Biopharmaceutical considerations in paediatrics with a view to the evaluation of orally administered drug products – a PEARRL review.

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Abstract

Objective

In this review, the current biopharmaceutical approaches for evaluation of oral formulation performance in paediatrics are discussed.

Key findings

The paediatric gastrointestinal (GI) tract undergoes numerous morphological and physiological changes throughout its development and growth. Some physiological parameters are yet to be investigated, limiting the use of the existing *in vitro* biopharmaceutical tools to predict the *in vivo* performance of paediatric formulations. Meals and frequencies of their administration evolve during childhood and affect oral drug absorption. Furthermore, the establishment of a paediatric Biopharmaceutics Classification System (pBCS), based on the adult Biopharmaceutics Classification System (BCS), requires criteria adjustments. The usefulness of computational simulation and modeling for extrapolation of adult data to paediatrics has been confirmed as a tool for predicting drug formulation performance. Despite the great number of successful physiologically based pharmacokinetic models to simulate drug disposition, the simulation of drug absorption from the GI tract is a complicating issue in paediatric populations.

Summary

The biopharmaceutics tools for investigation of oral drug absorption in paediatrics need further development, refinement and validation. A combination of *in vitro* and *in silico* methods could compensate for the uncertainties accompanying each method on its own.

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Abbreviation list

ADME Absorption Distribution Metabolism and Excretion

AUC Area under the curve

BCS Biopharmaceutics Classification System

BW Body weight

BSA Body surface area

C_{max} Maximum plasma concentration

CYP Cytochrome P450

d Days

EMA European Medicines Agency

EFSA European Food Safety Agency

f_a Fraction absorbed

FDA Food and Drug Administration

GE Gastric emptying

GI Gastrointestinal

GST Glutathione S-transferase

ICH International Conference on Harmonisation

K_e Rate constant of elimination

MMC Migrating motility complex

NAT N-acetyltransferases

mo Months

pBCS Paediatric Biopharmaceutics Classification System

PEARRL Pharmaceutical Education And Research with Regulatory Links

PBPK Physiologically based pharmacokinetics

$t_{1/2}$ Half-life time

TIM TNO Gastro-Intestinal Model

t_{max} Time at which C_{max} is reached

PSA Parameter sensitivity analysis

SI Small intestine

SITT Small intestinal transit times

SULT Sulfotransferase

UGT Uridine 5'-diphosphate-glucuronosyltransferase

yr Years

wk Weeks

WHO World Health Organization

1. Introduction

In recent years, there has been an increased effort to improve safety and effectiveness of medicines that are specifically designed for paediatric patients [1-3]. Not only is it important to develop age appropriate medicines, it is also crucial to establish methodologies for evaluating the performance of a formulation as a function of age [1]. Understanding of the physiological and anatomical development of the human gastrointestinal (GI) tract is a demanding task and crucial for understanding the pharmacokinetics (PK) [1]. Absorption, Distribution, Metabolism and Excretion (ADME) can all be affected by the transformations that occur throughout childhood, hence in order to design better and more appropriate paediatric medicines, changes occurring from birth to adulthood need to be taken into consideration [4].

The International Conference on Harmonisation (ICH) has previously subdivided the paediatric population in several age groups (**Table 1)**. The ICH aims to harmonise guidance for regulatory agencies and industry. Europe, United States of America and Japan are regulatory founders of this initiative. The European Medicines Agency (EMA) follows the age subdivision proposed by the ICH, and further classifies children into pre-school children and school children. US Food and Drug Administration (FDA) endorses ICH age classification as one of the possible classifications, however, small differences in paediatric age groups can be found across literature including information from regulatory partners and health organisations. FDA's new draft guideline presents a different classification according to Centre for Drug Evaluation and Research [5]. A separate classification is also presented by World Health Organization (WHO) [6]. Differences between these classifications are small and reside on the days (d) until the sub-population "newborn" is considered, *i.e.* 27 days versus one month (mo). Other differences reside in how a child can be sub-classified and how the end of adolescence is described, *i.e.* 16, 18 or 20 years (yr). All paediatric subpopulations need to be considered in the drug development process. The more traditional methods for paediatric dosing, also

known as allometric scaling, are based on algorithms that allow estimation of doses by scaling adult values, based on comparison of parameters such as body weight (BW), age, and body surface area (BSA) [7]. These approaches do not account for maturation changes, such as ontogeny of enzymes and transporters [7], in comparison to more complex mathematical models, *e.g*. physiologically based pharmacokinetic (PBPK) modeling, which in certain cases might deliver a more adequate prediction of the appropriate paediatric dose.

BW and BSA differences between paediatric age groups and adults are presented in **Table 1.** Paediatric BW was retrieved from the 50th percentile boys and girls values in the Centre for Disease Control and Prevention (CDC) growth charts for paediatrics; adult 50th percentile BW values were obtained from clinical charts that include multiple races and a wide range of ages in U.S [8]. BSA values were calculated using the Mosteller formula ($BSA = \left(\frac{Weight \times Height}{3600}\right)^{\frac{1}{2}}$) [9]. Body height used for the calculations was retrieved from the same source as the respective BW. Newborns and infants are the age groups that show the highest differences compared to the adult population in terms of BW and BSA. The younger subpopulations show large differences in terms of physiological and anatomical factors. The absorption process in the younger subpopulations is highly influenced by the type of food ingested and the co-administration of medicine with food. The definition of a fasted state in newborns and infants is a difficult task and should be addressed with care in the design of *in vitro* experiments.

In this review, the parameters concerning paediatric oral drug absorption are explored. The current knowledge and considerations for the biopharmaceutical evaluation of orally administered drug products for paediatrics and the *in vitro* and *in silico* tools to help guide the development of appropriate paediatric medicines are discussed.

Please place Table 1 here

2. Paediatric nutrition

Nutrition represents a major determinant in body development, and maturation in paediatrics; moreover, certain nutritional patterns (*e.g.* duration of breastfeeding) have been associated with long-term health consequences, such as cardio-vascular disease prevalence [12]. Therefore, food components should be adjusted to the specific needs of each body developmental stage and health status, *e.g.* presence of chronic or acute diseases that alter the metabolic state, malabsorption of nutrient components, or food allergies and intolerances [12; 13]. Accordingly, meal properties and portions vary amongst the paediatric age groups. Eminent nutritional changes occurring during growth and maturation of healthy paediatric populations are addressed in the following section [14].

2.1. Age-dependent feeding: recommendations and practice

The most heterogeneous groups with regards to the meal type appear to be newborns and infants. International and national guidelines aim to harmonise global feeding practices, which can vary depending on food availability and cultural factors [15]. According to the WHO [16; 17], the European and the British guidelines [15; 18], newborns and infants younger than 6 months, should be exclusively breastfed or receive formula milk. A complementary meal should be added during the 6th month, followed by the introduction of "finger foods" by the 8th month. In contrast, according to the American and the French authorities weaning should begin between the 4th and 6th month, as the 4-month-old GI tract is able to assimilate soft foods [15; 19]. Food consistency increases along with the infant's ability to "munch" and chew. By the 12th month of age, infants can usually consume minced or chopped family foods and meal transition to common "adult" food should be completed by the age of two years [16]. Milk and dairy products remain an essential meal component throughout infancy [14; 17]. In practice, introduction of complementary food begins before the 6th month [20; 21]. Diverse studies report earlier access to solid or semi-solid foods, accompanied by usual overfeeding and disregarding recommendations on food composition [22-24].

2.2. Paediatric energy needs and feeding frequency

Average energy requirements for healthy individuals are derived from total energy expenditure, which is defined as the product of energy spent on activities and the resting energy expenditure. Equations obtained from regression analysis of measured resting energy expenditure from various subject groups are utilised for its prediction [25; 26]. Growth processes require additional energy for synthesis and deposition of new tissues. This parameter has been shown to have the highest relative contribution to total energy requirements in the first month of life (40%) and decreases to 3% during the 12^{th} month [25]. The European guidelines utilise the equations for resting energy expenditure for paediatrics proposed by Henry *et al.* [27]. Ultimately, different levels of physical activity are assigned to the paediatric groups: light, moderate, or heavy activity. The recommended daily caloric intake for European and American paediatric populations is shown in **Figure 1** [18; 26; 28; 29]. The non-linearity of the energy requirements as a function of age can be explained by the BW-based nature of the calculations behind them. The caloric needs of paediatric subpopulations increase with age towards adult values, and factors such as gender and physical activity, become more and more relevant over time [26]. According to the European Food and Safety Authority (EFSA) newborns, infants, and children up to four years of age are more likely to have a sedentary level of activity (**Figure 1A**), whereas older children and adolescents tend to show higher activity level (**Figure 1B**) [18]. The aforementioned energy requirements are estimated for average healthy individuals [26]; various health conditions, *e.g.* severe infections, fever, diarrhoea etc., would demand special treatment also with regard to nutritional amount and composition [30].

Please place Figure 1 here

The required number of meals depends on their caloric density [17]. Newborns should be breastfed at least 8 times during the day and night for 4 weeks (wk), starting at birth [31]. This frequency is also

reflected in current practice, whereby breastfeeding occurs 8 to 10 times daily [32]. Bergman *et al.* suggest a feeding interval of one hour, which may not be easily applicable in everyday life [33]. The recommended mother's milk or formula milk volumes and feeding intervals for infants are shown in **Figure 2** [12]. The feeding intervals for formula feeding and breastfeeding show differences until the second month of life, with shorter intervals being attributed to breastfeeding [33]. Infants receive complementary meals in addition to milk beginning in the 6th month (EU recommendations) [15; 34]. This would result in a narrower feeding interval for general feeds in comparison to the shown data, which only depicts milk feedings. The number of meals decreases with advancing age; adult meal frequency is recommended for children and adolescents: a three-times daily meal, accompanied by one snack [16]. Recently, the following feeding frequencies for paediatrics were reported by Johnson and colleagues: from birth to six months individuals receive six feedings daily, from six months to one year - five feeds, and beyond one year of age four feeds [35].

Please place Figure 2 here

2.3. Water and fluid intake

Water (fluid) intake is required in order to maintain normal hydration status through compensating for body water losses; these occur mainly by urinal and faecal excretion and evaporation via skin and lungs [36]. Newborns and infants differ from children and adults in their water needs due to their tissue composition, *e.g.* greater total body water contents, greater BSA/BW ratio, lower sweating capacity and limited concentrating ability of the kidneys. Higher daily fluid volumes normalised per BW are attributed to younger age-groups compared to older children and adults [35]. The younger populations obtain water mostly through the consumed food [37]. During the first days after birth, a healthy newborn receives only breast milk. Measurements of urine osmolality have shown adequate hydration status in *ad libitum* breastfed newborns and infants without a necessity for additional water [38; 39]. On the contrary, formula-fed newborns and infants require 400 - 600 mL of water per day in addition

to the water consumed from milk; these needs can be explained by the greater renal solute load of cow's milk infant formulae compared to human breast milk, 97 mOsmol/kg and 307 mOsmol/kg for breast milk and cow's milk, respectively. European recommendations on water intake are based on water needs per consumed calories and observations of water intake in populations with adequate urine osmolality values. Water intake reference values for healthy individuals from the paediatric population as reported by EFSA are presented in **Figure 3** [36]; the reported amounts include water present in foods and other fluids administered throughout the day. Higher water intake is attributed to males compared to females beginning at the age of 9 years.

Please place Figure 3 here

Although juices can be introduced to infants at the age of 1 year, intake should be limited [40; 41]. In France, the fluid consumption of children and adolescents amounts to 1.0 - 1.1 L/day, with water being the most common drink, followed by dairy drinks and juices [42]. Water requirement in adolescents and adult populations are mainly shaped by the physical activity level and health status [36]. Paediatric daily fluid requirements in a hospitalised setting tend to be lower than those for healthy populations; fluid reference values are usually acquired by the Holliday-Seger method (calculation that takes basic metabolic caloric expenditure, caloric exhaustion determined by the physical activity level under hospitalised conditions, corrected by urinary and insensible water loss into account). Paediatric populations undergo dynamic physiological development; this is taken into account by dividing the fluid requirements according to three BW bands: patients under ten kilograms, up to and beyond twenty kilograms of BW [43].

2.4. Food composition

Human breast milk undoubtedly offers the optimal macro- and micronutrients composition for newborns and infants [17]. The composition of breast milk changes rapidly: the first milk, colostrum, undergoes compositional alterations from the fifth to fifteenth day postpartum (intermediate milk) to reach mature milk composition in the third week after birth [44; 45]. The major differences between colostrum and mature milk are the notably decreased protein content and increased fat fraction, as indicated in **Table 2** [44]. The high protein content measured in human breast milk (14% from the total caloric content) might not be of nutritional value, as it has been previously reported to contain high levels of non-digestible lactoferrin and IgA [44; 45]. A great variability with regard to macronutrient contents and amounts have been observed for breast milk in relation to the maternal health background and diet [46; 47]. Formula milk development is based on the properties of human breast milk. Accordingly, these two types of milk exhibit similar macronutrient composition, which is shown in **Table 2** [45; 47]. Furthermore, regulations ensure the appropriateness of the essential macro and micronutrients in marketed infant formulae in the EU [45]. The proportions of casein to whey-proteins, lipid composition, fat-globule structure and size, and milk origin, (*e.g*. soy or cow's milk) are variable among different formulae and not equal when compared to human breast milk [48; 49]. The presence of bile salts in human breast milk, but not in formula milk, should be considered as an additional potential factor that might affect oral drug absorption [48]. Unmodified cow's milk contains higher protein fraction than human breast milk, hence the earliest administration of fortified full-fat cow's milk should only occur after the first year of age [38]. It is interesting to note that proteins account for less than 10% of the calories in human breast milk and infant formula milk. Carbohydrates represent the main energy source in complementary foods, while fats contribute less to the total caloric content when compared to breast milk. The protein fraction in infants' weaning foods depends on the meal type (**Table 2**). From children to adults, the meal protein content increases, while the fat content

291 decreases. Carbohydrates reach adult recommended levels already in the meals for infants (45 - 65%)
292 (**Table 2**).

293 *Please place Table 2 here*
294

295 **2.5. Physicochemical properties of meals and beverages**

296 Foods for infants differ from adult meals regarding their texture and physicochemical properties. The
297 properties of 15 commonly used soft foods, juices, and suspensions (vehicles) have been investigated
298 for their physicochemical characteristics (**Figure 4**) [55]. Formula milk exhibits greater viscosity than
299 juices and cow's milk. The viscosity of meals for different paediatric populations becomes greater with
300 increasing age, *i.e.* milk formula versus soft foods. Juices and "fruity vehicles" show acidic pH values,
301 which in some cases can compromise drug stability [55; 56]. Milk types exhibit different buffer
302 capacity and osmolality, which might result from addition of excipients (*e.g.* sugars, lecithin) in
303 flavoured milk compared to cow's milk (**Figure 4B and 4C**). In agreement with the similar
304 macronutrient composition of human breast milk and formula milk, similar pH and osmolality values
305 were found in the literature for human breast milk, pH of 6.8 and osmolality of 290 - 299 mOsmol/kg
306 [57], when compared to the values presented in **Figure 4**. Recently, the physicochemical properties of
307 26 types of soft foods and beverages available on the EU and USA market were investigated [56]. A
308 significant difference among formula milk types was reported for the surface tension of the three tested
309 products (Formula First Milk, Formula Soya Infasoy®, and Formula Soya Wysoy®) [56]. Differences
310 among milk types and yogurts, *e.g.* soy, plain product, and flavoured product, were observed for the
311 measured buffer capacity, osmolality, surface tension, and viscosity. Variability among different
312 brands of applesauce and blackcurrant squash available on different markets (*i.e.* UK, Germany, and
313 USA) was shown in their buffer capacity, osmolality, surface tension and viscosity; some of these
314 reported differences are probably related to the different amount of sugars added to the products [56].
315 Currently, food-effect bioavailability and fed state bioequivalence studies for paediatric drug product
316 are performed in adults, under conditions that comply with the recommendations provided by the US

FDA and EMA with a high-calorie, high-fat standard adult breakfast as a meal for the fed state investigation [52; 53]. The physicochemical properties of the FDA/EMA standard breakfast (**Table 2**) [58] deviate from the physicochemical properties of the tested vehicles for paediatric use in terms of pH values, viscosity, and osmolality (**Figure 4**). Although some trends can be observed from the available data for the reported soft foods and drinks, e.g. fruit juices, dairy products, formula milk and milk types, further investigation of the product variability between different brands with focus on their physicochemical characterisation might be of interest.

Please place Figure 4 here

3. Physiological and anatomical changes in paediatrics

Growth and maturation continuously take place from birth to adulthood. These processes, which govern paediatric development, are fastest in the youngest paediatric subpopulations (newborns and infants). As previously mentioned, BSA and BW increase significantly during the first year of life **(Table 1).** Furthermore, changes in body composition take place. A decrease of body water and an increase of lipid and protein are seen throughout development [60; 61]. Therefore, younger populations, such as newborns and younger infants, present higher extracellular water contents [60]. Physiological and anatomical age-related changes in the GI tract are capable of influencing oral drug absorption processes, such as rate and extent of drug absorption [61-64]. In the following sections, the main changes in the GI tract that may influence the pharmacokinetics following oral drug administration in paediatric populations will be discussed.

3.1. Gastrointestinal volumes

Gastric volumes in the fasted state are most often reported as a function of BW (**Table 3**), with similar volume values reported across the different ages. Values of gastric volumes were selected if no clear

fluids (e.g. water, tea, clear apple juice) had been administered for at least 2 h or more, and constraint of solid food/semi-solid food/other fluids lasted for a minimum of 4 h prior to the gastric volume measurement. Nevertheless, studies have shown that small volumes (less than 2 mL/kg) of clear fluids (such as water, tea and others) are not expected to affect measurements of gastric volume within a 2 h period [65]. Literature studies have evaluated the fasted gastric volume across the paediatric subpopulations, and no clear age distinction among the studied subpopulations (newborns, infants and children) is reported. Maekawa *et al.* also reported that ingestion of higher volumes (10 mL/kg of BW) of fluids (apple juice) ingested up to 2 h before measurements are not expected to affect gastric volume [66].

