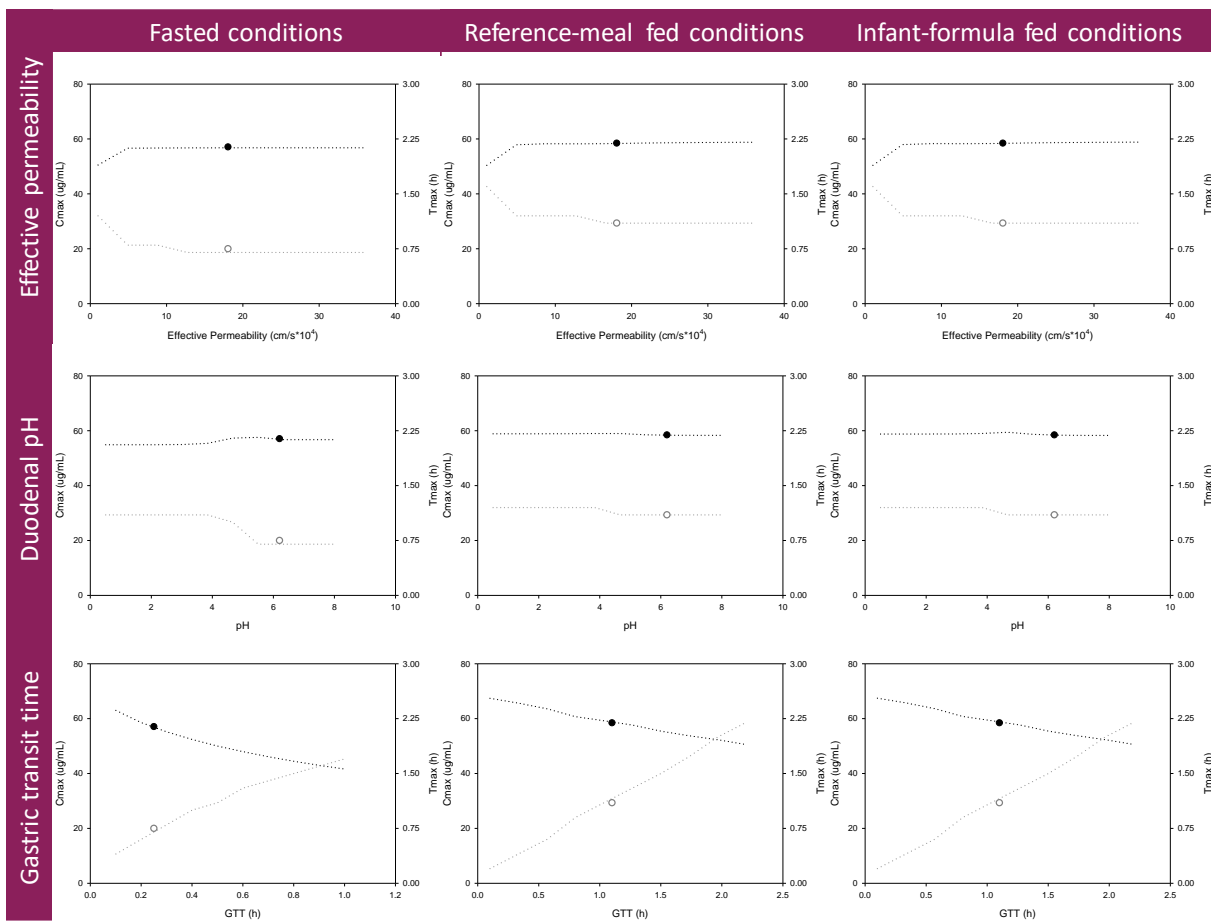
Figure B-S9 Ibuprofen parameter sensitivity analysis performed for effective permeability (upper panel), duodenal pH (middle panel) and gastric transit times (lower panel) in a beagle (male, 10 kg) under fasted state conditions, reference-meal fed state conditions, and infant-formula fed state conditions. Black lines depict C_{max} values (left Y-axis) and grey lines depict T_{max} values (right Y-axis). Symbols denote the parameter value (baseline) employed in the performed beagle simulations.
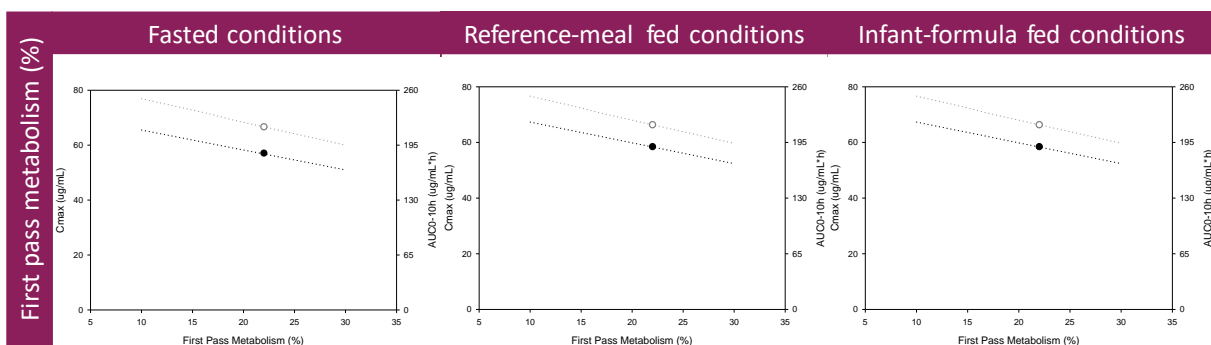