Please place Table 3 here

In the paediatric population, it is more likely that the medication is dosed with food. Considering that the youngest subpopulations are mainly in the postprandial state, due to the higher frequency of food intake, food will most likely already be available in the stomach [48]. Following the ingestion of food, the stomach content can increase significantly (up to 50 fold), and stomach capacity volumes can range from 10 to 100 mL in newborns, 90 to 500 mL in infants, 750 to 960 mL in children, and 1500 to 2000 mL in adolescents and 3000 mL in adults [78]. For the youngest sub-populations, the gastric volume in the fed state will be mainly represented by the volume of the food ingested [35]. Gastric volume in children measured 3 h after administration of drinks (orange squash, maximum 200 mL) and of drinks and biscuits (orange squash, maximum 200 mL and two plain biscuits) was 0.39 mL/kg and 0.46 mL/kg, respectively (compared to 0.25 mL/kg measured after 7 h fasting) [70].

Roman *et al.* investigated the effect of gastric secretions on gastric volumes in premature newborns (n = 9, ~5 wk postnatal age), by assessing the difference between residual meal volumes, and total gastric content volumes after ingestion of human milk and infant formula [79]. Volumes of gastric contents were determined by aspiration from 0 - 180 min after meal ingestion, and residual meal

368 volumes were calculated by the difference between initial meal volume and gastric emptying (GE).
369 Gastric secretions were a significant contributing factor of gastric contents in the fed state: 32%, 28%,
370 and 43% v/v at 30, 60, and 90 min following feeding, respectively. A separate study showed that
371 volumes of gastric secretions corresponded to 2.0 ± 1.4 mL/kg BW in newborns (n = 8, 4 - 24 wk) in
372 the first postprandial hour [80]. Smaller contributions of gastric secretions to total gastric volume
373 (1 mL/kg in 30 min following meal intake) have also been reported in premature newborns (n = 10,
374 1 - 9 wk postnatal age) [81].
375 The gastric volume after administration of three types of food (*i.e.* human milk 18.4 ± 0.5 mL/kg;
376 SMA-SP® formula 17.4 ± 0.5 mL/kg; and Similac SC® formula 17.0 ± 0.7 mL/kg) to newborns and
377 infants (1 - 11 wk) was measured at 10, 30, and 50 min after food intake [82]. Ten minutes after feeding
378 the volume ranged from 10 to 13.5 mL/kg and after 50 minutes there was still a volume of 4 to 6 mL/kg
379 present in the stomach [82]. Based on these studies, a mean feeding volume of newborns and young
380 infants of 23.5 ± 4.2 mL/kg has been suggested [48]. No information was found on intestinal volumes
381 across paediatric subpopulations.
382
383 **3.2. Gastrointestinal fluid composition**
384 In paediatrics, fasted gastric pH is widely described as being neutral moments after birth, ranging from
385 values of 6 to 8, mainly due to amniotic fluid ingestion [83; 84]. Contradictory information has been
386 reported with regards to the time after birth which is needed to reach acidic pH values. Nevertheless,
387 reviews of original reports show that fasted gastric acidic pH values of 1.5 to 3 are reached hours after
388 birth, up to the first two weeks of life [48; 63; 85; 86]. A summary of the pH values of GI contents of
389 paediatric population and of adults is presented in **Figure 5**.
390
391 *Please place Figure 5 here*
392

Newborns and young infants are mainly fed with milk, whether it is breast milk or different types of formulae, which can have an impact on several characteristics, including fed gastric pH. Studies have reported that pH values over 4 were detected more frequently in newborns and infants than in older children [79; 106; 107], mainly due to feeding patterns in this subpopulation and the high buffer capacity of breast milk and formulae [106; 108]. Comparison of two separate studies (adults vs. newborns) of continuously monitoring of the fed gastric pH showed that 2 h after a meal, higher fed gastric pH values (0.7 - 1.8 units) were found in newborns (2 - 15 d) [109]. The meal ingested by adults consisted of a standard solid meal (1000 Kcal), opposed to newborns where formula milk was ingested (14.5 - 29.0 mL/kg per feeding) [98; 99]. It should be noted that the interpretation of pH in the fed state is difficult, as differences might simply arise as a function of meal composition, or the time interval after intake of the meal and the measurement.

Available data on fasted and fed intestinal pH indicates high variability of measured values, for both adults and paediatric age groups, and that similar intestinal pH values are seen in the two groups (**Figure 5**). Children and adolescents (n = 12, 8 - 14 yr) present similar fasted intestinal pH, ranging from 6.4 - 7.4 [94], and similar mean fed intestinal pH values of 6.3 (n = 16, 7 - 16 yr) [105]. Fasted intestinal pH in newborns (n = 10, 1 - 25 d) has been studied by Fallinborg *et al.*, and mean pH values were 6.5 [94]. Newborns and infants (2 wk - 3 mo, breastfed and formula-fed) also seem to present similar fed intestinal pH profiles compared to adults, with values ranging from 6 to 7 in the duodenum [110]. Nevertheless, studies concerning intestinal pH in both fasted and fed states are scarce, especially for newborns and infants, and limit conclusions. Furthermore, the variety of techniques used to measure the pH (*i.e*. pH electrode measurements of enteric aspirates, in situ pH electrode measurements, or radio transmitting pH-sensitive capsule), could attribute to the observed variability of the measurements.

418 The concentration and composition of bile salts vary with age. Total duodenal bile salts concentrations
419 [48; 109] are usually reported as a small pool of bile salts in newborns and infants when compared to
420 adults, and lack in secondary bile salts [48; 111]. In the younger populations (newborns and young
421 infants), tauro-conjugation of bile acids is predominantly detected, with glycol-conjugation and
422 glycine conjugates reaching adult levels by 7 to 12 months of age [112]
423 High variability with respect to fasted bile salt levels in the small intestine (SI) of newborns and young
424 infants has been identified [48; 109]. Fasted bile salt levels in duodenal aspirates have been shown to
425 increase continuously during the first 60 days of life in breastfed infants, from 2 mM to 8 mM (n = 41,
426 mean 4.4 ± 2.0 mM) [48]. The effect of breastfeeding compared to formula supplemented with
427 different amounts of taurine and cholesterol has been investigated [113]. Total bile salt concentrations
428 were evaluated in the fasted state, in duodenal aspirates of 65 pre-term newborns
429 (31 - 36 gestational age), while higher bile salt concentrations were found in breastfed newborns. In
430 breastfed newborns, the concentrations increased from ~5 mM (1 wk postnatal) to ~8 mM (5 wk
431 postnatal) [113]. Signer *et al.* found that premature newborns (n = 9, 14 d) fed with cow's milk
432 formula, exhibited higher total bile acid concentration in duodenal samples, when compared to
433 breastfed newborns (n = 9, 14 d), in both the fasted (8.8 mM vs 3.8 mM) and fed state 60 min following
434 feeding (4.4 mM vs 1.9 mM). Nevertheless, this was attributed to the difference in gestational age
435 between the two groups (breastfed: 35 wk vs. cow's milk formula: 37 wk) [114]. Investigation of the
436 effect of administration of a test meal [carbohydrate (4%), protein (4%), and fat (4%)] was performed
437 by Harries *et al.*, duodenal aspirates were collected 2 h after administration of a meal to 13 infants and
438 children (1.3 - 16.3 yr, mean 3.3 yr), and revealed fed total bile salt concentration values of 7.4 mM
439 (range of 3.0 - 16.0 mM) [115]. Comparison of total bile salts concentration between pre-term
440 newborns (2 wk postnatal age) and infants/children (3 mo - 6 yr), revealed lower concentrations of bile
441 salts in the younger groups. Newborns were divided into two groups, where different types of milk
442 were administered (evaporated milk vs modified milk), and older children received a test liquid feed

443 (containing corn oil, glucose, polyethylene glycol-4000 and water). Fed total bile salt concentration
444 was measured in duodenal aspirates and values were ~1 mM (evaporated milk) and ~0.5 mM (modified
445 milk), and ~5.9 mM in the older group [116]. A linear trend was recently established between the
446 logarithm of age and bile salt concentration data collected from available studies of fed state duodenal
447 bile salts concentration of newborns and infants (R^2 = 0.54, 7 paediatric studies and 5 adult studies)
448 [109]. Based on this, mean fed intestinal bile acid concentration was found to be approximately
449 2.5 mM for newborns and 7.5 mM for infants.
450
451 The role and importance of digestive enzymes in newborns and infants has been described in a recent
452 review [48]. A summary highlighting the differences of relevant digestive enzymes between adults and
453 paediatrics will be discussed in this review. The following enzymes have been proven to be essential
454 for the digestion and lipolysis in newborns and infants: human gastric lipase, pancreatic triglyceride
455 lipase (and colipase), carboxylester hydrolase, pancreatic lipase-related protein 2, and bile salt-
456 stimulated lipase [48]. Human gastric lipase is a pre-duodenal lipase which is responsible for
457 intragastric lipolysis in newborns, its expression is fully matured at birth and its activity in the stomach
458 is similar to adults [48]. Pancreatic triglyceride lipase plays a major role in the lipid lipolysis process
459 in adults. Its activity in the fed state has been shown to be lower in newborns, possibly due to dilution
460 of enzyme levels in response to high frequent feedings in the younger subpopulations, contrary to what
461 happens in adults, where enzyme secretion is stimulated by the presence of macronutrients [48]. The
462 expression of carboxylester hydrolase and pancreatic lipase-related protein 2 is not fully developed at
463 birth [48].
464
465 Pepsin is a protease secreted by the stomach and its expression is not fully matured at birth [48]. Lower
466 pepsin secretions have been reported in younger cohorts, such as newborns and infants less than one
467 year of age, compared to older children and adults [92]. Fasted gastric pepsin concentrations in younger

newborns (birth and 8 d of postnatal age) appear to be approximately 15% of adult values, while older newborns (10 - 32 d) and infants (67 - 110 d) express similar mean concentrations of approximately 41% of the adult values [109]. Similarly to pepsin, trypsin expression is not matured at birth, and lower concentrations have been reported in newborns and infants when compared to children and adults [48]. In summary, pancreatic enzyme concentrations are lower at birth and appear to reach mature levels by one year of age [63].

Limited information is available on osmolality and buffer capacity of paediatric GI fluids. A positive linear correlation has been reported between the osmolality of the diet as a function of the osmolality observed in the stomach and duodenum in 15 low-birth-weight newborns monitored for three hours after food ingestion [117]. Maharaj *et al.* built a linear regression model for a 60 min postprandial period (R^2 = 0.95, n = 8 separate feeds) to predict neonatal fed gastric osmolality based on results obtained from Billeud *et al.* [109; 117]. The predictions were compared with a separate study in which osmolality was measured after three separate breast milk feeds fortified with minerals/supplements [118]. As an example, after a feed with an osmolality of 344 mOsmol/kg, the corresponding measured fed gastric osmolality at 60 min was of 354 mOsmol/kg, and the predicted osmolality was 327 mOsmol/kg, with 7.6% under-prediction error. The developed model predicted fed gastric osmolality within one hour after feeding, whereby the time period was selected to reflect the high frequency of feeding in paediatric populations. The same approach was used to predict fed state duodenal osmolality (R^2 = 0.92, n = 8 separate feeds). Due to scarcity of data in paediatrics, predictions were validated against two adult studies reported by Kalantzi *et al.* and Clarysse *et al.* Measured duodenal osmolality values were 405 and 392 mOsmol/kg, 60 min following administration of liquid meals characterised by an osmolality of 610 and 670 mOsmol/kg, and predicted osmolality were adequate with values of 430 (6% over-prediction) and 454 (16% over-prediction) mOsmol/kg respectively [97; 119]. In newborns and young infants, buffer capacity of the fed gastric fluids is likely to be similar to the buffer

capacity of the administered food, as the volume of fasting gastric contents is small, and therefore unlikely to have an impact on the buffer capacity of the fed gastric fluids [109]; especially in the younger cohorts, where the frequency of meals is higher when compared to older children and adults.

3.3. Gastric emptying

Newborns and young infants have slower GE rates when compared to older children and adults [64; 84; 120]. In the fasted state, migrating motility complex (MMC) is responsible for the regulation of the GE rate [121]. Non-nutrient liquids do not normally interfere with the MMC [122]. The gastric emptying half-life ($GEt_{1/2}$), is reported to be 6.9 min for a liquid non-caloric meal (5 mL/Kg) in newborns (1 – 8 d), measured by epigastric impedance using four electrodes [123]. The use of other techniques for the measurement of GE of liquids have shown higher values, Euler and Byrne measured emptying rate of distilled water by the dilution marker technique and reported the mean $GEt_{1/2}$ to be 15 minutes after administration of 20 mL/kg of water to infants (2 - 24 mo) [124]. Administration of 20 mL/kg of tap water to children (mean age 8.25 ± 2.24 yr) led to a mean $GEt_{1/2}$ of 27.1 min when measured by the ultrasound technique [124].

In the fed state, the dependency of GE on meal type and composition, meal volume and osmotic pressure has been described [84; 85; 125; 126]. In a recent meta-analysis of mean gastric residence time studies showed that GE was not affected by age and confirmed the importance of food in influencing GE rates [121]. Aqueous solutions (without calories) empty faster than liquids containing fat or protein, such as milk. Milk, the main food type for newborns and infants, empties faster than common solid foods that are ingested by older children and adults. It should be noted, that newborns and infants are the paediatric populations most likely to show differences in the fed state when compared to adults, due to the differences in meal types, but also because of the high frequency of feedings in the youngest subpopulations. Differences in composition of breast milk and formula result in faster GE of breast milk [121]. $GEt_{1/2}$ was affected by administration of equal volumes of breast

518 milk compared to infant formula in newborns and infants (4 wk - 6 mo) [127], where $GEt_{1/2}$ was 48 ±
519 15 min, and 78 ± 14 min, respectively, indicating that infant formula empties at slower rates than breast
520 milk. The faster emptying of breast-milk was also reported by Ewer *et al.* who compared $GEt_{1/2}$ of
521 breast-milk (36 min) and formula milk (72 min) in pre-term newborns (n = 14, postnatal age 4 - 26 d)
522 [128]. Staelens *et al.* compared GE in infants (n = 17, 2 d - 3 mo) fed with intact protein formula (Nan
523 1, Nestle®), a partially hydrolysed formula (Nan H.A.1, Nestle®), and an extensively hydrolysed
524 formula (experimental formula); $GEt_{1/2}$ was 55, 53 and 46 min, respectively [49], confirming that faster
525 fed GE was observed following ingestion of protein hydrolysate formula, when compared with a
526 formula containing native cow's milk protein, and also that the extent of dairy protein hydrolysis may
527 affect GE. Casein-predominant feeds (typical for cow's milk products) have also been showed to
528 empty slower than feeds with a greater whey fraction, but the authors highlighted that different
529 methodology, food compositions and patient groups, limit the validity of the conclusions [129]. A
530 summary of $GEt_{1/2}$ studies is presented in **Figure 6**. The use of various techniques for the $GEt_{1/2}$
531 measurement may be associated with the observed variation. Increments of GE variability as a function
532 of age in **Figure 6,** can be attributed to a broader spectrum of food types ingested by the older
533 populations (*i.e.* caloric density).
534
535 *Please place Figure 6 here*
536
537 **3.4. Small intestinal transit times**
538 Analysis of available literature concerning small intestinal transit times (SITT) as a function of age,
539 indicates that there are no significant differences in SITT across ages and that the measurement
540 technique can have an impact on the estimated SITT value [134]. A limiting factor from the study
541 resides in the low number of paediatric patients included in the analysis; namely only one newborn
542 (0 - 30 d); one infant (1 mo - 2 yr); three young children (2 - 5 yr); 10 children (6 - 12 yr); and one

543 adolescent (12 - 18 yr) were present from a total of 52 subjects (16 paediatric subjects compared to 36
544 adults). Therefore, conclusions might change if data from a greater number of newborns and infants
545 was available to be included in the analysis [134].
546 The International Commission on Radiological Protection (ICRP) publication 89 also reports SITT to
547 be independent of age and type of meal ingested with a mean value of 3.9 ± 1.5 hours and recommends
548 the adoption of a reference value of 4 h for males and females of all ages. These results were obtained
549 from a meta-analysis of data derived where several techniques were used [135]. In conclusion,
550 although differences between measuring techniques have been previously reported [84; 134], SITT is
551 generally considered independent of age [48; 85].
552
553 **3.5. Intestinal surface area**
554 The intestinal surface area is related to both radius and length of the intestinal segment [84]. The length
555 of the intestine changes with growth, ranging from approximately 275 cm at birth, 380 cm at 1 year,
556 450 cm at 5 years, 500 cm at 10 years, and 575 cm at 20 years [136]. The radius of the SI also naturally
557 increases with age, and ranges from approximated values of 1.2 - 2.6 cm in newborns, compared to
558 values of 3 to 6 cm in adults [135]. Since both intestinal length and radius increase with paediatric
559 development, the functional surface area can increase significantly [137]. Furthermore, specific
560 morphological features on the luminal surface, such as folds, villi and microvilli, naturally increase the
561 surface area available for absorption [138]. SI villous patterns start developing at an early stage of
562 gestation. The growth of these features occurs by crypt hyperplasia and crypt fission (a process where
563 the crypts unzip and duplicate). Cummins *et al.* studied these mechanisms and showed that crypt
564 fission occurred predominantly during infancy, and crypt hyperplasia occurred during both infancy
565 and childhood [139; 140]. Mean crypt fission rates in newborns, infants, children and adults were
566 7.8%, 15%, 4.9%, and 1.7%, respectively. The peak of crypt fission was found to be 18% in 5 infants
567 from 6 to 12 months of age. Villus height, measured in biopsies of younger children, exhibits lower
568 values compared to healthy adults, while the crypt depth has been shown to be greater in young

children [63; 141]. Newborns show elongated small finger-shaped villi and small crypts, with leaf-shaped villi appearing from one month after birth [140]. Feeding has been described as a modulating factor of differences in villi structure between newborns and infants, where smaller crypts have been described for those fed with breast milk, when compared to those fed with formula milk [140], whereas other literature has described villi as single projections in children younger than three years, with development of leaf or finger-shaped villi above this age [84]. Reports concerning the development of these features in early childhood are conflicting and provide a rather qualitative type of information [84]. Overall, comparison of newborns and infants with older children and adults, shows presence of lower intestinal surface area, with differences in both structure and quantity of the villi [84].

3.6. Intestinal permeability

Intestinal permeability is high at birth for preterm infants, with a decrease to adult values over the first week of postnatal life [142-144]. Nevertheless, both decreases and increases in permeability during the first month after birth have been reported, which might be attributed to several factors, such as differences in gestational age, clinical condition, feeding regimen, and postnatal age at the time of assessment [145]. It is unclear at which age full maturation of permeability processes is reached [142]. Children over 2 years of age present similar permeability values to adults [83; 146; 147]. Additionally, processes involved in passive and active transport are fully developed in infants by ~ 4 months old [137; 142]. Growth factors, hormones, breast milk and changes in the thickness and viscosity of the intestinal mucus, have been described as factors underpinning the development of permeability processes [145].

Intestinal permeability and influence of the type of feeding, have been evaluated with dual sugar test, lactulose and mannitol, and creatinine. No differences in intestinal permeability were found between infants fed with breast milk, and standard cow's milk formula, nor when different types of formulae

were compared [148]. Lower permeability is often linked to ingestion of human milk, due to the presence of bioactives [145]. Stratiki *et al.* showed that infant cow's milk formula supplemented with bifidobacteria tended to decrease intestinal permeability [149; 150].

Recently, intestinal influx oligopeptide transporter peptide transporter 1 (PEPT1) was studied to understand how the disposition of substrates of this transporter changes with age. The expression and tissue localisation across the paediatric age range were investigated by analysing intestinal samples (n = 20 newborns/infants, n = 2 children, n = 4 adolescents). Lower mRNA expression levels of PEPT1 was observed in newborns/infants opposed to older children, nevertheless, the difference was small and the distribution in intestinal tissue of the transporter was similar. Therefore, similar absorption profiles with respect to PEPT1 transporter substrates are expected in the paediatric subpopulations and adults [151].

Contradictory literature can be found on the ontogeny of the efflux transporter P-glycoprotein (Pgp), also referred to as multidrug resistance protein-1 (MDR1) [137; 142]. Mooij *et al.* studied the gene expression of several hepatic and intestinal drug transporters. Intestinal mRNA expression of MDR1, MRP2, and OATP2B1 was determined in surgical small bowel samples (newborns, n = 15; infants, n = 3; adults, n = 14), and expression values for MDR1 and MRP2 were similar to the values in adults. Intestinal OATP2B1 expression in newborns was significantly higher than in adults [152]. The methodology should be considered and results should be carefully interpreted with regard to mRNA data, which may not be entirely representative of transporters' protein expression or activity [153].

Quantitative data on paediatric intestinal permeability is limited [48; 142; 146]. The need for further research in the field of drug transporters in the paediatric populations has been highlighted [154]. Some of the factors that may interfere with studies on drug transporter activity are disease, drug-gene interactions, drug-drug interactions, food-drug interactions, and exposures to environmental chemicals [154]. Access to high-quality tissue samples in the paediatric population is limited. Current tissue sources include left-over tissue from surgery and biopsies and post-mortem tissue from organ

transplants and autopsies. Issues arising from the current samples used are the periods between sample collection and death of the subjects as well as the available sample size. Additionally, acquiring parent's consent for autopsy is challenging. Development of methodologies, which will enable quantitative measurement of transporter proteins using small biologic samples, would contribute to gain insight into ontogeny trajectories of various transporters [155]. Furthermore, the development of a paediatric biobank of healthy tissues would improve research on the ontogeny of transporters and metabolic enzymes [156].

3.7. Metabolism

The intestine and liver are the two main sites for metabolism of drugs. The activity of drug metabolising enzymes is low at birth and reaches adult levels by early childhood [142]. In older children, due to a larger liver size and higher hepatic blood flow, when normalized per BW, increased hepatic clearance is observed, even if enzyme activity is described as similar to adults [142].

Drug metabolism in the gut lumen is characterised by the presence of intestinal microbiota, with changes in bacterial colonisation affecting drug absorption [63; 157]. Microbiota is present right after birth [142]. A wide variety of factors influence the patterns and extent of microbiota colonisation of the gut, including gestational and postnatal age, mode of birth, type of food, *etc.* [63; 158]. The intestinal microflora of the infants' intestine start to resemble adults' one at the end of the first year of age [145], but full maturation is only reached between 2 and 4 years of age.

Ontogeny of intestinal wall metabolism requires further investigation [142], with infants and children being the age groups with less information available [63; 142]. Reports of enzyme ontogeny describe changes in mRNA, protein, and activity levels [106]. In adults, cytochrome P-450 enzymes (CYPs) are mainly represented by the CYP3A4 and CYP3A5 [142]. In paediatrics, more information is needed about CYP intestinal enzymes to draw a conclusion. The mRNA expression of CYP3A4 and CYP3A5 decreases with age, although protein expression increases significantly with age [106]. Ontogeny of

these enzymes remains to be elucidated [63]. Age-dependent changes of other metabolic enzymes responsible for gut wall metabolism have been reported [142]; for example, the intestinal activity of Glutathione S-transferase alpha 1 (GSTA1-1) is significantly greater in paediatric patients younger than 5 years (as estimated by intestinal biopsies) compared to adults and older children. Sulfotransferase (SULT) mean activity values were three times higher in foetal intestinal tissues compared to adults [142]. However, not all metabolic enzymes are reported to change as function of change, for example intestinal alcohol dehydrogenases maintain the same expression levels throughout infancy and adulthood [142].

The ontogeny of hepatic metabolic enzymes has been studied more broadly than intestinal metabolism. Regarding CYPs, low levels are seen in younger paediatric subpopulations. Adult values start to be reported from 1 - 5 years depending on the isoform [142].A recent examination of CYPs' hepatic expression, activity and abundance as a function of age have reported greater enzyme activity and abundance for enzymes of the CYPA1-3 families after birth, except for the isoform CYP3A7 [159]. When compared to postnatal samples, a different trend is seen, in which activity is higher than abundance [159]. The evaluated samples represented the subpopulations of newborns and infants (< 1 yr, n = 6), a juvenile group (1 - 18 yr, n = 10), and the adult population (>18 yr, n = 9); the lack of differentiation among the juvenile group, hinders the formation of a firm conclusion on age-dependent metabolic activity in this group [159]. In general, infants and juvenile groups, displayed high enzymatic abundance accompanied by a lower activity, when compared to adults [159]. Moreover, other hepatic metabolic enzymes have shown age-dependency, such as Uridine 5'-diphosphate-glucuronosyltransferase; SULT; N-acetyltransferases.

More research in the field of the ontogeny of metabolic enzymes is still required. More paediatric subpopulations should be addressed, such as infants and children. Intestinal gut metabolism should be further studied in order to give clarity on how gut wall enzymes change with age. Changes in enzyme expression and activity can result in profound differences in production of metabolites that are not

obligatory encountered in adults [142]. As for permeability, measurement techniques should be considered when interpreting the results, as mRNA information might not be able to predict changes in levels of activity and protein expression. Literature reports should, therefore, be interpreted carefully, and methods such as protein quantification, such as targeted liquid chromatography-tandem mass spectrometry, and functional assays with *ex vivo* material should be preferred [63; 153].

4. Paediatric Biopharmaceutics Classification Systems (pBCS)

The introduction of the Biopharmaceutics Classification System (BCS) by Amidon *et al.* in which drugs are divided into four categories based on their solubility an permeability, set the foundation for evaluation of oral drug absorption in the fasted state [160]. Since its establishment, the BCS' role has evolved into a useful regulatory framework, which allows extrapolation of drug product bioequivalence, in specific cases, based on *in vitro* dissolution experiments, and the correlation to *in vivo* drug product performance, also known as BCS-based biowaiver [142; 161]. Additionally, the key role of BCS in early drug development is undeniable as part of the decision making on salts and polymorph form selection and timing of dedicated studies, support of formulation decisions in pre-clinical animal models, and drug formulations intended for humans [162].

A recent survey, conducted among experts in the field of paediatric biopharmaceutics, confirmed the need of a Paediatric Biopharmaceutics Classification System (pBCS), outlined current trends, possible criteria for its establishment, and prioritised the areas of insufficient knowledge that need to be further explored [147]. Division of the paediatric population into 4 - 7 subpopulations has been proposed, with the question of the appropriateness of a further breakdown of the covered age rages [156; 163]. The challenges towards the pBCS criteria establishment and the possible approaches for setting the classification criteria will be discussed in the following subsections.

694 **4.1. pBCS solubility classification criteria**

695 The three key factors that define the solubility classification of a drug (the highest dose strength, the
696 initial gastric volume which is available upon drug arrival, and the solubility of the drug) vary amongst
697 all paediatric subpopulations. Paediatric dose determination can be based on various calculations
698 *(i.e.* allometric or isometric scaling) or on clinical observations [164; 165] and can, therefore, result in
699 different recommendations for each specific paediatric subset.

700

701 The paediatric initial gastric volumes have been calculated by a BW-extrapolation method based on
702 the initial gastric volume found in adults (250 mL, corresponding to a glass of water administered in
703 adult bioequivalence studies) and a paediatric fasted gastric fluid volume of 0.56 mL/kg [65; 146; 147;
704 163]. Slight variation of the initial gastric volume for paediatric subpopulations is observed depending
705 on the average weight reference values selected for the same paediatric age group **(Figure 7)** [146;
706 163]. The calculation of paediatric initial gastric volumes by BSA-extrapolation function based on the
707 adult initial gastric volume (*i.e.* 250 mL) and adult BSA of 1.73 m^2 has also been reported and results
708 in a greater volume estimated for paediatric subpopulations compared to BW-based
709 extrapolations (**Figure 7)** [164].
710 Although newborns and young infants typically receive none or only small amounts of water, the BW
711 or BSA-based extrapolations of the volumes based on adult water intake with a medicine may be
712 applicable to other typical fluids for these subpopulations, *e.g.* breast milk or formula milk. The down-
713 scaling of the recommended administered volumes in adults to children may slightly overestimate the
714 "real-life" administered volumes, as the adult value of 250 mL utilised in the extrapolation to
715 paediatrics has been reported to overestimate "real-life" administered volumes in adults [166].

716

717 *Please place Figure 7 here*

718

719 Another reasonable approach for determining the initial gastric volumes for the pBCS might be to
720 investigate the administered fluid volumes, considered representative for each paediatric sub-group,
721 and establish the limits on an empirical basis [147]. In a recent study, it was found that the majority of
722 infants and young children take no additional fluids to facilitate oral drug administration, the authors
723 explained these results with the fact that liquid formulations were commonly administered to these age
724 groups and that no additional fluid is required to facilitate drug intake [166]. In this case, the only
725 available fluid for drug dissolution would be the volume of the administered formulation, adding up to
726 5 mL for a liquid preparation [167], plus the available fluid in the fasted stomach. When fluids were
727 used to enable medication administration, water and milk were preferred for these age groups [166].
728 Liquids for drug intake by the older paediatric participants were usually reported as half a glass of
729 water, juices or soda [166]. For adults, the recommended volume to administer oral medication consists
730 of a glass of water (250 mL), whereas "real-life" studies report that only half of this volume is used
731 for medicine intake [166]. Generally, the volumes of consumed liquids increase with advancing age.
732 Evidence-based appropriate fluid volumes for drug administration throughout the paediatric subgroups
733 are insufficient to underpin a limit for the reference volume and could beneficially be investigated
734 further to provide guidelines [147]. Ultimately, it should be noted that drug administration with
735 beverages other than water has been reported to affect the drug's bioavailability [168].
736 Further investigation is required on the need of matching dose strength to initial gastric volume for
737 each paediatric subset [142]. In the case that a default dose of the drug is not set for the subpopulation
738 of interest, an individual body-weight or BSA-based dose calculation in the phase of fast growth
739 (*e.g.* a child of 7 years of age versus a child of 11 years of age) might lead to a BCS class change, if
740 the dose is doubled, while the values for solubility and initial volume remain constant [146].
741
742 For the dose/solubility-ratio, the lowest measured thermodynamic solubility of the drug in the pH range
743 1.2 - 6.8 has been proposed [160]. In the context of a pBCS, the choice of a relevant pH-range for the

744 solubility assessment requires more reliable data on paediatric GI fluid characterisation for the separate
745 paediatric subpopulations, as outlined in Section 3.2. [147]. The majority of the paediatric
746 biopharmaceutics experts surveyed by Batchelor *et al.* considered the adult pH range for solubility and
747 dissolution appropriate for the pBCS [147].
748
749 **4.2. pBCS permeability classification criteria**
750 Permeability values have been derived from absolute bioavailability data in paediatric patients [164];
751 due to the limited pharmacokinetic data generated in paediatrics, alternative determination methods
752 need to be examined. Calculated log P values guided the provisional classification of the drugs
753 included in the WHO list of essential drugs for children with view to drug permeability [146].
754 Calculated log P values showed a high linear correlation with experimentally established log P values
755 for selected compounds (R^2 = 0.92, n = 35) and were therefore utilised for the BCS classification of
756 drugs regarding their permeability [163]. Although several publications have reported log P and
757 calculated log P to correlate to adult SI permeability, which might be applicable to paediatric groups
758 over 2 years of age, the appropriateness of these parameters for newborns and infants remains
759 unknown [146; 163]. In the aforementioned expert survey, the determination of the permeability limit
760 for school children and adolescents was set as equal to the criteria of the adult BCS [147]. A PBPK
761 modeling approach has been proposed as a means to detect the sensitivity of the cumulative fraction
762 absorbed (f_a) to a permeability decrease in children, results show that fluconazole would remain a
763 Class I drug regardless of its permeability in children [125]. The controversial nature of the available
764 information on permeability in newborns and infants poses a hurdle towards establishing meaningful
765 permeability criteria for these subpopulations.
766
767
768

4.3. Challenges for the pBCS criteria determination

In spite of recent advances in the field of paediatric biopharmaceutics, significant knowledge gaps concerning absorption processes, maturation and growth of the GI tract impede the establishment of solid, evidence-based pBCS criteria. One more challenge towards the establishment of the pBCS originates in the developmental heterogeneity of the paediatric subpopulations. The necessity of a subdivision of the paediatric subpopulations has been highlighted several times; the selected groups should account sufficiently for growth and maturation changes [142; 147; 164; 169]. On one hand, the pBCS should discriminate as many paediatric age groups as needed, but on the other hand, it should not be overcomplicated and deprived of its universal and simplistic character. In order to establish distinct and adequate pBCS criteria, further research in the area of paediatric physiology and anatomy is needed, of which permeability of the SI as a function of age has been given the highest priority by the majority of paediatric biopharmaceutics experts surveyed by Batchelor *et al.* [147]. Biorelevant media and dissolution tests for paediatric formulations require further improvement, in order to establish appropriate pBCS dissolution test criteria for a potential pBCS-based biowaiver [147]. Another raised concern is whether the development of a pBCS is meaningful with respect to the available paediatric formulations. Although conventional tablets are not the formulation of choice for the youngest paediatric groups, other solid formulations (*e.g.* chewable tablets, mini-tablets, multiparticulate formulations, orally disintegrating tablets or films, lingual tablets, dispersible tablets) are gaining further popularity for low-solubility drugs [170].

Early biopharmaceutical risk assessment in paediatric drug development is crucial [171] and a simple system such as pBCS, compared to more complex tools like PBPK modeling, can offer a satisfactory estimation of the oral drug absorption and help troubleshoot potential limiting parameters [169]. A pBCS establishment would contribute to formulation bridging, line extensions, and minimising clinical trial and regulatory burden [169].

5. Food effects on oral drug absorption in paediatrics

Oral delivery continues to be the route of choice for administration of most drugs both in adult and paediatric populations. A review of submitted Paediatric Investigation Plans (PIPs) to the EMA in 2009, shows that 73% of pharmaceutical dosage forms developed for paediatric use were oral dosage forms [172]. EMA defends that if possible, the formulation should be available in more than one oral dosage form (solid and liquid) in order to facilitate administration and improve acceptability [10]. Liquid formulations are likely to be the most appropriate oral formulations from birth to 5 years due to swallowability and dose flexibility. Supporting evidence shows that with support and training younger children, *i.e.* below 6 years, can learn to take solid dosage forms such as tablets and capsules. The definition of an ideal formulation for all paediatric age-groups is challenging due to individual preferences and specific characteristics of patients [168]. An algorithm was proposed to guide the development of age-appropriate medicines with a focus on acceptability in every age subpopulation [173]. For newborns, liquid formulations and appropriate 2 mm mini monolithic tablets were suggested. For infants, more options become available, including liquids, mini monolithic tablets, multi particulates and orodispersible tablets. In children from 2 - 5 years, in addition to the formulations mentioned above, chewable tablets become an option [173]. Off-label drugs are widely used in paediatrics, most of the times due to lack of an appropriate paediatric oral formulation. Frequently, the most commonly used formulations in adults are modified and administered to children; crushing tablets or opening capsules to facilitate dosing are not uncommon practice [168]. Martir *et al.* reviewed the recommendations for administration of oral drugs by the British National Formulary for children and showed that the most common formulation administered to newborns are capsules, which are meant to be opened, and sprinkled or mixed with food and beverages [168]. In infants, a wider selection of formulations is recommended to be mixed with food, but capsules remain the most frequently used formulation (30%). The following section outlines the current regulations for drug administration after a whole meal or when mixed with small amounts of food or beverages and focuses

on the adjusted pharmacokinetic investigation approaches for paediatric formulations. Additionally, the food effect, seen from the perspective of paediatric drug formulation will be discussed.

5.1. Regulations and current practice: administration after a meal

The EMA and FDA guidelines provide a precise framework for the conduct and evaluation of food-effect bioequivalence studies in adults [52; 53]. The need of investigating drug pharmacokinetics in the paediatric population has been acknowledged by regulators through the issuing of relevant guidelines, while no specific regulations on food effect evaluation in paediatrics have been published [5; 174; 175].

In order to estimate the current trends regarding bioavailability studies for paediatric formulations, a search of the EU Clinical Trials Register was performed (status November 2017). The platform includes 31465 clinical trials with a EudraCTprotocol (16 % of which were paediatric clinical trials) and additional 18700 paediatric clinical trial reports. The search yielded 32 completed and ongoing bioavailability investigations, 16 of the studied formulations were intended for the oral administration route. Three of the studies investigated food effects; all of them were performed in an adult study population with a standardised high-caloric, high-fat breakfast. The tendency that food effects on the bioavailability of paediatric drug formulations is usually investigated in adult populations has recently been reported by Elder *et al.* [169]. In the context of food effect studies, age-adjusted meals were sometimes taken into consideration: milk was a common meal option for formulations intended for infants and younger children, whereas a breakfast was used for older children [176]. The study design should aim to investigate the maximum effect, which the meal can have on the formulation of interest [176].