Figure B-S10 Ibuprofen parameter sensitivity analysis performed for first pass metabolism changes between 10 - 30 % in a beagle (male, 10 kg) under fasted state conditions, reference-meal fed state conditions, and infant-formula fed state conditions. Black lines depict C_{max} values (left Y-axis) and grey lines depict AUC_{0-10h} values (right Y-axis). Symbols denote the parameter value (baseline) employed in the performed beagle simulations.
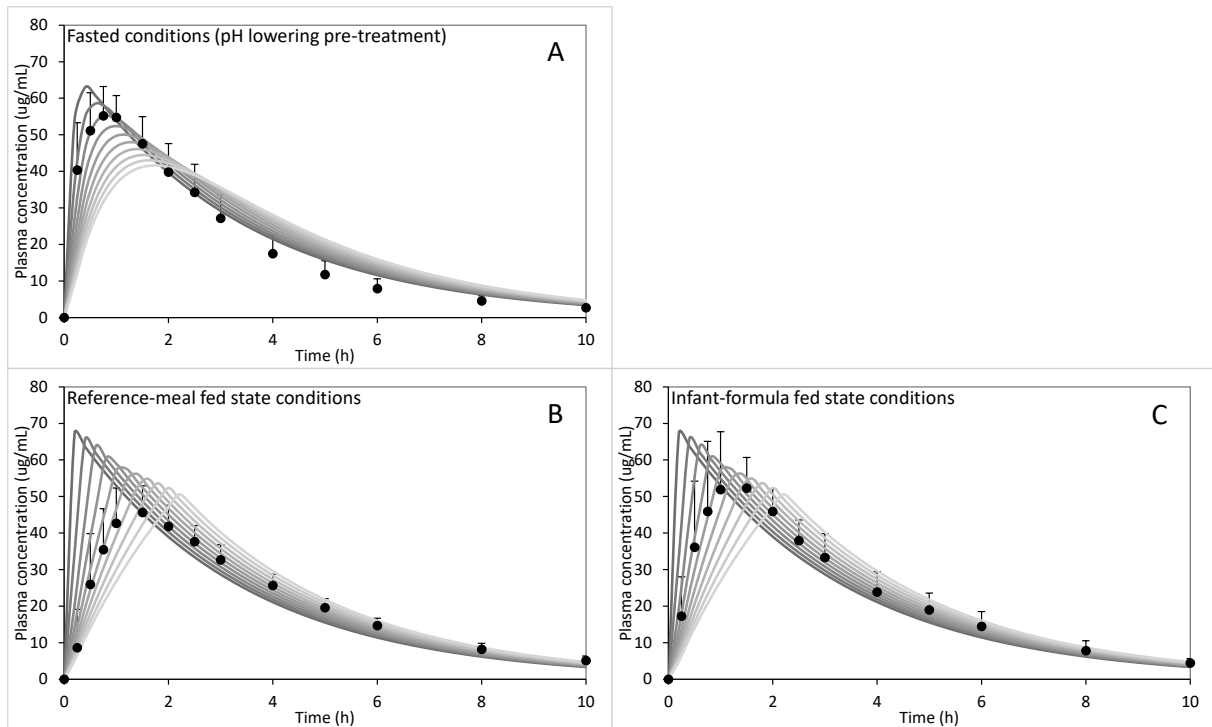
Figure B-S11 Ibuprofen simulations investigating sensitivity to gastric transit time (GTT) changes under fasted state conditions: range 0.1 h to 1 h (A), reference-meal fed state conditions: range 0.1 h to 2.2 h (B), and infant-formula fed state conditions: range 0.1 h to 2.2 h (C). Grey lines denote simulated plasma concentration-time profiles, with dark to light color gradient denoting rapid to slow GTT values (10 steps), symbols and error bars denote mean concentrations and standard deviations observed in the canine study.
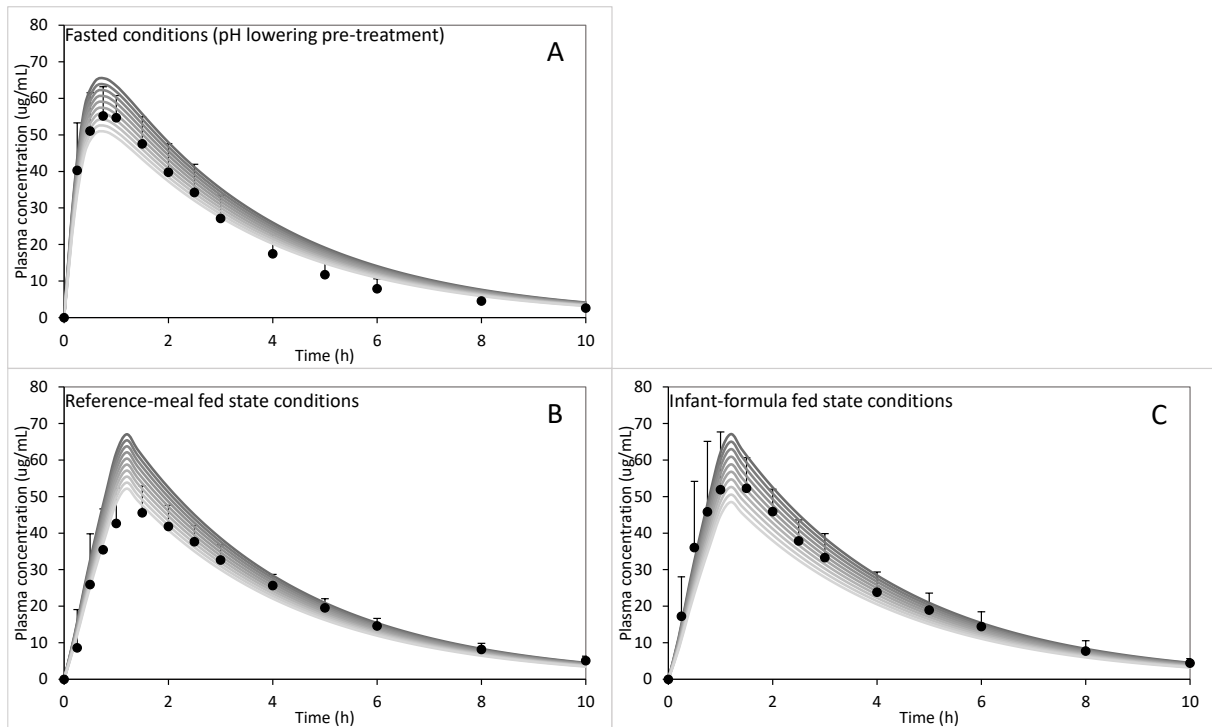