Milk is not only the key energy source in the early life stages, but it additionally offers a caloric breakdown similar to the FDA standard breakfast **(Table 2)**. The type of milk should be chosen carefully, as the various infant formula types and cow's milk has different composition and

physicochemical properties (Section 2.5.) and exhibit different GE-rate in infants and newborns when administered with a similar energy amount (Section 3.3.) [49; 117]. To the best of our knowledge, the effects of different milk, and formula milk types on adults GE has not been studied; the potential impact should be considered if whole cow's milk is used instead of breast milk or formula milk when conducting bioavailability or bioequivalence studies for paediatric populations in adults.

Food effects on drug absorption following a meal in paediatric patients have been reported [176-185]. Drugs with reported food effects in adult populations showed no significant bioavailability changes in paediatric populations in the fasted versus fed state [177; 178; 181; 183; 184; 186], as it was observed for formulations of desmopressin, cefpodoxime proxetil, and methotrexate. On the contrary, food effects in paediatrics were observed for amoxicillin and ampicillin, while adult studies showed no significant food influence on the extent of drug absorption [182; 187]. Therefore, a food effect bioequivalence study in adults, following the design recommended for adult drug products, might not always be considered a reliable predictive tool for formulation performance under fed conditions in the paediatric population [176].

Some of the inconsistencies (*e.g.* significant and non-significant differences in drugs bioavailability due to distinct prandial state) might be explained by heterogeneous, lenient or indefinite requirements or reporting concerning the fasting time prior to drug administration (*e.g.* 30 - 120 minutes among different studies), food and fluid consumption at the time of administration, and meal standardisation. Whereas the majority of paediatric studies were based on real-life dosing conditions with regard to meal type and quantity, adult studies investigate the maximum food impact on the formulation's bioavailability. In contrast to paediatrics, adult food effect studies were usually conducted according to relevant guidelines. Although the adoption of such a guideline for paediatrics would ensure a unified approach and comparability of the investigations, ethical and recruitment issues may pose a challenge in guideline's development and applicability.

5.2. Regulations and current practice: co-administration of formulation with food/ drinks

Small amounts of soft foods and juices are used for improving acceptability and palatability of formulations in the paediatric population. Previous cases reporting significant drug bioavailability alterations have raised safety concerns [59; 188-190]. As a result, vehicles (discussed in Section 2.5.) which are considered safe or inappropriate to be mixed with the formulation, should be included in the product information supported by relevant *in vivo* or *in vitro* studies. The amount of soft food or beverage for co-administration is crucial for the study outcome, and a "small portion (*e.g.* one spoon) or otherwise justified quantity of the food or drinks" is recommended by the EMA [167]. There is a lack of guidance on what an exact age-appropriate amount is. EMA guideline on pharmaceutical development of paediatric medicines [167], suggests an optional *in vivo* study, which can be a separate bioequivalence study in adults [191], alternatively paediatric clinical trials can be conducted with the vehicle(s) of choice, as reported for omeprazole and montelukast paediatric formulations [192; 193]. On the other hand, the sprinkling of formulations on soft food is referred in the FDA guidance on Food-Effect Bioavailability and Fed Bioequivalence Studies. In the case of investigation of formulations that are meant to be sprinkled on foods, a study in healthy adult volunteers is usually requested by regulatory authorities [53]. Investigation of the vehicle(s), as part of the paediatric clinical trial, would provide the highest reliability in terms of product safety and efficacy, although it might further complicate the trial design (through introduction of additional drug administration conditions), execution (*e.g.* patient recruitment difficulties), and outcome interpretation [169; 194].

The type and quantity of studied foods or beverages varied in adult studies investigating the administration of paediatric formulations mixed with small amounts of vehicles. Quantities from one tablespoon to 120 mL were reported for the commonly used soft foods and typical fluids were investigated in volumes ranging from 5 to 240 mL [176; 190]. Possible food-drug interactions may occur with the commonly used applesauce and apple juice, *e.g.* for fexofenadine inhibition of OATP transporters in the GI tract have been reported with influence on the pharmacokinetic

profile [195]. A recent study reported by Batchelor *et al.* described how *in vivo, in vitro* and *in silico* investigations were adjusted to the previous knowledge available for two model drugs categorised as BCS class II and III [196]. Briefly, the stability of each drug in various vehicles was confirmed and possible vehicles for co-administration were selected; this was followed by a combination of *in vitro* dissolution and solubility studies and *in silico* modeling [196; 197].

Although the regulatory bodies acknowledge the importance of conducting paediatric studies, the paediatric trials should provide benefit for the patients and should not be unnecessary [198]. Studies performed in adults are accepted and the applicability of the results to the paediatric population should be discussed; additionally, *in vitro* and *in silico* tests are accepted as supportive evidence [167]. Finally, a regulatory statement concerning the appropriate volumes for product testing would provide valued information and ensure a more unified approach to the dedicated studies.

5.3. Food effects and paediatric dosage forms

The type of dosage form can contribute to the occurrence and extent of food effects. Formulation-related food effects are generally regarded as less common for oral liquid formulations, because of the liquids' greater mobility in the adult GI tract and less variable GE rate in the fasted and fed state [199]. Cases of absorption delay have been reported for suspensions, solutions and powder for reconstitution [185; 200-202]. The presence of food in the stomach limited gastric disintegration and dissolution of a solid dosage form in adults, leading to delayed absorption of fosamprenavir [203]. This effect might not be relevant for younger paediatric patients who are not able to swallow a whole tablet but should be considered in formulation development for school children and adolescents.

Drug absorption from innovative paediatric solid formulations, which are usually formulated into a hard capsule, such as multiparticulates and mini-tablets, show less dependency on the time needed for disintegration, compared to the intact formulation. Differences in the pharmacokinetic profiles have been observed after administration of a capsule and sprinkled formulation in the fed state, achieved by

the two formulations in an adult study [204]. McLean *et al.* compared the performance of administration of an intact carbamazepine controlled-release formulation in the fasted and fed states and sprinkling of the contents in applesauce [205]. The different treatments showed bioequivalence, although the extent of absorption in the fed state was slightly higher than in the fasted state for the intact formulation and for the sprinkled formulation administered with applesauce. The sprinkled formulation achieved slightly greater extent of absorption compared to the intact formulation in the fasted state; it remains unclear if this difference might be due to the presence of soft food used for the administration or to the drug product itself (intact capsule or sprinkled contents). The increased absorption in the presence of food was explained by the drug's properties and was not formulation-associated [205]

The process of formulation transfer into the SI could explain further formulation-related food effects. Small particles pass into the SI together with the chyme during the GE of the meal. In contrast, non-disintegrating dosage forms with a diameter greater than 2 mm [176] are commonly cleared into the SI during MMC Phase III (in the fasted prandial state) and less frequently through isolated distal antral contractions [206]. Generally, such formulations (matrix tablets or coated tablets) would arrive in the SI earlier in the fasted state than in the fed state, as the MMC only occurs in the fasted state [206].

Monolithic non-disintegrating formulations can usually be considered for paediatric patients older than 6 years of age mainly for swallowability reasons [10]. The solid monolithic formulation behaviour in the presence of food is dependent on multiple factors, *e.g.* properties of the coating agent and stability in different pH media, type of matrix material used, breaking force of the tablet, and general formulation robustness when exposed to different GI fluids. Investigations performed in adults report remarkable differences between formulations, with positive food effects (an increase of exposure up to 50%) with or without absorption delay, or significantly reduced drug absorption, or no influence of the prandial state [207; 208]. Formulation-related food effects for theophylline in paediatric patients aged between 4 and 14 years revealed great variability after drug formulation administration after a

standardised breakfast, consisting of approximately 20% fats, 70% of carbohydrates, and up to 13% of proteins; the total caloric count was normalised per BW 10 - 15 Kcal/kg [209]. One formulation (Somophyllin®, sustained release sprinkle product) showed no changes regarding the extent of absorption, but a delayed absorption. A second sustained-release formulation (Theo-Dur® sprinkle) showed less variable *in vivo* performance in the fasted state compared to the fed state; this sprinkled formulation performed similarly in adults and paediatric patients, although the negative food effect was more pronounced in the paediatric group [207; 209]. The exposure achieved by the monolithic theophylline formulation (Uniphyllin®, sustained-release tablet) in the fed state was doubled compared to the fasted state, due to dose dumping, which occurred in 50% of the population. GI transfer delay might not only result in an unfavourable impact on the timing of the drug effect when rapid drug onset is required, but it can have an impact on drug bioavailability for drugs with narrow absorption windows, as observed for pregabalin controlled-release tablets in adults [210]. In order to ensure that the extrapolation of food effects for non-disintegrating or controlled-release formulations from adults to paediatrics is reliable, further accurate knowledge about the MMC process, size of particles that can pass through the pylorus sphincter, GI motility, and transit times across the GI is essential.

6. *In vitro* evaluation of drug products for paediatrics

GI developmental changes must be addressed in the design of *in vitro* models to achieve adequate predictions of oral drug absorption as a function of age. In the following subsections, recently proposed *in vitro* methodologies will be presented.

6.1. Paediatric biorelevant media

Compositional differences in GI fluids for the development of biorelevant media, representative of newborns and infants in the fasted and fed state, have recently been addressed by Maharaj *et al.* [109].

The proposed media gathered information on physiological relevant components of GI fluids, such as pepsin concentrations, food type for fed state media, bile salt concentration, pH, osmolality, and others (**Table 4)** [109]. The paediatric biorelevant media were developed for the youngest subpopulations, newborns and infants (1 - 12 mo), and were based on the adult biorelevant media composition [109]. As discussed above, these age groups show the highest degree of developmental differences, when compared with adults. Values reflecting the physiological conditions (where available) were set in order to simulate more closely the GI composition of fluids in newborns and infants. Solubility studies of seven BCS class II drugs were performed in the paediatric biorelevant media. The solubility changes in paediatric media, compared to the solubility in adult biorelevant media, was evaluated based on risk assessment (risk set when values were outside the 80 to 125% range) [109]. The impact of age-related alterations in GI fluid composition on compound solubility was revealed, as for 6 of the 7 BCS Class II compounds investigated the solubility in at least one of the developed paediatric media fell outside the 80 to 125% range compared to the solubility in adult media [109].

Kamstrup *et al.* performed a literature review of relevant physiological components and proposed a composition of physiologically relevant medium for newborns and young infants (0 – 2 mo) representative of the fasted and fed state. Biorelevant components addressed included bile salts concentration, the ratio of bile salts to phospholipids, and digestive enzymes (pepsin, human gastric lipase, and pancreatic triglyceride lipase). The media were developed with the purpose of being used for an *in vitro* lipolysis method, and it has been applied to study the *in vitro* lipolysis of furosemide, which will be discussed in the next section [48].

Please place Table 4 here

6.2. Evaluation of drug products characteristics

In vitro dissolution testing is a standard method used for the characterisation of drug products. Questions regarding the relevance of dissolution tests within paediatrics have been raised in a recent review since dissolution testing mainly aims to characterise solid oral dosage forms, and its applicability to commonly used paediatric formulations as liquids, semisolids, or orally disintegrating tablets is debatable [169]. Nevertheless, as mentioned in Section 4.2., paediatric solid formulations (*e.g.* chewable tablets, mini-tablets, multiparticulates, *etc.*) are gaining further popularity for low-solubility drugs [170]. The mini-paddle apparatus, that is based on the phrmacopoeia paddle apparatus (USP II apparatus with scaled down dimensions), and the flow-through cell apparatus (USP IV apparatus) have been acknowledged as superior to USP I and II apparatus, in terms of simulating paediatric conditions [169].

New paediatric dissolution setups have been proposed by Karkossa *et al.*, which investigated different dosing scenarios of a paediatric formulation of sodium valproate (BCS class I compound; pKa = 4.8 and log P = 2.75) extended-release mini tablets formulation (Orfiril long®) [211]. Two scenarios were investigated: i) impact of gastric pH on drug release, in a new dissolution apparatus (proposed in the study as a modified USP III vessel (shortened height and glass ring in outer surface) in a water bath with stirring provided by a magnetic stirrer (550 rpm), and ii) impact of co-administration of different vehicles in a mini-paddle apparatus with a subsequent transfer to a new dissolution apparatus. Residence times for the simulation of each stage of GI tract were 30 min for the gastric compartment, 240 minutes for the SI and 480 min for the proximal colon [216]. Gastric fluids were simulated by mixing 10 mL of simulated gastric fluid (pH range 1.8 - 4.0), and 50 mL of water. After 30 min, simulated gastric contents were transferred to a second vessel where 110 mL of simulated small intestinal fluid (pH 6.8 bicarbonate based simulated intestinal fluid, 50 mL) was present. Results showed that gastric pH had no impact on overall drug release. During the short-simulated fasted gastric

residence time of 30 min, almost no drug was released. Approximately 50 - 60% of the dose was released during simulated small intestinal residence time, and drug release was complete at the end of the simulated passage through the SI and proximal/mid colon. The impact of co-administration of dosing vehicles on drug release was investigated with a two-stage dissolution model. Gastric residence of the administered formulation with water, apple juice or soft foods (applesauce, yoghurt, or pudding) was performed in the mini-paddle apparatus (170 mL; 30 min; 75 rpm). After the first 30 minutes, 60 mL of the simulated gastric contents together with the tablets were transferred into the modified USP III vessel, with the addition of 50 mL of bicarbonate-based simulated intestinal fluid, in order to simulate the intestinal conditions. Drug release under these conditions was screened for 12 h representing residence time in the SI and proximal colon. These release studies revealed that administration of the formulation with other beverages, and soft foods should not affect bioavailability and confirmed the appropriateness of the paediatric dosing recommendation for this formulation [211]. *In vitro* release profiles from experiments simulating co-administration with different soft foods (applesauce, yoghurt, and pudding) were similar to those obtained in water and apple juice, suggesting that co-administration of soft food will not affect bioavailability of the extended-release formulation.

Brassine and Fotaki investigated the effect of age-related physiological parameters, the effect of dose, and the effect of hydrodynamics on the performance of carbamazepine (BCS class II; non-ionisable in the physiological pH range; log P = 2) for paediatric use. Biorelevant media, with adjusted bile salt concentration, were incorporated in an *in vitro* dissolution testing to evaluate the effect of age on dissolution and release of carbamazepine pellets prepared by extrusion-spheronisation [212]. The dissolution study was conducted with the dissolution USP IV, and parameters were adjusted (flow rate and residence time) to simulate GI physiological parameters in paediatric groups (newborns, infants and children) and adults. Furthermore, the effects of the hydrodynamics on the dissolution was studied by setting the closed-loop mode (for simulation of gastric conditions) followed by the intestinal conditions simulated with the open-loop mode. Results showed a slower release of carbamazepine

under all paediatric-simulated conditions when compared to the conditions used for the adults; nevertheless, no significant differences were revealed for the release of carbamazepine between the investigated paediatric groups [212].

The same USP IV biorelevant setup for the fasted state was performed to investigate age-related differences in the dissolution performance of Tegretol® 200 mg tablets [213]. Paediatric biorelevant media developed by Maharaj *et al.* were used. Results showed that carbamazepine was not completely dissolved in all of the tested conditions. An age-dependent dissolution profile of carbamazepine from Tegretol® tablet was observed in the two studied paediatric groups revealing the impact of the GI differences (fluid composition and transition times) between the age groups on dissolution. Furthermore, the use of the closed-loop mode for the simulation of dissolution in the gastric compartment resulted in a higher discrimination of the dissolution profiles between the two age groups [213].

Non-compendial apparatus for the evaluation of paediatric formulations have also been proposed [169]. A TNO Gastro-Intestinal Model (TIM) paediatric setup (TIMpaediatric) has been developed, which simulates conditions in the GI tract determined by four interactive factors: i) degree of maturation of the age groups (term newborns; infant; or toddler), ii) food type, iii) health status and vi) co-medications [214]. The TIMpaediatric was applied to investigate age-related effect of co-administration of food matrices with paracetamol (BCS class I; pKa = 9.5; log P = 0.2), diclofenac (BCS class II; pKa = 4.15; log P = 4.51), and esomeprazole (BCS class II; pKa = 4.78; log P = 0.6), where bioaccessibility curves were constructed (amount of drug available when sampling). Selected dosage forms were tested in the *in vitro* TIMpaediatric by taking into consideration the simulation of daily practices used for administration of paediatric medicines, including crushing of tablets, mixing drugs with appropriate amounts of food (simulations performed for administration with formula milk vs. water), and simulation of the co-administration with proton pump inhibitor were simulated

(simulations performed under high gastric pH conditions (pH 6.7 to 6.0). A validation experiment of TIMpaediatric was performed by comparing *in vitro* bioaccessibility profile with *in vivo* clinical data for Calpol® syrup suspension (containing paracetamol) mixed with food, under term-newborn, infant and toddler GI conditions, and similar bioaccessible amounts were found when compared to plasma concentration profiles, demonstrating the quality of the predictions obtained from the TIMpaediatric. Further experiments were then performed, paracetamol formulations investigated were Sinaspril® syrup, Sinaspril® tablets (crushed), and Marel® tablets (crushed, also contain caffeine) and results showed that paracetamol concentration available for intestinal absorption was independent of the different GI conditions of the age-groups, the tested dosage forms, the food matrix, and the co-administration of a proton pump inhibitor. Two brands of enteric-coated diclofenac tablets were tested (Voltaren® vs. Diclofenac Sodium Teva®), results showed that diclofenac available for absorption of is not influenced by co-administration of a proton pump inhibitor, but the administration of a crushed tablet with infant food showed a significant positive effect on diclofenac bioaccessibility. The investigated formulation of esomeprazole formulation was Nexium® enteric coated tablets (crushed), and results showed after a first dose of a crushed tablet to infants was low, but increases after repeated dosing due to a higher gastric pH by the proton pump inhibitor [214].

A recent literature review has been performed with the intention of developing an *in vitro* digestion model for newborns and infants (0 - 2 mo) based on a previous lipolysis model for adults [48]. Considerations were taken to represent changes during the feeding cycle of newborns and infants, which is approximately 3 h. The *in vitro* digestion model was argued to be more appropriate than other *in vitro* predictive tools, due to the frequent feeding of newborns. Since newborns are mainly in the fed state, this can ultimately affect the composition of the fluids and hydrodynamics available for drug dissolution and solubilisation processes. For the design of the *in vitro* setup, several physiological factors were reviewed including GE, SITT, gastric volumes *etc*, and suggested flow rates for the

transfer of GI fluids under fed state conditions. A two-step model was proposed as more appropriate, comprising a gastric phase and an intestinal phase, where the duration of each phase, and the transfer between the two phases, should be reflective of GE and SITT in newborns/young infants. The performance of Furix® 20 mg furosemide (BCS class II compound) tablets, in the newborn and infant GI tract was investigated with this setup [215]. Fasted and fed states were simulated to represent feeding patterns in the studied population; therefore, the fasted state assumed the presence of small amounts of milk. The physiological relevant media used were composed of a chosen appropriate milk (Nan 1, Nestle®), and the inclusion of digestive enzymes (*i.e*. pancreatic triglyceride lipase and pepsin and human gastric lipase). Two *in vitro* models simulating the GI transfer were utilised. In the immediate transfer model, a concentrated intestinal medium was added in a single step at a designated time point, altering the digestion medium from gastric to intestinal medium instantaneously. In the continuous model, digestion medium was continuously pumped from a gastric to an intestinal compartment, where the concentrated medium simulating the SI fluid was present. The results suggested that the oral bioavailability of furosemide in this subpopulation increased in the presence of food [215]. In contrast, parameter manipulation, such as simulation of food digestion and crushing of the tablets seemed to cause no alterations in the oral performance of furosemide [215]. The entire furosemide dose was completely soluble in the aqueous phase of the simulated postprandial state, which led the authors to conclude a high bioavailability of the drug in the presence of food [215]. GI digestion of food ingested showed no effect on the amount of furosemide solubilised, nor did the administration of the pure powder form of furosemide, which indicates that the dosage form does not influence the oral performance of furosemide. The results suggest that presence of food in newborns and young infants is affected by the pH at fed state and volume available for drug solubilisation, which allows the that the entire dose of furosemide is solubilised in the digestion studies without being affected by excipients and digestion. On the contrary, In order to further evaluate and validate these results and usefulness of the *in vitro* models, *in vivo* data is required [215].

A considerable amount of progress has been made in the development of paediatric *in vitro* dissolution tests. Compendial and non-compendial apparatus have been used, and biorelevant setups have been proposed. Nevertheless, further research is required to better characterise GI physiological and anatomical changes in paediatrics, in both the fasted and fed state, which will inevitably allow optimisation and proposal of more biorelevant models. Validation of the *in vitro* setups with clinical data would be helpful to establish confidence in these methods so that they can be used to inform the development of more complex and innovative paediatric dosage forms. Furthermore, a combination of biorelevant *in vitro* tests with paediatric PBPK models is expected to improve knowledge and understanding of oral drug absorption in paediatrics [169].

7. *In silico* evaluation of drug products for paediatrics

Regulatory frameworks allow investigators to use existing adult clinical data as supporting evidence for efficacy in paediatric populations [216; 217] assuming that disease progression and exposure-response in both populations are expected to be similar. A significant number of conducted pharmacokinetic and efficacy studies in the paediatric population did not achieve labelling for various reasons, such as poor study design planning or inappropriate dose determination, indicating the need of robust and reliable approaches for interpreting and benefiting from already available clinical data [218].

Predicting *in vivo* drug performance relies on the estimation of the drug's ADME properties and the understanding of the physiological processes influencing pharmacokinetic parameters. Scaling of parameters for different organisms can be facilitated by calculations using isometric or allometric functions, or be performed on a more complex level such as PBPK modeling [219].

7.1. Allometric scaling

Paediatric parameters are calculated as a function of the normalized *BW* or BSA and a specific allometric coefficient [220]. For example, a fixed allometric coefficient of 0.75 is used for clearance scaling, whereas a value of one is used for the down-scaling of the volume of distribution [220]. Mahmood *et al*. reported that drug clearance calculated by allometric scaling with an adjusted allometric exponent, and clearance predicted via PBPK modeling achieved similar results for newborns and infants < 3 months of age; the studied drugs were mainly cleared by glucuronidation [221]. The prediction accuracy for newborns and infants is expected to be compromised for drugs undergoing more complex metabolism, due to variable enzyme ontogeny, maturation processes, and alternative metabolic pathways. The use of fixed-coefficient allometric scaling is recommended after 2 - 5 years of age when the maturation processes can be considered completed [220; 222-225]. The method's simplicity and unproblematic utilisation contribute to its widespread application in clinical settings.

7.2. PBPK modeling

While allometric functions are still useful for scaling ADME properties, PBPK modeling would be preferred, if more complex processes need to be studied [226]. PBPK modeling is an *in silico* biopharmaceutical tool describing the pharmacokinetics of a compound while taking the drug properties and drug product characteristics into consideration when introduced to a specific system (*e.g.* healthy adult body) according to a pre-defined study design (*e.g.* administered formulation). In adults, PBPK modeling is often used to predict drug product performance [227]. In paediatrics its use has increased the last decade, recognised by the EMA and FDA by publishing guiding documents on the appropriate use of previous knowledge (*e.g.* adults) in paediatric medicines development and by PBPK modeling guideline [216; 228; 229].

Two modeling strategies may be used to construct a PBPK model, depending on the input used for the system. The "top-down" approach is based on observed clinical data as a model for the system (human body), followed by an investigation of the components and occurring processes (*e.g.* parameter estimation from plasma drug concentration-time profiles). In contrast, a model that is based solely on a combination of physiological processes parameters and *in vitro* experiments, generating numerous connected compartments, which represent an organ or the whole body, is regarded as a "bottom-up" approach (usual PBPK model). While the latter depends on absolute knowledge of details, which contribute to drug performance in order to predict pharmacokinetics and pharmacodynamics *a priori*, the former relies completely on already obtained clinical data but may not be able to provide the necessary detail in each case. A "middle-out" concept that benefits from the combination of the two approaches might offer a sensible compromise when some parameters have not been reliably estimated yet or need refinement through already available clinical data [230; 231]. Several software platforms enable the building of PBPK models for adults (*e.g.* GI-Sim®, PK-SIM®, Stella®, MATLAB®), while some of them do not provide an integrated detailed model of oral absorption (MATLAB®) [227]. Additionally, commercially available software platforms, such as, GastroPlus® (Simulations Plus Inc. [232]), and Simcyp® (Simcyp Ltd., Sheffield, UK [233]), facilitate the development of whole-body PBPK models and models focused on oral drug absorption for adults and their further extrapolation to the paediatric population [234].

7.2.1. Paediatric PBPK models: current status

A search in PubMed with the keywords "Paediatric PBPK" OR, "PBPK model Paediatric" AND, "infants", "newborns", "children", "adolescents" OR, "PBPK paediatric modeling", OR "mechanistic model paediatric pharmacokinetics" identified 405 relevant entries, including reviews and original articles (status August 2017). A snowball sampling of the review articles for potentially mentioned

articles, complying with the focus of the search was performed and the papers, which reported a developed PBPK model for paediatric populations, were selected (n = 93; **Figure 8**).

Please place Figure 8 here

Pre-term and term newborns were found to be less studied (**Figure 8A)** – a trend also reported in clinical trials performed in paediatrics. Over 80% of the paediatric PBPK models were developed based on a PBPK model for adults **(Figure 8B)**. Evaluation of the aims of the models developed showed numerous successful mechanistic clearance and drug-disposition models for intravenous (IV) administered drugs. Twenty nine percent of PBPK models following oral drug administration have been established until now (**Figure 8C**). A similar trend was observed for the adult PBPK models, where modeling oral drug absorption accounted for only 12% of the developed PBPK models [235]. The biggest part of the PBPK models was built with the help of a commercially available software platform, whereby Simcyp® appeared to be the most frequently used one (**Figure 8D**). Additionally, the BCS classes of the orally administered drugs, used for modeling were analysed (**Figure 9**). A preference of PBPK model development for highly soluble drugs might be related to the fact that these would usually not introduce further solubility or dissolution complications in addition to the model uncertainties originating in the complexity of the oral drug absorption processes itself [7]. The low number of medicines modeled containing BCS IV compounds can be explained by the great number of uncertainties accompanying both permeability and solubility of these compounds in paediatric populations.

Please place Figure 9 here

7.2.2. Building a PBPK model

The most common approach in constructing a paediatric PBPK model is to build first the adult disposition PBPK model (**Figure 10, Step 1**), and after ensuring reliability of the intravenous model, oral administration can be incorporated (**Figure 10, Step 2)** [236].

If the adult PBPK model provides an adequate prediction of the available clinical data in adults, the scaling to the paediatric population could proceed [237]. By selecting a specific paediatric population as the study population in the software platform, default age-dependent changes and parameters of physiology and anatomy are incorporated into the paediatric model.

Please place Figure 10 here

Step 1: Building drug disposition PBPK model for adults

For the development of a PBPK model, system-dependent and compound-dependent parameters are needed [7; 169; 236; 238-240]. System-dependent components (*i.e.* organ sizes, blood flow, and tissue composition) are incorporated in the commercially available software platform for the species of interest (*e.g.* human, dog, mouse). Drug-dependent parameter values are derived from literature or experimental data. Parameters describing the drugs physicochemical properties (*i.e.* molecular weight, log P, pKa, compound type, and pH-dependent solubility) are used. Drug parameter values that depend on the drug and the adult human physiology (fraction unbound, permeability, plasma/blood-partitioning, intrinsic clearance) may require further investigations and adjustment for the modeled system or special population [240].

The human body is represented as a network of organs and tissues, linked by an arterial and venous blood, with attributed specific blood flows. The disposition model is based on differential equations that describe the distribution of the drug into the different tissue compartments and organs [7; 227; 235]. A simulation takes place when the input parameters and the study design (*e.g.* selecting study

population, age, sex, dose strength, dosing conditions, duration of infusion, *etc*.) have been defined. If the pharmacokinetic simulations of the model incorporating predicted values for clearance or volume of distribution mismatch the observed clinical intravenous data, model optimisation can be achieved by informing the model with clinical data (if available). Once the predictions forecast the observed data from IV administration, the modeling of oral drug absorption can be undertaken [236; 237].

Step 2: Building oral absorption PBPK model for adults

The oral absorption of a drug can be modeled in detail using the relevant available commercial software oral models, such as ACAT™ model (GastroPlus®), or ADAM™ model (Simcyp®). In both models, the GI tract is divided into sequentially connected transit compartments, beginning with the stomach, which gives the input for the SI according to a specific emptying-rate. The SI is further divided into sub-compartments (representing the duodenum, upper and lower jejunum, and upper and lower ileum) and it is linked subsequently to the colon. Each compartment exhibits different surface area, luminal fluid composition and volumes, and metabolising luminal enzymes. In addition to the mass-balance differential equations, the model considers the local pH-dependent solubility by the incorporation of the Henderson-Hasselbalch equation and calculates the dissolution behaviour with *e.g.* Noyes-Whitney kinetics [227; 240]. In this step, the drug formulation, which is to be investigated, is incorporated. If relevant, available dissolution data from biorelevant *in vitro* tests can be used to inform the model [227]. Ultimately, drug dissolution, precipitation, or supersaturation are considered if relevant for the drug/drug formulation; hence the absorbed, degraded, or metabolised drug fraction are taken into account simultaneously [227].

The permeability of a drug can be derived from *in vivo* or *in vitro* studies or estimated via the utilised software. In case that active transporters are involved in the drug uptake, the kinetic parameters (*i.e*. Michaelis Menten constant (K_m) and maximum rate achieved at saturating substrate concentration (V_{max})) of the substrate, the transporter availability, and activity, at the sites of interest are needed and an adequate estimation of permeability-limited transport through the cell membranes should be

included [239]. If relevant information is not available in the literature or from *in vitro* studies performed, a model fitting based on *in vivo* data from oral drug administration studies can be applied [240]. The accuracy of the model's prediction needs to be confirmed and refinements should be undertaken if needed before application to other populations can proceed.

Step 3: PBPK model conversion to the paediatric population

The GastroPlus® platform (PBPKPlus™ module) generates physiological parameters for the model by its feature Population Estimates for Age-Related Physiology (PEAR®). It takes the population (e.g. American/Western Japanese, and Chinese), gender (male/female) age, gestational age (including pre-mature newborns), BW, height, body-mass index, percent body fat into account and adjusts tissue volumes and perfusion rates accordingly [241]. Correspondingly, in the Simcyp population-based simulator (Simcyp®), physiological parameters are adjusted by converting to the available module Simcyp® Paediatric [237]. Age-dependent changes are introduced to the full PBPK model, *e.g.* adjustments of compartment volumes, blood perfusion rates, tissue compositions, specific partition coefficients for tissues. In addition to these adjustments, a model with focus on oral drug absorption in paediatrics addresses GI specific physiological parameters such as GE rates, SITT, fluid volumes throughout the GI tract, composition of the GI fluids, GI hydrodynamics, and size of the separate compartments of the GI tract; all of these parameters influence drug movement through the GI tract, drug dissolution and absorption rates, and therefore drug product performance following oral administration [125; 242].

In the ACAT Model (GastroPlus®), GI organs and their respective blood flows change dependent on age, intestinal length and radius are calculated according to intestinal growth data and are based on the assumption that proportional growth occurs throughout the SI [242]. Age-adjusted SITT values are incorporated in the model, although it should be noted that the data used for this assumption is highly dependent on the method utilised for the measurement (Section 3.4), thus introducing a level of model

uncertainty [242]. Furthermore, fluid secretion volumes are scaled as a function of age for the paediatric population in the ACAT™ model (GastroPlus® version 9.0) [243]. Adult values are adopted for the gastric and intestinal pH and GE in the model. The villi structure is also reflected, as for adults, due to the qualitative nature of the information available (Section 3.5); this leads to a large uncertainty for the estimation of passive absorption of drugs, especially for the youngest populations < 3 years of age [242]. Due to the scarcity of data found for bile salt composition and site of reabsorption, adult parameter values are adopted; model inaccuracies can be expected for compounds that exhibit great solubility and permeability dependency on bile salts. Ultimately, intestinal enzyme levels for CYP3A4 are implemented in the modeling platform according to age, based on paediatric *in vivo* data, but for less well-characterised intestinal enzymes and transporters adult values are utilised. Since expression density and ontogeny are expected to show differences in newborns and infants compared to adults, the user has the option to modify the default values of enzyme/transporter expression levels per intestinal compartment based on surface area, and the enzyme/transporter density in adults [242].

Within the Simcyp® platform, the intestinal diameter, length and surface area are scaled according to age by using BSA-based functions; here it should be noted that no correction is incorporated for the potentially additional available surface area created by villi and microvilli with increasing age [35]. Fasted gastric pH for paediatrics is assigned similar values as for adults, except for the age groups of newborns and infants. For these paediatric subpopulations, higher values are considered appropriate in order to simulate the more frequently administered meals and the absence of a 'true' fasted state [35]. Salivary secretion is described by a BW-based function and is further incorporated in the calculation of the fasted gastric volume. The fed gastric volume is calculated according to BW and is characterised for 3 age groups, based on the different daily fluid requirements and the feeding frequency [35]. Fluid secretion volumes are scaled based on BSA-functions. Intestinal pH values observed in adult populations are designated to all paediatric subpopulations [35]. GE is described as a function of meal

type, the user is given a choice of simulating the effects of liquid, semi-solid or solid meal ingestion; the SITT values for paediatrics are adopted from the adult model [35]. Ultimately, the ontogeny and presence of metabolising luminal enzymes of the CYP and UGT families are calculated in the same pattern as the well-defined CYP3A4 in paediatrics. The enzyme abundance follows a BSA-dependent function, specifically assigned to the different intestinal segments. Assumptions are needed for some less investigated parameters, such as intestinal transport proteins, for which adult values are adopted [35; 244]

Simulation in paediatric subpopulations usually begins in the subpopulation most similar to adults, *e.g.* adolescents or children, proceeding gradually to the younger subpopulations [236]. Throughout the process, confirmation, validation, and if necessary, refinement steps are undertaken. The gradual adaption of the model facilitates easier detection of probable refinement demand [236]. Mismatches between the predicted and observed paediatric clinical data should be further investigated through parameter sensitivity analysis (PSA) [35; 125; 236]. This is also a useful approach for investigating "what-if" scenarios related to the assumptions and uncertainties which were included in the model throughout development [216].

7.2.3. Examples of paediatric PBPK models: focus on oral drug absorption

Prediction of oral drug exposure to sotalol was built over the entire paediatric age range (*i.e.* newborns, infants, children and adolescents) and adults, by Khalil *et al.*, with the utilisation of two modeling software platforms, Simcyp® (version 12.1) and PK-SIM® (version 4.2.2) [238]. Sotalol is an amphoteric compound (pKa values: 8.3 and 9.7) with hydrophilic characteristics (log P of 0.37). Firstly, the adult disposition model was developed. Parameters from the model after IV administration were kept constant, and parameters relevant to oral drug absorption were adjusted. Lastly, age-specific anatomical and physiological changes, which are part of the paediatric module of the software, were

taken into account. Adult values were used for several parameters, such as gastric and intestinal pH, GE, SITT, intestinal enzyme ontogeny/abundance, and intestinal transporter ontogeny/abundance. Drug-specific parameters, including solubility, remained unchanged throughout all age groups regardless of the utilised software. Information on the sotalol formulations investigated with the PBPK models, was not provided. Further complications arose from the data scarcity of neonatal and infant pharmacokinetic data, which are needed in order to validate the PBPK models. Simulations from both paediatric models (Simcyp® and PK-SIM®) were comparable and showed acceptable adequate description in adults, adolescents, children and infants, when compared with *in vivo* clinical data. For newborns, the predictions generated with the Simcyp® simulator successfully reflected the time at which C_{max} is reached (t_{max}), and rate of elimination (k_e) when compared with the clinical *in vivo* data, but were inadequate in the forecasting area under the curve (AUC) AUC_{last} in newborns, and maximum plasma concentration reached (C_{max}) in newborns; moreover the model tended to under-predict drug plasma levels in all paediatric subpopulations ((for AUC_{last}, C_{max}, and t_{max} for all of the paediatric populations studied: mean observed/predicted ratios >1). Results obtained with the modeling platform PK-SIM® successfully predicted AUC_{last}, C_{max} and k_e, although the pre-defined two-fold error range was exceeded for t_{max} in newborns and infants (<1 yr). The results from this study confirm the importance of gaining deeper insight into intestinal paracellular permeability, transporter ontogeny, intestinal fluid dynamics, and characteristics of the intestinal unstirred boundary layer in order to develop a reliable PBPK model for oral drug administration [238].