Figure B-S12 Ibuprofen simulations investigating sensitivity to liver first pass metabolism (%) changes 10-30 % under fasted state conditions (A), reference-meal fed state conditions (B), and under infant-formula fed state conditions (C). Grey lines denote simulations, with dark to light color gradient denoting low to high first pass metabolism values (10 steps), symbols and error bars denote mean and standard deviations observed in the *in vivo* study in beagles.
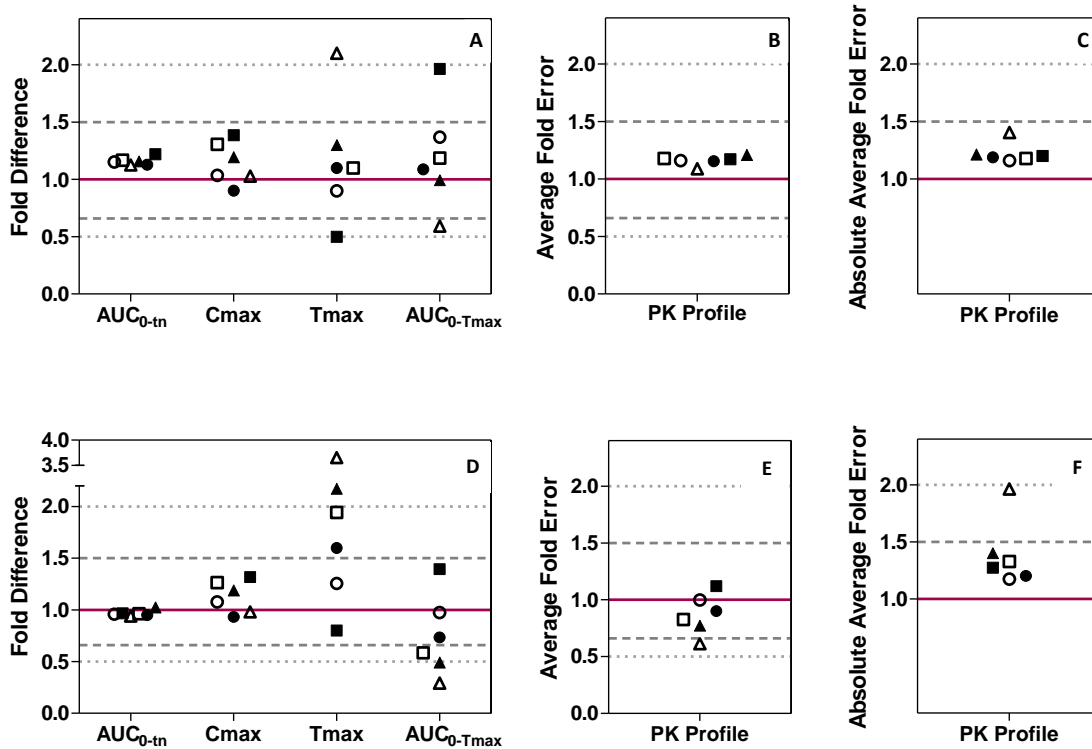
Figure B-S13 Fold difference (simulated/observed) for AUC_{0-8h}, C_{max}, T_{max} (A, D), Average Fold Error (B, E), and Absolute Average Fold Error (C, F) calculated from the simulated paracetamol profiles with PBPK model based on human adult bioavailability data (closed symbols, (42)) or based on beagle bioavailability data (open symbols, present study). Upper panel (A, B, C) depicts model performance according to the study by Hopkins et al., 1990 (43) (study mean age 4 months); lower panel (D, E, F) study by Walson et al., 2013 (44) (study mean age 10 months). Adjusted fasted state conditions (circles), adjusted reference-meal fed state conditions (squares), and adjusted infant-formula fed state conditions (triangles). The solid line represents the line of unity, grey dashed lines 0.66-1.5 range indicating successful simulations, and grey dotted lines the 0.5-2 range indicating adequate simulations.

Figure B-S14 Fold difference (simulated/observed) for AUC_{0-8h}, C_{max}, T_{max} (A, D), Average Fold Error (B, E), and Absolute Average Fold Error (C, F) calculated from the mean simulated ibuprofen profiles with PBPK model based on human adult bioavailability data (closed symbols, (45)) or based on beagle bioavailability data (open symbols, present study). Upper panel (A, B, C) depicts model performance according to the study by Brown et al., 1992 (46) (study age range 0.3-12 years); lower panel (D, E, F) study by Walson et al., 1989 (47) (study age range 2-11 years). Adjusted fasted state conditions (circles), adjusted reference-meal fed state conditions (squares), and adjusted reference-meal fed state conditions (> 2.5 years) and adjusted infant-formula fed state conditions (< 2.5 years) (triangles). The solid line represents the line of unity, grey dashed lines 0.66 -1.5 range indicating successful simulations, and grey dotted lines the 0.5-2 range indicating adequate simulations.
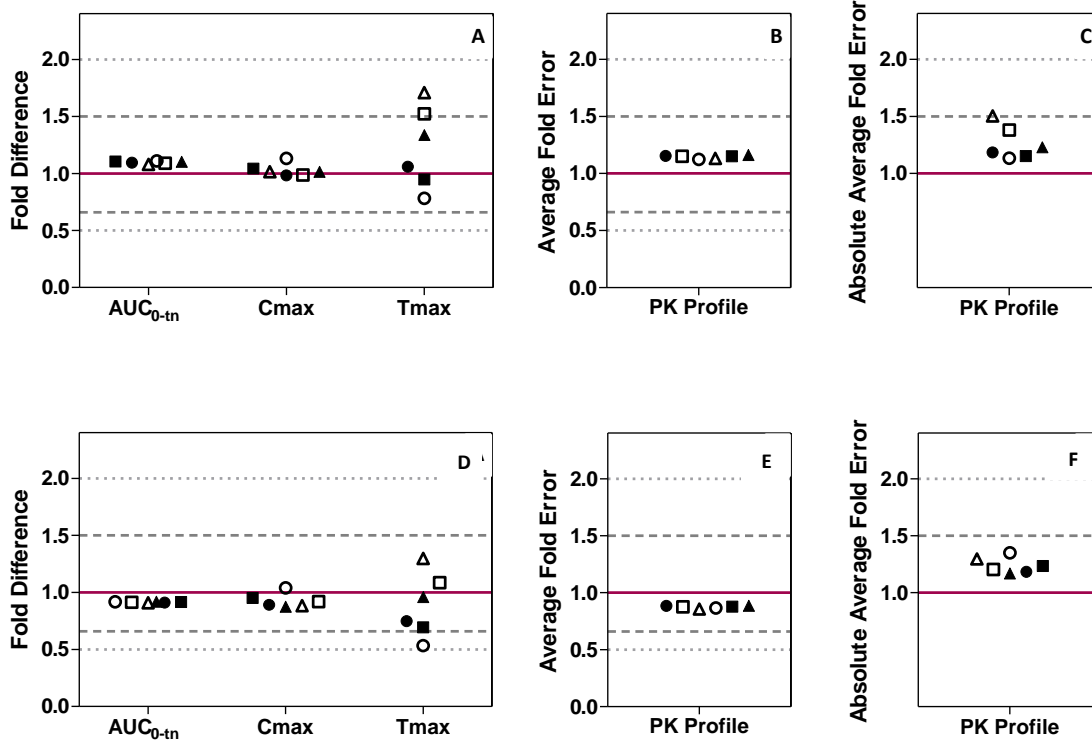
References