Paediatric PBPK models have been developed (GastroPlus® version not mentioned) for two highly soluble, and highly permeable compounds (sotalol and paracetamol) by Villiger *et al.* [236]. As previously described, Sotalol is an amphoteric compound, and paracetamol is a hydrophilic weak acid (pKa = 9.5; log P = 0.2). The same approach for model building was used as in the first example, where a drug disposition model was developed to simulate the IV profiles in adults, followed by the

adjustment of parameters for oral administration in adults. Secondly, after attaining confidence in the adult models, the paediatric oral model was built in a stepwise approach. In this study, *in vitro* dissolution testing was performed for immediate-release formulations, Sotalex® tablets (containing sotalol) and Dafalgan® powder-filled sachets (containing paracetamol), in order to investigate the formulation performance and understand drug release in the GI tract [236]. For the *in vitro* tests, conditions more closely reflecting newborn physiology were simulated by adjusting GI volumes to 5 mL and the use of formula milk as dissolution medium, in comparison to an adult setup, represented by 250 mL of adult biorelevant media. Results showed that the described age-adjusted conditions did not influence dissolution of both test drugs. Dissolution information was not used to inform the model building, and further information on the formulations and their incorporation into the models was not reported for the performed simulations. PSA revealed that slower mean gastric transit times led to slower absorption rate of sotalol and paracetamol in newborns and infants when compared to older children and adults [236]. Good predictions were observed after scaling age-dependent factors incorporated in the software used (Gastroplus®), for children 2 - 11 years, but discrepancies were again seen by Villiger *et al.* for younger populations with under-prediction of C_{max} and over-prediction of t_{max} (newborns and infants) [236]. As previously described in the first example, Khalil *et al*. also obtained good predictions for other age-groups, except for newborns [238]. Interestingly Khalil *et al.* did not conduct PSA, but Villiger *et al.* took advantage of PSA to understand the critical parameters of oral drug absorption for these compounds, and subsequent improvement of the models predictions was possible, demonstrating the importance of conducting such analysis [236]. Adjustments of mean gastric transit times (default value of 0.25 h for all age groups) was performed by incorporating prolonged times. Sotalol simulations were improved by changing mean gastric transit time from 2.3 to 2.5 h in both infants and newborns, while for paracetamol, a prolonged mean gastric transit time of 0.8 to 1.5 h in infants and 0.1 to 0.8 h in newborns gave the best predictions. Improvements of C_{max} and t_{max} (Observed/Predicted ratios) were seen for the simulations in newborns and infants.

A mechanistic absorption model for predicting formulation performance in paediatric subjects has been described for paracetamol and theophylline (BCS class I compounds), and ketoconazole, (BCS class II compound) for the fasted and fed state using the ADAM™ module of the Simcyp® software paediatric (version 15.1) [35]. Theophylline simulations were developed for the oral administration of an oral solution to newborns, infants, and adults; the aqueous drug solubility was used for the model. Although the investigated paracetamol formulation was a suspension and required the incorporation of a dissolution model within ADAM™, no further dissolution testing was performed as previous studies have reported that drug dissolution was not the absorption rate-limiting step [35; 236]; again, the aqueous drug solubility value was incorporated in the model. Ketoconazole is a drug with a highly pH-dependent aqueous solubility; hence, reference solubility values at physiologically relevant pH range 3.3 - 7.5 were used to inform the model; dissolution data were not included as an input parameter. Additionally, the model considers further processes such as intraluminal supersaturation and precipitation and bile salt mediated solubility. Paracetamol and ketoconazole simulations were developed for the oral administration of a suspension to newborns, infants, children and young adults. Theophylline plasma profiles were predicted with good accuracy (observed/predicted ratio: 0.85 - 1.25 range); the accuracy of the predictions for paracetamol and ketoconazole was evaluated as reasonable (observed/predicted ratios: 0.82 - 1.33-fold for paracetamol) [35]. The prediction for full-term newborns failed to predict the observed pharmacokinetic data for pre-term newborns. PSA revealed that extremely prolonged GE times, resulting from the absence of enteral feeding, could lead to a low systemic exposure as observed *in vivo* (*i.e.* decrease of C_{max} in the range GE 2 - 20 h), and that elevated gastric pH values (*i.e.* values higher than 4) are less likely to cause low plasma drug levels. The f_a for paracetamol and theophylline was similar in the fasted and fed state, while t_{max} was shown to be slower in the fed state. For both drugs, the slowest absorption rate among the age groups studied was the newborns. For all three compounds, t_{max} values in the fed state were greater for all ages and showed a trend towards an increase with advancing age; a slightly shorter t_{max} was demonstrated for liquid foods

compared to semi-solid or solid meals. For ketoconazole, increasing age was related to a longer t_{max} and lower f_a. Higher f_a values were observed in the fed state compared to the fasted state in all ages and no difference was observed between solid and semi-solid foods [35].

A PBPK model was developed for montelukast (BCS class II/I; log P 8.79; pKa 2.7 and 5.8) in Simcyp® for adults and paediatric patients. Montelukast is an amphiphilic drug with a high lipophilicity [245]. The simulations were first built for adults after IV and oral administration of a solution (no information about food state), and film-coated tablets in the fasted and fed state. Following validation of the adult model, scaling was performed to simulate the administration in paediatric populations after administration of oral granules in infants, and film-coated tablets in children/adolescents, but no information was given about food state in paediatrics. The model building included the experimental *in vitro* measurements of particle size and solubility in fasted simulated gastric and intestinal fluid, and the dispersion type of the different formulations. Visually, the absorption profiles were not well described for any of the paediatric age groups and mismatches of observed vs. predicted pharmacokinetic profiles could be seen for infants after administration of granules and children. Based on the model building process where parameterisation was based on sub-models, and what information was known for each age-group, predictions of plasma concentration profiles were regarded as reasonable, which in most cases appeared to be within two-fold of the observed values (no ratios of observed/predicted were provided) [245].

An adult and paediatric disease PBPK model for oral administration of carvedilol, a BCS class II drug, has been developed for patients with heart failure [246]. Carvedilol is a weak base with a pKa of 7.97 and log P of 4.19. The model was used to investigate the oral pharmacokinetics in infants, children, adolescents (oral suspension) and adults (capsules and oral suspension). Changes in hepatic and renal blood flows were incorporated in the model to simulate more accurately the physiology of chronic

heart failure patients and the accuracy of the predicted (mean ratio observed vs. predicted) pharmacokinetic parameters were improved in adults with chronic heart failure after oral administration of a capsule or a suspension. The paediatric model for carvedilol was then constructed with the pharmacokinetic parameters of carvedilol scaled to the paediatric patients by using the paediatric module of Simcyp® (version 13.1). The predictions of the exposure of carvedilol in the paediatric patients did not show as good correlations as for adults, except for patients above 17 years of age. The limitations of the applied paediatric ADAM™ model was attributed to the lack of information on anatomical and physiological changes, such as information on gastric and intestinal pH, bile secretion, transporters, and gut fluid dynamics [246].

A PBPK model was developed to investigate the age dependency in oral absorption of the poorly soluble lipophilic compound, carbamazepine (non-ionisable in the physiological pH range; BCS class II; log P of 2) [243]. The model was developed to simulate administration of different formulations in the separate age groups: administration of tablets children/adolescents, suspension prepared from crushed tablets administered to newborns and infants, and administration of oral solution, suspension and Tegretol® tablets to adults. After the development of the adult model for oral administration of different formulations, doses and food status, adjustment of clearance (to take into account patient characteristics and co-medication), the model was scaled to paediatric patients using the default parameters of Gastroplus® (version 9.0) paediatric physiology adjusted module. *In vitro* experiments were conducted to investigate biorelevant solubility and dissolution (µDISS Profiler®) in adult and paediatric biorelevant media developed by Maharaj *et al.* [109]. The dissolution experimental setups for adults and paediatrics were performed with Tegretol® tablets (or weighted fraction) added to 20 mL of the pre-heated dissolution medium (37° C). Samples were stirred at 100 rpm and the amount of dissolved drug was determined over 2 h. Dissolution experiments did not show any specific media-mediated influence on carbamazepine dissolution, more than 80% dissolved in 20 min for

almost all tested media, and for all tested media in 30 min. Despite this, neither dissolution experiments, nor solubility in paediatric biorelevant media were used as parameters for building the models. Simulated dissolution and f_a profiles were compared, and as expected for a BCS class II compound, permeation was not found to be a rate-limiting step for absorption. Nevertheless, aqueous solubility and solubility in adult fasted and fed intestinal simulated fluids were used in the model building process. Interestingly, PSA revealed that solubility and dose were the most sensitive parameters for carbamazepine f_a. Particle radius, SITT, fraction of small intestinal fluid volume, SI length and radius, permeability and bile salt solubilisation ratio, showed an impact at higher doses of carbamazepine, but only a minor impact at low doses. The prandial state was also shown to be critical for absorption of higher doses, where increases in the extent of absorption were observed for simulations in the fed state. With the exception of one study in paediatrics, the pharmacokinetic data used for the validation of the simulations did not specify food status of the patients. Nevertheless, both fasted and fed states were investigated. Interestingly, accuracy of the simulations in newborns was improved when assuming fed state conditions when compared to fasted state simulations, which supports the common assumption that newborns and young infants are mainly in fed state due to the high frequency of feedings. Fraction absorbed of carbamazepine was shown to be dose-dependent, at high doses f_a was sensitive to intestinal length and transit time, while simulations for lower doses of carbamazepine resulted in complete absorption, for a wide range of simulated intestinal lengths, and transit times [243]. The authors highlighted that this dose-dependency of carbamazepine is an important factor to take into account, as paediatric patients can sometimes require higher doses per BW. Finally, it was shown that age could influence both rate and extent of oral absorption. Low carbamazepine doses (children dose 9 mg/kg and newborns 5 mg/kg) was associated with complete absorption within 4 to 6 h after drug administration, in all age-groups, however a slower rate of absorption was seen for newborns in comparison with the older age-groups, moreover, high

carbamazepine doses (19 and 17 mg/kg respectively) were related to incomplete absorption in children and newborns [243].

The examples provided above (excluding Johnson *et al*., 2018) demonstrate the general approach followed when building the PBPK oral absorption models, as previously discussed in the Section 7.2.2. In all of the examples, knowledge gaps concerning physiological and anatomical changes in paediatrics, relevant to oral drug absorption, were pointed out as limiting factors of the models predictions. Furthermore, in most examples, several details concerning study design and formulation were lacking. The *in vitro* dissolution of the compounds was evaluated in three out of the eight examples, with two of these compounds being highly soluble ones. Moreover the dissolution data were not incorporated (as an input parameter) in the PBPK models, since no discrepancies in dissolution-adjusted conditions for paediatrics were observed for the compounds/formulations investigated so far. In future studies, it would be interesting to investigate the absorption of other classes of BCS compounds, especially poorly soluble (BCS II and IV). The prandial state in paediatric simulations has been explored in one of the examples, in most cases no information was provided for the simulations performed, which might be a result of lack of quality in clinical data for paediatrics that is used for validation of the predictions. Furthermore, the paediatric data sets used for the validation of the PBPK models, applied a sub-division of the paediatric population according to the common sub-groups. The majority of the examples were able to generate appropriate predictions for older paediatric populations (*i.e*. children) while simulations in newborns and infants were more challenging. There is still a long way to go in terms of paediatric PBPK absorption modeling, the examples of the models developed so far, are useful to generate knowledge about oral drug absorption modeling.

7.2.4. Challenges in the paediatric oral drug absorption model

The determination of organ/tissue sizes (*e.g.* volume), tissue blood flow and tissue composition estimations introduce a model uncertainty. Typically, due to lack of clinical data, relevant parameters,

e.g. length and diameter of GI tract, are extrapolated from adult data, based on BSA function for the paediatric populations and assume a proportional growth of the organs [125; 242]. The determination of GE rates and luminal composition (including the pH) in newborns and infants is challenging, due to frequent meal administration, therefore, food-related physiological responses in paediatrics is difficult to define [236]. Although biorelevant media for newborns and infants have recently been proposed [109], drug solubility estimations under conditions reflecting the luminal composition are challenging due to the limited information in the various paediatric populations and the unclear fasted vs. fed state, especially in newborns and infants. Intestinal permeability in paediatrics has been the subject of a number of studies, nevertheless, no precise values or methods have been reported; therefore the intestinal permeability for paediatric virtual populations is usually adjusted from the permeability parameter for adults (Caco-2 permeability or *in situ* permeability studies) [137; 169]. In the case of transporter involvement in the uptake or excretion of the drug, in addition to the parameters used for the adult model, the transporter availability and functionality in the paediatrics need to be confirmed and adjusted accordingly. Alternative influx and efflux routes only relevant in paediatrics populations and their contribution to the absorption process should be further investigated for the age range of interest, as shown in the process of building a PBPK model for valganciclovir, a substrate of the transporter PEPT1 [239]. In addition to the accuracy of the parameters used to describe paediatric physiology, a reasonable parameter variability value needs to be introduced in order to ensure that the generated predictions would match real-life heterogeneity among the paediatric population [227]. This can be challenging due to the nature of available paediatric data. For some of the presented examples of paediatric models in Section 7.2.3., possible formulation influence on the absorption processes was taken into consideration, although solubility and dissolution tests were not always performed, thus outlining further aspects that should be the subject of future evaluation. The established model requires validation towards clinical data acquired in the target population. Due to the lack of published high-quality clinical data in specific paediatric populations, confirmation of the developed paediatric PBPK

models has not always been possible. Finally, great importance has been assigned to the comparison of the model-predicted outcomes to clinical paediatric *in vivo* data by the EMA in the "Guidelines on the qualification and reporting of PBPK modeling and simulation" and a "Reflection paper on the use of extrapolation in the development of medicines for paediatrics" [216; 229].

8. Conclusions

Despite ongoing advances in the paediatric biopharmaceutics field, detailed knowledge on physiological differences among paediatric subpopulations and between adults is still lacking. While there have been many study outcomes reported on physiological parameters such as gastric fasted pH levels, GE times, and hepatic drug metabolism, other areas, such as GI fluid composition and SITT, intestinal metabolism, drug transporters and permeability, have been investigated to a very limited extent. Inconsistencies amongst meal types and frequencies throughout paediatric studies result in a complex definition of the paediatric prandial state, which further complicates the prediction of drug and formulation performance. Specific guidance by regulatory agencies on bioequivalence studies and age-specific definitions of fasted and fed state conditions for paediatrics is lacking, which make the development of solid evidence-based pBCS criteria quite challenging. Common background knowledge is needed for the development and validation of age-specific *in vitro* and *in silico* biopharmaceutics tools. A combination of both methods, *in vitro*/PBPK, can be utilised to obtain information that is able to compensate for the uncertainties of the single tool on its own.

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References

1. Preis M, Breitkreutz J. Pediatric Drug Development and Dosage Form Design. *AAPS PharmSciTech* 2017; 18(2): 239-40.
2. European Medicines Agency (EMA). 10-year Report to the European Commission: General report on the experience acquired as a result of the application of the Paediatric Regulation. *EMA/231225/2015.* 2017.
3. Salunke S et al. European Paediatric Formulation Initiative (EuPFI) — Formulating Ideas for Better Medicines for Children. *AAPS PharmSciTech* 2017; 18(2): 257-62.
4. Barrett JS et al. Physiologically based pharmacokinetic (PBPK) modeling in children. *Clin Pharmacol Ther* 2012; 92(1): 40-9.
5. Food and Drug Administration (FDA). General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products Guidance for Industry (draft guidance). 2014.
6. World Health Organization (WHO). Position Paper: Paediatric Age Categories to be Used in Differentiating Between Listing on a Model Essential Medicines List for Children. World Health Organization (WHO), 2007.
7. Maharaj AR, Edginton AN. Physiologically Based Pharmacokinetic Modeling and Simulation in Pediatric Drug Development. CPT Pharmacometrics Syst Pharmacol 2014; 3(11):1-13.
8. Centers for Disease Control and Prevention (CDC). CDC Growth Charts. Atlanta, GA: Centers for Disease Control and Prevention, Department of Health and Human Services, 2000.
9. Verbraecken J et al. Body surface area in normal-weight, overweight, and obese adults. A comparison study. *Metabolism* 2006; 55(4): 515-24.
10. European Medicines Agency (EMA). Reflection Paper: Formulations of choice for the paediatric population *EMEA/CHMP/PEG/194810/2005.* 2006.
11. European Medicines Agency (EMA). ICH Topic E 11 Clinical Investigation of Medicinal Products in the Paediatric Population. *PMP/ICH/2711/99.* 2001.
12. DiMaggio DM et al. Updates in Infant Nutrition. *Pediatrics in review* 2017; 38(10): 449-62.
13. Noimark L, Cox HE. Nutritional problems related to food allergy in childhood. *Pediatr Allergy Immunol* 2008; 19(2): 188-95.
14. Dewey KG, Brown KH. Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food Nutr Bull* 2003; 24(1): 5-28.
15. Schwartz C et al. Development of healthy eating habits early in life. Review of recent evidence and selected guidelines. *Appetite* 2011; 57(3): 796-807.
16. Gidding S et al. Dietary recommendations for children and adolescents: a guide for practitioners. *Pediatrics* 2006; 117(2): 544-559.
17. Dewey K. Guiding principles for complementary feeding of the breastfed child. PAN American Health organization, World Health Organization. Washington D.C. 2002.
18. European Food Safety Authority (EFSA). Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. *EFSA J* 2013; 11(10): 3408-511.
19. Butte N et al. The Start Healthy Feeding Guidelines for Infants and Toddlers. *J Am Diet Assoc.* 2004; 04(3): 442-54.
20. Harrison M et al. A qualitative systematic review of maternal infant feeding practices in transitioning from milk feeds to family foods. *Matern Child Nutr* 2016; 13(2): e12360
21. Schiess S et al. Introduction of complementary feeding in 5 European countries. *J Pediatr Gastroenterol Nutr* 2010; 50(1): 92-8
22. Anderson AS et al. Rattling the plate-reasons and rationales for early weaning. *Health Educ Res* 2001; 16(4): 471-9.
23. Heinig MJ et al. Barriers to compliance with infant-feeding recommendations among low-income women. *J Hum Lact* 2006; 1: 27-38.