1. Vertzoni M V., Archontaki HA, Galanopoulou P. Development and optimization of a reversed-phase high-performance liquid chromatographic method for the determination of acetaminophen and its major metabolites in rabbit plasma and urine after a toxic dose. J Pharm Biomed Anal. 2003;32(3):487–93.

2. Lalande M, Wilson DL, Mcgilveray IJ. Rapid high-performance in human plasma. J Chromatogr B. 1986;377:410–4.

3. Statelova M, Goumas K, Fotaki N, Holm R, Symillides M, Reppas C, et al. On the Design of Food Effect Studies in Adults for Extrapolating Oral Drug Absorption Data to Infants : an Exploratory Study Highlighting the Importance of Infant Food. AAPS J. 2020;22(6):1–11.

4. Kalantzi L, Reppas C, Dressman JB, Amidon GL, Junginger HE, Midha KK, et al. Biowaiver monographs for immediate release solid oral dosage forms: Acetaminophen (Paracetamol). J Pharm Sci. 2006;95(1):4–14.

5. Jiang X-L, Zhao P, Barrett JS, Lesko LJ, Schmidt S. Application of physiologically based pharmacokinetic modeling to predict acetaminophen metabolism and pharmacokinetics in children. CPT pharmacometrics Syst Pharmacol. 2013;2(August):e80.

6. Ladumor MK, Bhatt DK, Gaedigk A, Sharma S, Thakur A, Pearce RE, et al. Ontogeny of hepatic sulfotransferases and prediction of age-dependent fractional contribution of sulfation in acetaminophen metabolism. Drug Metab Dispos. 2019;47(8):818–31.

7. Johnson TN, Bonner JJ, Tucker GT, Turner DB, Jamei M. Development and application of a physiologically-based model of paediatric oral drug absorption. Eur J Pharm Sci. 2018;

8. Sun D, Lennernas H, Welage LS, Barnett JL, Landowski CP, Foster D, et al. Comparison of human duodenum and Caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs. Pharm Res. 2002;19(10):1400–16.

9. Yamashita S, Furubayashi T, Kataoka M, Sakane T, Sezaki H, Tokuda H. Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. Eur J Pharm Sci. 2000;10(3):195–204.

10. SimulationsPlus Inc. GastroPlus Manual. 2020.

11. Strougo A, Eissing T, Yassen A, Willmann S, Danhof M, Freijer J. First dose in children: Physiological insights into pharmacokinetic scaling approaches and their implications in paediatric drug development. J Pharmacokinet Pharmacodyn. 2012;39(2):195–203.

12. Pharmacopoiea E. Ibuprofenum. Ph Eur. 2008;7(1):2225–7.

13. Avdeef A, Berger CM, Brownell C. pH-metric solubility. 2: Correlation between the acid-base titration and the saturation shake-flask solubility-pH methods. Pharm Res. 2000;17(1):85–9.

14. Potthast H, Dressman JB, Junginger HE, Midha KK, Oeser H, Shah VP, et al. Biowaiver monographs for immediate release solid oral dosage forms: Ibuprofen. J Pharm Sci. 2005;94(10):2121–31.

15. Lu ATK, Frisella ME, Johnson KC. Dissolution Modeling: Factors Affecting the Dissolution Rates of Polydisperse Powders. Pharm Res. 1993;10(9):1308–14.

16. Yee S. In vitro permeability across Caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man - fact or myth. Pharm Res. 1997;14(6):763–6.

17. Cristofoletti R, Dressman JB. Use of Physiologically Based Pharmacokinetic Models Coupled with Pharmacodynamic Models to Assess the Clinical Relevance of Current Bioequivalence Criteria for Generic Drug Products Containing Ibuprofen. J Pharm Sci. 2014;103:3263–75.

18. Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennernäs H, Hussain AS, et al. Molecular Properties of WHO Essential Drugs and Provisional Biopharmaceutical Classification. Mol Pharm. 2004;1(1):85–96.

19. Avdeef A, Box KJ, Comer JEA, Gilges M, Hadley M, Hibbert C, et al. PH-metric log P 11 . pK a determination of water-insoluble drugs in organic solvent – water mixtures. J Pharm Biomed Anal. 1999;20:631–41.

20. Prescott L. Kinetics and metabolism of paracetamol and phenacetin. Br J Clin Pharmacol. 1980;10(2 S):291S-298S.

21. Rodgers T, Rowland M. Physiologically Based Pharmacokinetic Modelling 2: Predicting the Tissue Distribution of Acids, Very Weak Bases, Neutrals and Zwitterions. J Pharm Sci. 2005;95(6):1238–57.

22. Kohlmann P, Stillhart C, Kuentz M, Parrott N. Investigating Oral Absorption of Carbamazepine in Pediatric Populations. AAPS J. 2017;19(6):1864–77.

23. Lukacova V, Parrott NJ, Lave T, Fraczkiewicz G, Bolger MB. Role of fraction unbound in plasma in calculation of tissue:plasmapartition coefficients. In: AAPS National meeting, Atlanta, November 15-20. 2008.

24. Clements J, Critchley J, Prescott L. The role of sulphate conjugation in the metabolism and disposition of oral and intravenous paracetamol in man. Br J Clin Pharmacol. 1984;18(4):481–5.

25. Laine JE, Auriola S, Pasanen M, Juvonen RO. Acetaminophen bioactivation by human cytochrome P450 enzymes and animal microsomes. Xenobiotica. 2009;39(1):11–21.

26. Mutlib AE, Goosen TC, Bauman JN, Williams JA, Kulkarni S, Kostrubsky S. Kinetics of acetaminophen glucuronidation by UDP-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15. Potential implications in acetaminophen-induced hepatotoxicity. Chem Res Toxicol. 2006;19(5):701–9.

27. Adjei AA, Gaedigk A, Simon SD, Weinshilboum RM, Leeder JS. Interindividual variability in acetaminophen sulfation by human fetal liver: Implications for pharmacogenetic investigations of drug-induced birth defects. Birth Defects Res Part A - Clin Mol Teratol. 2008;82(3):155–65.

28. Aarons L, Grennan DM, Siddiqui M. The binding of ibuprofen to plasma proteins. Eur J Clin Pharmacol. 1983;25(6):815–8.

29.   Obach RS. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and nonspecific binding to microsomes. Drug Metab Dispos. 1999;27(11):1350–9.