24. Horodynski M et al. Low-income mothers' decisions regarding when and why to introduce solid foods to their infants: influencing factors. *J Community Health Nurs* 2007; 24(2): 101-18.
25. Butte NF. Energy requirements of infants. *Public Health Nutr 2005; 8(7A)*: 935-67.
26. European Food Safety Authority (EFSA). Scientific Opinion on Dietary Reference Values for energy. *EFSA J* 2013; 11(1): 3005-115.
27. Henry C. Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutr* 2005; 8(7a): 1133-52.
28. U.S. Department of Agriculture, U.S. Department of Health and Human Services. Dietary Guidelines for Americans. Washington, DC: U.S. Government Wrinting Offce, 2010.
29. U.S. Deparment of Agriculture. Infant Nutrition and Feeding: A guide for use in the WIC and CSF programs. 2009: 51-96.
30. World Health Organization (WHO). Management of the child with a serious infection or severe malnutrition : guidelines for care at the first-referral level in developing countries. *WHO/FCH/CAH/001.* Geneva, Switzerland: WHO, 2000.
31. World Health Organization (WHO). Caring for newborns and children in the community: Caring for the newborn at home. Geneva, Switzerland: WHO, 2015.
32. Dewey KG et al. Risk factors for suboptimal infant breastfeeding behavior, delayed onset of lactation, and excess neonatal weight loss. *Pediatrics* 2003; 112(3 Pt 1): 607-19.
33. Bergman NJ. Neonatal stomach volume and physiology suggest feeding at 1-h intervals. *Acta paediatrica* 2013; 102(8): 773-7.
34. European Food Safety Authority (EFSA). Opinion on complementary feeding of infants. *EFSA J* 2009; 7(12): 1423-61.
35. Johnson TN et al. Development and application of a physiologically-based model of paediatric oral drug absorption. *Eur J Pharm Sci.* 2018; 115:57-67.
36. European Food Safety Authority (EFSA). Scientific Opinion on Dietary Reference Values for water. *EFSA Journal* 2010; 3: 1459-1461.
37. World Health Organization (WHO). Guiding principles for feeding non-breastfed children 6-24 months of age. Geneva, Switzerland: WHO, 2005.
38. Cattaneo A et al. Infant and young child feeding: standard recommendations for the European Union In: European Network for Public Health Nutrition: Networking, M., Training, I. a. eds., 2006: 540-6.
39. Sachdev HP et al. Water supplementation in exclusively breastfed infants during summer in the tropics. *Lancet* 1991; 337(8747): 929-33.
40. Heyman M, Abrams S. Fruit Juice in Infants, Children, and Adolescents: Current Recommendations. *Pediatrics* 2017. 139(6): e20170967.
41. Abrams SA, Daniels SR. Fruit Juice and Child Health. *Pediatrics* 2017; 139(4): e20170041.
42. Bellisle F et al. A study of fluid intake from beverages in a sample of healthy French children, adolescents and adults. *Eur J Clin Nutr* 2010; 64(4): 350-55.
43. Meyers RS. Pediatric Fluid and Electrolyte Therapy. *J Pediatr Pharmacol Ther* 2009; 14(4): 204-11.
44. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC pediatrics* 2014;14(1): 216.
45. European Food Safety Authority (EFSA). Scientific Opinion on the essential composition of infant and follow-on formulae. *EFSA Journal* 2014; 7: 3760-864.
46. Keikha M et al. Macro- and Micronutrients of Human Milk Composition: Are They Related to Maternal Diet? A Comprehensive Systematic Review. *Breastfeed Med* 2017; 12(9): 517-27.
47. Michaelsen KF et al. Variation in macronutrients in human bank milk: influencing factors and implications for human milk banking. *J Pediatr Gastroenterol Nutr* 1990; 11(2): 229-39.
48. Kamstrup D et al. In Vitro Model Simulating Gastro-Intestinal Digestion in the Pediatric Population (Neonates and Young Infants). *AAPS Pharm Sci Tech* 2017; 18(2): 317-329.

49. Staelens S et al. Gastric emptying in healthy newborns fed an intact protein formula, a partially and an extensively hydrolysed formula. *Clin nutr* 2008; 27(2): 264-8.
50. Michaelsen K. Feeding and nutrition of infants and young children: guidelines for the WHO European region, with emphasis on the former Soviet countries. WHO Regional Publications, European Series No. 87. 2000.
51. European Parliament. Regulation (EU) No 609/2013 of the European Parliament and of the Council on Food Intended for Infants and Young Children, Food for Special Medical Purposes, and Total Diet Replacement for Weight Control. Official Journal of the European Union 2012, 2012: 35-56.
52. European Medicines Agency (EMA). Guideline on the investigation of drug interactions. *EMA/CHMP/458101/2016*, 2012
53. Food and Drug Administration (FDA). Guidance for Industry Food-Effect Bioavailability and Fed Bioequivalence Studies. 2002.
54. U.S. Department of Health and Human Services, National Institure of Health. *Keep the Beat? Recipes: Deliciously Healthy Family Meals*, NIH Publication No. 10-7531, 2010.
55. Kersten E et al. Physicochemical characterisation of fluids and soft foods frequently mixed with oral drug formulations prior to administration to children. *Die Pharmazie* 2016; 71(3): 122-7.
56. Martir J et al. Characterisation of the physicochemical properties of food and drinks used for the co-administration of drugs in the paediatric populations. *APPS Published abstracts*. Available from http://abstracts.aaps.org/published/, AAPS annual meeting, San Diego, 2017.
57. Neville MC, Jensen RG. E - The Physical Properties of Human and Bovine Milks. *Handbook of Milk* 57. Neville MC, Jensen RG. E - The Physical Properties of Human and Bovine Milks. *Handbook of Milk Composition.* San Diego: Academic Press, 1995: 81-85.
58. Klein S et al. Media to simulate the postprandial stomach I. Matching the physicochemical characteristics of standard breakfasts. *J Pharm Pharmacol* 2004; 56(5): 605-10.
59. Manrique YJ et al. Crushed tablets: does the administration of food vehicles and thickened fluids to aid medication swallowing alter drug release? *J Pharm Pharm Sci* 2014; 17(2): 207-19.
60. Jong GT. Pediatric Development: Physiology. Enzymes, Drug Metabolism, Pharmacokinetics and Pharmacodynamics. In: Bar-Shalom, D., Rose, K. eds. *Pediatric Formulations: A Roadmap.* New York, NY: Springer New York, 2014: 9-23.
61. Lu H, Rosenbaum S. Developmental Pharmacokinetics in Pediatric Populations. *J Pediatr Pharmacol Ther* 2014; 19(4): 262-76.
62. Ku LC, Smith PB. Dosing in neonates: special considerations in physiology and trial design. *Pediatr Res* 2015; 77(0): 2-9.
63. Nicolas JM et al. Oral drug absorption in pediatrics: the intestinal wall, its developmental changes and current tools for predictions. *Biopharm Drug Dispos* 2016; 38(3): 209-30.
64. Batchelor HK. Paediatric Development: Gastrointestinal. In: Bar-Shalom, D., Rose, K. eds. *Pediatric Formulations: A Roadmap*. New York, NY: Springer New York, 2014: 43-54.
65. Crawford M et al. Effects of duration of fasting on gastric fluid pH and volume in healthy children. *Anesth Analg* 1990; 71(4): 400-3.
66. Maekawa N et al. Effects of 2-, 4- and 12-hour fasting intervals on preoperative gastric fluid pH and volume, and plasma glucose and lipid homeostasis in children. *Acta Anaesthesiol Scand* 1993; 37(8): 783-7.
67. Schwartz DA et al. Gastric contents in children presenting for upper endoscopy. *Anesth Analg* 1998; 87(4): 757-60.
68. Nicolson SC et al. Shortened preanesthetic fasting interval in pediatric cardiac surgical patients. *Anesth Analg* 1992; 74(5): 694-697.
69. Manchikanti L et al. Assessment of age-related acid aspiration risk factors in pediatric, adult, and geriatric patients. *Anesth Analg* 1985; 64(1): 11-17.

70. Meakin G et al. Effects of fasting and oral premedication on the pH and volume of gastric aspirate in children. *Br J Anaesth* 1987; 59(6): 678-82.
71. Sandhar BK et al. Effect of oral liquids and ranitidine on gastric fluid volume and pH in children undergoing outpatient surgery. *Anesthesiology* 1989; 71(3): 327-330.
72. Schmidt AR et al. Gastric pH and residual volume after 1 and 2 h fasting time for clear fluids in childrendagger. *Br J Anaesth* 2015; 114(3): 477-82.
73. Schreiner MS et al. Ingestion of liquids compared with preoperative fasting in pediatric outpatients. *Anesthesiology* 1990; 72(4): 593-7.
74. Splinter WM et al. Clear fluids three hours before surgery do not affect the gastric fluid contents of children. *Can J Anaesth* 1990; 37(5): 498-501.
75. Splinter WM et al. The effect of preoperative apple juice on gastric contents, thirst, and hunger in children. *Can J Anaesth* 1989; 36(1): 55-8.
76. Splinter WM et al. Large volumes of apple juice preoperatively do not affect gastric pH and volume in children. *Can J Anaesth* 1990; 37(1): 36-9.
77. Splinter W, Schaefer J. Ingestion of clear fluids is safe for adolescents up to 3 h before anaesthesia. *Br J Anaesth* 1991; 66(1): 48-52.
78. Kaye JL. Review of paediatric gastrointestinal physiology data relevant to oral drug delivery. *Int J Clin Pharm* 2011; 33(1): 20-4
79. Roman C et al. Quantitative and qualitative study of gastric lipolysis in premature infants: do MCT-enriched infant formulas improve fat digestion? *Pediatr Res* 2007; 61(1): 83-8.
80. Cavell B. Postprandial gastric acid secretion in infants. *Acta Paediatr Scand* 1983; 72(6): 857-60.
81. Siegel M et al. Gastric emptying in prematures of isocaloric feedings with differing osmolalities. *Pediatr Res* 1982; 16(2): 141-7.
82. Armand M et al. Effect of Human Milk or Formula on Gastric Function and Fat Digestion in the Premature Infant. *Pediatr Res* 1996; 40(3): 429-37.
83. Batchelor HK et al. Application of in vitro biopharmaceutical methods in development of immediate release oral dosage forms intended for paediatric patients. *Eur J Pharm Sci* 2013; 85(3), Part B: 833-42.
84. Edginton AN, Fotaki N. Oral drug absorption in pediatric populations. In: Informa Healthcare USA, I. ed. *Oral Drug Absorption: Prediction and Assessment (Dressman JB and Reppas C).* New York, 2010: 108–26.
85. Yu G et al. Similarities and Differences in Gastrointestinal Physiology Between Neonates and Adults: a Physiologically Based Pharmacokinetic Modeling Perspective. *AAPS J* 2014; 16(6): 1162-66.
86. De Zwart LL et al. Pharmacokinetics of Ingested Xenobiotics in Children: a Comparison with Adults. RIVM Report 623860011, 2002
87. Kelly EJ et al. The effect of intravenous ranitidine on the intragastric pH of preterm infants receiving dexamethasone. *Arch Dis Child* 1993; 69(1 Spec No): 37-9.
88. Kelly EJ et al. Gastric acid secretion in preterm infants. *Early human development* 1993; 35(3): 215-20.
89. Omari TI, Davidson GP. Multipoint measurement of intragastric pH in healthy preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2003; 88(6): F517-20.
90. Miller B et al. Gastric residual volume in infants and children following a 3-hour fast. *J Clin Anesth* 1990; 2(5): 301-305.
91. Wolman IJ. Gastric phase of milk digestion in childhood: A study of the fasting secretions and of the physiologic responses to "hard curd" (pasteurized) and "soft curd" (homogenized) milks. *Am J Dis Child* 1946; 71(4): 394-422.
92. Gharpure V et al. Indicators of postpyloric feeding tube placement in children. *Crit Care Med* 2000; 28(8): 2962-6.

93. Metheny NA et al. Clinical Research: Indicators of Feeding-Tube Placement in Neonates. *Nutr Clin Pract* 1999; 14(6):307-14.
94. Fallingborg J et al. Measurement of Gastrointestinal pH and Regional Transit Times in Normal Children. *J Pediatr Gastroenterol Nutr* 1990; 11(2): 211-4.
95. Westhus N. Methods to test feeding tube placement in children. *MCN Am J Matern Child Nurs* 2004; 29(5): 282-7.
96. Di Maio S, Carrier RL. Gastrointestinal contents in fasted state and post-lipid ingestion: in vivo measurements and in vitro models for studying oral drug delivery. *J Control Release* 2011; 151(2): 110-22.
97. Kalantzi L et al. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm Res* 2006; 23(1): 165-76.
98. Dressman JB et al. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm Res* 7(7): 756-61.
99. Sondheimer JM et al. Continuous gastric pH measurement in young and older healthy preterm infants receiving formula and clear liquid feedings. *J Pediatr Gastroenterol Nutr* 1985; 4(3): 352-5.
100. Litman RS et al. Gastric volume and pH in infants fed clear liquids and breast milk prior to surgery. *Anesth Analg* 1994; 79(3): 482-5.
101. Fuchs A, Dressman JB. Composition and physicochemical properties of fasted-state human duodenal and jejunal fluid: a critical evaluation of the available data. *J Pharm Sci* 2014; 103(11): 3398-411.
102. Boehm G et al. Postnatal adaptation of lipase- and trypsin-activities in duodenal juice of premature infants appropriate for gestational age. *Biomed Biochimic Acta* 1990; 49(5): 369-73.
103. Fredrikzon B, Olivecrona T. Decrease of lipase and esterase activities in intestinal contents of newborn infants during test meals. *Pediatr Res* 1978; 12(5): 631-4.
104. Rune SJ, Viskum K. Duodenal pH values in normal controls and in patients with duodenal ulcer. *Gut* 1969; 10(7): 569-71.
105. Robinson PJ et al. Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci* 1990; 35(10): 1299-304.
106. Mooij MG et al. Ontogeny of oral drug absorption processes in children. *Expert Opin Drug Metab Toxicol* 2012; 8(10): 1293-303.
107. Mason S. Some Aspects of Gastric Function in the Newborn. *Arch Dis Child* 1962; 37(194): 387–391.
108. De Koning BAE et al. Developmental Changes in the Processes Governing Oral Drug Absorption. In: Bar-Shalom, D., Rose, K. eds. Pediatric Formulations: A Roadmap. New York, NY: Springer New York, 2014: 25-42.
109. Maharaj AR et al. Assessment of Age-Related Changes in Pediatric Gastrointestinal Solubility. *Pharm Res* 2016; 33(1): 52-71.
110. Barbero GJ et al. Investigations on the bacterial flora, pH, and sugar content in the intestinal tract of infants. *J Pediatr* 1952; 40(2): 152-63.
111. Abrahamse E et al. Development of the Digestive System— Experimental Challenges and Approaches of Infant Lipid Digestion. *Food Dig* 2012; 3(1-3): 63-77.
112. Bourlieu C et al. Specificity of infant digestive conditions: some clues for developing relevant in vitro models. *Crit Rev Food Sci Nutr* 2014; 54(11): 1427-57.
113. Jarvenpaa AL et al. Feeding the low-birth-weight infant. III. Diet influences bile acid metabolism. *Pediatrics* 1983; 72(5): 677-83.
114. Signer E et al. Role of bile salts in fat malabsorption of premature infants. *Arch Dis Child* 1974; 49(3): 174-80.
115. Harries JT et al. Intestinal bile salts in cystic fibrosis: studies in the patient and experimental animal. *Arch Dis Child* 1979; 54(1): 19-24.
116. Glasgow JF et al. A comprehensive study of duodenal bile salts in newborn infants and their relationship to fat absorption. *Ir J Med Sci* 1980; 149(9):346-56.

117. Billeaud C et al. Gastric emptying in infants with or without gastro-oesophageal reflux according to the type of milk. *Eur J Clin Nutr* 1990; 44(8): 577-83.
118. Thatrimontrichai A, Janjindamai W. Postprandial osmolality of gastric contents in very low-birth-weight infants fed expressed breast milk with additives. *Southeast Asian J Trop Med Public Health* 2009; 40(5): 1080-6.
119. Clarysse S et al. Postprandial evolution in composition and characteristics of human duodenal fluids in different nutritional states. *J Pharm Sci* 2009; 98(3): 1177-92.
120. Bowles A et al. Specific aspects of gastro-intestinal transit in children for drug delivery design. *Int J Pharm* 2010 16;395(1-2):37-43.
121. Bonner JJ et al. Does age affect gastric emptying time? A model-based meta-analysis of data from premature neonates through to adults. *Biopharm Drug Dispos* 2015; 36(4): 245-57
122. Mudie DM et al. Physiological Parameters for Oral Delivery and In vitro Testing. *Mol Pharm* 2010; 7(5): 1388-405.
123. Lange A et al. Gastric emptying patterns of a liquid meal in newborn infants measured by epigastric impedance. *Neurogastroenterol Motil* 1997; 9(2): 55-62.
124. Hauser B et al. Gastric Emptying of Liquids in Children. *J Pediatr Gastroenterol Nutr* 2016; 62(3): 403-8.
125. Cristofoletti R et al. Exploratory Investigation of the Limiting Steps of Oral Absorption of Fluconazole and Ketoconazole in Children Using an In Silico Pediatric Absorption Model. *J Pharm Sci* 2016; 105(9): 2794-803.
126. Van Den Driessche M, Veereman-Wauters G. Gastric emptying in infants and children. *Acta Gastroenterol Belg* 2003; 66(4): 274-82.
127. Cavell B. Gastric emptying in infants fed human milk or infant formula. *Acta Paediatr Scand* 1981; 70(5):639-41.
128. Ewer AK et al. Gastric emptying in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* 1994; 71(1):F24-7.
129. Meyer R et al. Systematic review of the impact of feed protein type and degree of hydrolysis on gastric emptying in children. *BMC gastroenterology* 2015; 137.
130. Hauser B et al. Gastric emptying of solids in children: reference values for the (13) C-octanoic acid breath test. *Neurogastroenterol Motil* 2016; 28(10): 1480-7.
131. Dressman JB et al. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharm Res* 1998; 15(1): 11-22.
132. Malik R et al. Assessment of gastric emptying in children: Establishment of control values utilizing a standardized vegetarian meal. *J Gastroenterol Hepatol* 2016; 31(2): 319-25.
133. Hardoff R et al. Gastric emptying time and gastric motility in patients with Parkinson's disease. *Mov Disord* 2001; 16(6): 1041-7
134. Maharaj AR, Edginton AN. Examining Small Intestinal Transit Time as a Function of Age: Is There Evidence to Support Age-Dependent Differences among Children? *Drug Metab Dispos* 2016; 44(7): 1080-9.
135. The International Commission on Radiological Protection (ICRP). Basic anatomical and physiological data for use in radiological protection: reference values: ICRP Publication 89. *Annals of the ICRP* 2002; 32(3–4): 1-277.
136. Weaver L et al. Small intestinal length: a factor essential for gut adaptation. *Gut* 1991; 32(11): 1321-23.
137. Batchelor HK, Marriott JF. Paediatric pharmacokinetics: key considerations. *Br J Clin Pharmacol* 2015; 79(3): 395-404.
138. Bai JPF et al. Literature Review of Gastrointestinal Physiology in the Elderly, in Pediatric Patients, and in Patients with Gastrointestinal Diseases. *J Pharm Sci* 2016; 105(2): 476-83.
139. Cummins AG et al. Crypt fission peaks early during infancy and crypt hyperplasia broadly peaks during infancy and childhood in the small intestine of humans. *J Pediatr Gastroenterol Nutr* 2008; 47(2): 153-7.