30.   Pavliv L, Voss B, Rock A. Pharmacokinetics, safety, and tolerability of a rapid infusion of i.v. ibuprofen in healthy adults. Am J Heal Pharm. 2011;68(1):47–51.

31.   Boase S, Miners JO. In vitro-in vivo correlations for drugs eliminated by glucuronidation: Investigations with the model substrate zidovudine. Br J Clin Pharmacol. 2002;54(5):493–503.

32.   Davies NM. Clinical pharmacokinetics of ibuprofen. The first 30 years. Clin Pharmacokinet. 1998;34(2):101–54.

33.   Cox SR. Effect of route of administration on the chiral inversion ov (R)-ibuprofen. Clin Pharmacol Ther. 1988;43(2):146.

34.   Cheng H, Rogers JD, Demetriades JL, Holland SD, Seibold JR, Depuy E. Pharmacokinetics and Bioinversion of Ibuprofen Enantiomers in Humans. Vol. 11, Pharmaceutical Research. 1994. p. 824–30.

35.   Hall SD, Rudy AC, Knight PM, Brater DC. Lack of presystemic inversion of (R)- to (S)-ibupreofen in humans. Clin Pharmacol Ther. 1993;53(4):393–400.

36.   Lockwood GF, Albert KS, Gillespie WR, Bole GG, Harkcom TM, Szpunar GJ, et al. Pharmacokinetics ibuprofen in man. I. Free and total area/dose relationships. Clin Pharmacol Ther. 1983;34(1):97–103.

37.   Abduljalil K, Pan X, Pansari A, Jamei M, Johnson TN. Preterm Physiologically Based Pharmacokinetic Model. Part II: Applications of the Model to Predict Drug Pharmacokinetics in the Preterm Population. Clin Pharmacokinet. 2019 Oct;

38.   Calvier EAM, Krekels EHJ, Johnson TN, Tibboel D, Knibbe CAJ. Scaling Drug Clearance from Adults to the Young Children for Drugs Undergoing Hepatic Metabolism : A Simulation Study to Search for the Simplest Scaling Method. AAPS J. 2019;21(38).

39.   Badée J, Qiu N, Collier AC, Takahashi RH, Forrest WF, Parrott N, et al. Characterization of the Ontogeny of Hepatic UDP-Glucuronosyltransferase Enzymes Based on Glucuronidation Activity Measured in Human Liver Microsomes. J Clin Pharmacol. 2019 Sep;59(S1):S42–55.

40.   Strougo A, Yassen A, Monnereau C, Danhof M, Freijer J. Predicting the "First Dose in Children" of CYP3A-Metabolized Drugs : Evaluation of Scaling Approaches and Insights Into the CYP3A7-CYP3A4 Switch at Young Ages. J Clin Pharmacol. 2014;54(9):1006–15.

41.   Edginton AN, Schmitt W, Willmann S. Development and evaluation of a generic physiologically based pharmacokinetic model for children. Clin Pharmacokinet. 2006;45(10):1013–34.

42.   Statelova M, Holm R, Fotaki N, Reppas C, Vertzoni M. 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. AAPS J. 2020;22(126):1–17.

43.   Hopkins CS, Underhill S, Booker PD. Pharmacokinetics of paracetamol after cardiac surgery. Arch Dis Child. 1990;65(9):971–6.

44. Walson PD, Halvorsen M, Edge J, Casavant MJ, Kelley MT. Pharmacokinetic Comparison of Acetaminophen Elixir Versus Suppositories in Vaccinated Infants (Aged 3 to 36 Months): A Single-Dose, Open-Label, Randomized, Parallel-Group Design. Clin Ther. 2013;35(2):135–40.

45. Statelova M, Holm R, Fotaki N, Reppas C, Vertzoni M. Factors affecting successful extrapolation of ibuprofen exposure from adults to paediatric populations after oral administration of a paediatric aqueous suspension. AAPS J. 2020; 22(146):1–17.

46. Brown RD, Wilson JT, Kearns GL, Eichler VF, Johnson VA, Bertrand KM. Single-dose pharmacokinetics of ibuprofen and acetaminophen in febrile children. J Clin Pharmacol. 1992;32:232–41.

47. Walson PD, Galletta G, Pharm D, Braden NT, Alexander L, Columbus BS. Ibuprofen, acetaminophen, and placebo treatment of febrile children. Clin Pharmacol Ther. 1989;46(1):9–17.