140. Cummins AG, Thompson FM. Effect of breast milk and weaning on epithelial growth of the small intestine in humans. *Gut* 2002; 51(5): 748-541.
141. Penna FJ et al. Jejunal mucosal morphometry in children with and without gut symptoms and in normal adults. *J Clin Pathol* 1981; 34(4): 386-92.
142. Batchelor HK et al. Paediatric oral biopharmaceutics: key considerations and current challenges. *Adv Drug Deliv Rev* 2014;73: 102-26.
143. Riezzo G et al. Maturation of gastric electrical activity, gastric emptying and intestinal permeability in preterm newborns during the first month of life. *Ital J Pediatr* 2009; 35(1)-6.
144. Van Elburg RM et al. Intestinal permeability in relation to birth weight and gestational and postnatal age. *Arch Dis Child Fetal Neonatal Ed* 2003; 88(1): F52-5.
145. Kerr CA et al. Early life events influence whole-of-life metabolic health via gut microflora and gut permeability. *Crit Rev Microbiol* 2015; 41(3): 326-40.
146. Batchelor HK. Paediatric biopharmaceutics classification system: Current status and future decisions. *Int J Pharm* 2014; 469(2): 251-3.
147. Batchelor HK et al. Towards the development of a paediatric biopharmaceutics classification system: Results of a survey of experts. *Int J Pharm* 2016; 469(2): 1151-7.
148. Colome G et al. Intestinal permeability in different feedings in infancy. *Acta paediatrica* 2007; 96(1): 69-72.
149. Akram G, Mullen AB. Paediatric nurses' knowledge and practice of mixing medication into foodstuff. *Int J Pharm Pract* 2012; 20(3): 191-8.
150. Stratiki Z et al. The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. *Early Hum Dev* 2007; 83(9): 575-9.
151. Mooij MG et al. Human Intestinal PEPT1 Transporter Expression and Localization in Preterm and Term Infants. *Drug Metab Dispos* 2016; 44(7): 1014-9.
152. Mooij MG et al. Ontogeny of human hepatic and intestinal transporter gene expression during childhood: age matters. *Drug Metab Dispos* 2014; 42(8): 1268-74.
153. Prasad B et al. The Promises of Quantitative Proteomics in Precision Medicine. *J Pharm Sci* 2017; 106(3): 738-44.
154. Brouwer KL et al. Human Ontogeny of Drug Transporters: Review and Recommendations of the Pediatric Transporter Working Group. *Clin Pharmacol Ther* 2015; 98(3): 266-87.
155. Elmorsi Y et al. Ontogeny of Hepatic Drug Transporters and Relevance to Drugs Used in Pediatrics. *Drug Metab Dispos* 2016; 44(7): 992-8.
156. Abdel-Rahman SM et al. Summary of the National Institute of Child Health and Human Development–Best Pharmaceuticals for Children Act Pediatric Formulation Initiatives Workshop–Pediatric Biopharmaceutics Classification System Working Group. *Clin Ther* 2012; 34(11): S11-24.
157. Quigley EMM. Microflora Modulation of Motility. *Neurogastroenterol Motil* 2011; 17(2): 140-7.
158. Merchant HA et al. Age-mediated changes in the gastrointestinal tract. *Int J Pharm* 2016; 512(2): 382-95.
159. Sadler NC et al. Hepatic Cytochrome P450 Activity, Abundance, and Expression Throughout Human Development. *Drug Metab Dispos* 2016; 44(7): 984-91.
160. Amidon G et al. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res* 1995; 3: 413-20.
161. Lennernäs H, Abrahamsson B. The use of biopharmaceutic classification of drugs in drug discovery and development: current status and future extension. *J Pharm Pharmacol* 2005; 57(3): 273-85.
162. Ku M. Use of the Biopharmaceutical Classification System in Early Drug Development. *AAPS J* 2008; 10(1): 208-12.
163. Shawahna R. Pediatric Biopharmaceutical Classification System: Using Age-Appropriate Initial Gastric Volume. *AAPS J* 2016; 18(3): 728-36.
164. Gandhi SV et al. Considerations for a Pediatric Biopharmaceutics Classification System (BCS): Application to Five Drugs. *AAPS PharmSciTech* 2014; 15(3): 601-11.

165. Mahmood I. Dosing in children: a critical review of the pharmacokinetic allometric scaling and modelling approaches in paediatric drug development and clinical settings. *Clin Pharmacokinet* 2014; 53(4): 327-46.
166. Hens B et al. Evaluation of real-life dosing of oral medicines with respect to fluid and food intake in a Dutch-speaking population. *J Clin Pharm Ther* 2017; 42(4): 467-74.
167. European Medicines Agency (EMA). Guideline on pharmaceutical development of medicines for paediatric use. *EMA/CHMP/QWP/805880/2012, Rev 2.* 2013.
168. Martir J et al. Recommended strategies for the oral administration of paediatric medicines with food and drinks in the context of their biopharmaceutical properties: a review. *J Pharm Pharmacol* 2016; 69(4): 384-97
169. Elder DP et al. Medicines for Pediatric Patients—Biopharmaceutical, Developmental, and Regulatory Considerations. *J Pharm Sci* 2017; 106(4): 950-60.
170. Boateng J. Drug Delivery Innovations to Address Global Health Challenges for Pediatric and Geriatric Populations (Through Improvements in Patient Compliance). *J Pharm Sci* 2017; 106(11): 3188-98.
171. Purohit V. Biopharmaceutic Planning in Pediatric Drug Development. *AAPS J* 2012; 14(3): 519-22.
172. Quijano Ruiz B et al. Pediatric formulation issues identified in Paediatric Investigation Plans. *Expert Rev Clin Pharmacol* 2014; 7(1): 25-30.
173. Mistry P et al. Evidence of acceptability of oral paediatric medicines: a review. *J Pharm Pharmacol* 2017; 69(4): 361-76.
174. European Medicines Agency (EMA). Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population. *EMEA/CHMP/EWP/147013/2004,* 2008
175. Food and Drug Administration (FDA). Guidance for Industry General Considerations for Pediatric Pharmacokinetic Studies for Drugs and Biological Products. 1998.
176. Batchelor H. Influence of Food on Paediatric Gastrointestinal Drug Absorption Following Oral Administration: A Review. *Children* 2015; 2(2): 244-71.
177. Lancaster DL et al. 6-Thioguanine in children with acute lymphoblastic leukaemia: influence of food on parent drug pharmacokinetics and 6-thioguanine nucleotide concentrations. *Br J Clin Pharmacol* 2001; 51(6): 531-9.
178. Borrmann S et al. The effect of food consumption on lumefantrine bioavailability in African children receiving artemether-lumefantrine crushed or dispersible tablets (Coartem) for acute uncomplicated Plasmodium falciparum malaria. *Trop Med Int Health* 2010; 15(4): 434-41.
179. Ginsburg CM et al. Effect of feeding on bioavailability of griseofulvin in children. *J Pediatr* 1983; 102(2): 309-11.
180. De Guchtenaere A et al. Pharmacokinetic data on oral desmopressin reducing dosage by changing to a new oral lyophilisate (melt) formulation. *J Urol* 2012; 187(4) Suppl: e301.
181. Lonnerholm G et al. Oral mercaptopurine in childhood leukemia: influence of food intake on bioavailability. *Pediatr Hematol Oncol* 1989; 6(2): 105-12.
182. Mccracken GH, Jr. et al. Pharmacologic evaluation of orally administered antibiotics in infants and children: effect of feeding on bioavailability. *Pediatrics* 1978; 62(5): 738-43.
183. Riccardi R et al. Influence of food intake on bioavailability of oral 6-mercaptopurine in children with acute lymphoblastic leukemia. *Pediatr Hematol Oncol* 1986; 3(4): 319-24.
184. Sofianou-Katsoulis A et al. Reduction in bioavailability of 6-mercaptopurine on simultaneous administration with cow's milk. *Pediatr Hematol Oncol* 2006; 23(6): 485-7.
185. Stevens RC et al. Effect of food and pharmacokinetic variability on didanosine systemic exposure in HIV-infected children. Pediatric AIDS Clinical Trials Group Protocol 144 Study Team. *AIDS Res Hum Retroviruses* 2000; 16(5): 415-21.
186. De Bruyne P et al. Pharmacokinetics of desmopressin administered as tablet and oral lyophilisate formulation in children with monosymptomatic nocturnal enuresis. *Eur J Pediatr* 2014; 173(2): 223-8.

187. Ginsburg CM et al. Comparative Pharmacokinetics of Amoxicillin and Ampicillin in Infants and Children. *Pediatrics* 1979; 64(5): 627-31.
188. Fleisher D et al. Drug, meal and formulation interactions influencing drug absorption after oral administration. Clinical implications. *Clin Pharmacokinet* 1999; 36(3): 233-54.
189. Jann MW et al. Interaction of dietary pudding with phenytoin. *Pediatrics* 1986; 78(5): 952-3.
190. Notterman DA et al. Effect of dose formulation on isoniazid absorption in two young children. *Pediatrics* 1986; 77(6): 850-2.
191. Tuleu C, Breitkreutz J. Educational paper: formulation-related issues in pediatric clinical pharmacology. *Eur J Pediatr* 2013; 172(6): 717-20.
192. Knorr B et al. Pharmacokinetics and safety of montelukast in children aged 3 to 6 months. *J Clin Pharmacol* 2006; 46(6): 620-7.
193. Andersson T et al. Pharmacokinetics of orally administered omeprazole in children. International Pediatric Omeprazole Pharmacokinetic Group. *Am J Gastroenterol* 2000; 95(11): 3101-6.
194. Turner MA et al. Paediatric drug development: the impact of evolving regulations. *Adv Drug Deliv Rev* 2014: 73(2-13).
195. Dresser GK et al. Fruit juices inhibit organic anion transporting polypeptide–mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* 2002; 71(1): 11-20.
196. Batchelor HK et al. Food effects in paediatric medicines development for products Co-administered with food. *Int J Pharm* 2017; 536(2): 530-5.
197. Strickley RG et al. Pediatric drugs-a review of commercially available oral formulations. *J Pharm Sci* 2008; 97(5): 1731-74.
198. European Commission. Medicinal Products for Paediatric use. *Regulation (EC) No 1901/2006*. Official Journal of the European Union, 2006.
199. Toothaker RD, Welling PG. The effect of food on drug bioavailability. *Annu Rev Pharmacol Toxicol* 1980; 20: 173-99.
200. Kakuda TN et al. Pharmacokinetics of darunavir after administration of an oral suspension with low-dose ritonavir and with or without food. *Clin Pharmacol Drug Dev* 2014; 3(5): 346-52.
201. Salem AH et al. A novel ritonavir paediatric powder formulation is bioequivalent to ritonavir oral solution with a similar food effect. *Antivir Ther* 2015; 20(4): 425-32.
202. Stampfuss J et al. The effect of food on the absorption and pharmacokinetics of rivaroxaban. *Int J Clin Pharmacol Ther* 2013; 51(7): 549-61.
203. Brouwers J et al. Parallel monitoring of plasma and intraluminal drug concentrations in man after oral administration of fosamprenavir in the fasted and fed state. *Pharm Res* 2007; 24(10): 1862-9.
204. Dohil R, Rioux P. Pharmacokinetic Studies of Cysteamine Bitartrate Delayed-Release. *Clin Pharmacol Drug Dev* 2013; 2(2): 178-85.
205. Mclean A et al. The influence of food on the bioavailability of a twice-daily controlled release carbamazepine formulation. *J Clin Pharmacol* 2001; 41(2): 183-6.
206. Cassilly D et al. Gastric emptying of a non-digestible solid: assessment with simultaneous SmartPill pH and pressure capsule, antroduodenal manometry, gastric emptying scintigraphy. *Neurogastroenterol Motil* 2008; 20(4): 311-9.
207. Karim A. Karim A. Effects of food on the bioavailability of theophylline from controlled-release products in adults. *J Allergy Clin Immunol* 1986; 78(4 Pt 2): 695-703.
208. Sips AP et al. Food does not effect in bioavailability of theophylline from Theolin Retard. *Eur J Clin Pharmacol* 1984; 26(3): 405-7.
209. Pedersen S. Effects of food on the absorption of theophylline in children. *J Allergy Clin Immunol* 1986; 78(4 Pt 2): 704-9.
210. Chew ML et al. Pharmacokinetics of pregabalin controlled-release in healthy volunteers: effect of food in five single-dose, randomized, clinical pharmacology studies. *Clin Drug Investig* 2014; 34(9): 617-26.

211. Karkossa F et al. Simulating Different Dosing Scenarios for a Child-Appropriate Valproate ER Formulation in a New Pediatric Two-Stage Dissolution Model. *AAPS PharmSciTech* 2017; 18(2): 309-316.
212. Fotaki N, Brassine C. Age related biorelevant dissolution testing for pediatric pellet formulations *APPS Published abstracts*. Available from http://abstracts.aaps.org/published/, AAPS annual meeting, San Antonio, 2013
213. Mencarelli G et al. Age related biorelevant dissolution testing for paediatric formulations *Int J Pharm* 2018; 536: 490-522.
214. Havenaar R et al. In vitro gastrointestinal model (TIM) with predictive power, even for infants and children? *Int J Pharm* 2013; 457(1): 327-32.
215. Klitgaard M et al. Studying furosemide solubilization using an in vitro model simulating gastrointestinal digestion and drug solubilization in neonates and young infants. *Eur J Pharm Sci*.2017; 109: 191-99.
216. European Medicines Agency (EMA). Draft reflection paper on the use of extrapolation in the development of medicines for paediatrics. *EMA/199678/2016*, 2017.
217. Food and Drug Administration (FDA). Leveraging Existing Clinical Data for Extrapolation to Pediatric Uses of Medical Devices: Guidance for Industry and Food and Drug Administration Staff. 2016
218. Dunne J et al. Extrapolation of adult data and other data in pediatric drug-development programs. *Pediatrics* 2011; 128(5): e1242-9.
219. Sharma V, Mcneill J. To scale or not to scale: the principles of dose extrapolation. *Br J Pharmacol* 2009; 157(6): 907-21.
220. Samant TS et al. Quantitative clinical pharmacology for size and age scaling in pediatric drug development: A systematic review. *J Clin Pharmacol* 2015; 55(11): 1207-17.
221. Mahmood I et al. 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.
222. Bjorkman S. Prediction of cytochrome p450-mediated hepatic drug clearance in neonates, infants and children : how accurate are available scaling methods? *Clin Pharmacokinet* 2006; 45(1): 1-11.
223. Calvier EA et al. Allometric Scaling of Clearance in Paediatric Patients: When Does the Magic of 0.75 Fade? *Clin Pharmacokinet* 2017; 56(3): 273-85.
224. Edginton AN et al. A mechanistic approach for the scaling of clearance in children. *Clin Pharmacokinet* 2006; 45(7): 683-704.
225. Jones H et al. A novel strategy for physiologically based predictions of human pharmacokinetics. *Clin Pharmacok* 2006; 45(5): 511-42.
226. Zhuang X, Lu C. PBPK modeling and simulation in drug research and development. *Acta Pharmaceutica Sinica B* 2016; 6(5): 430-40.
227. Kostewicz ES et al. PBPK models for the prediction of in vivo performance of oral dosage forms. *Eur J Pharm Sci* 2014; 57: 300-21.
228. Food and Drug Administration (FDA). Physiologically Based Pharmacokinetic Analyses — Format and Content Guidance for Industry (draft guidance). 2016.
229. European Medicines Agency (EMA). Guideline on the qualification and reporting of 4 physiologically based pharmacokinetic (PBPK) modelling 5 and simulation. *EMA/CHMP/458101/2016*, 2016.
230. Upton RN et al. An introduction to physiologically-based pharmacokinetic models. *Paediatr Anaesth* 2016; 26(11): 1036-46.
231. Tsamandouras N et al. Combining the 'bottom up' and 'top down' approaches in pharmacokinetic modelling: fitting PBPK models to observed clinical data. *Br J Clin Pharmacol* 2015; 79(1): 48-55.
232. Simulations Plus Inc., 2017. https://www.simulations-plus.com/.
233. Certara USA I 2011-2018. https://www.certara.com/.
234. Bouzom F et al. Physiologically based pharmacokinetic (PBPK) modelling tools: how to fit with our needs? *Biopharm Drug Dispos* 2012; 33(2): 55-71.

235. Jamei M. Recent advances in development and application of physiologically-based pharmacokinetic (PBPK) models: a transition from academic curiosity to regulatory acceptance. *Pharmacol Rep* 2016; 2(3): 161-69.
236. Villiger A et al. Using Physiologically Based Pharmacokinetic (PBPK) Modelling to Gain Insights into the Effect of Physiological Factors on Oral Absorption in Paediatric Populations. *AAPS J* 2016;18(4): 933-47.
237. Maharaj AR et al. A Workflow Example of PBPK Modeling to Support Pediatric Research and Development: Case Study with Lorazepam. *AAPS J* 2013; 15(2): 455-64.
238. Khalil F, Läer S. Physiologically Based Pharmacokinetic Models in the Prediction of Oral Drug Exposure Over the Entire Pediatric Age Range—Sotalol as a Model Drug. *AAPS J* 2014; 16(2): 226-39.
239. Lukacova V et al. A Physiologically Based Pharmacokinetic Model for Ganciclovir and Its Prodrug Valganciclovir in Adults and Children. *AAPS J* 2016; 18(6): 1453-63.
240. Kuepfer L et al. Applied Concepts in PBPK Modeling: How to Build a PBPK/PD Model. *CPT Pharmacometrics Syst Pharmacol* 2016; 5(10): 516-31.
241. Simulations Plus Inc. Gastroplus PBPK Modeling Software... from Discovery through Development. 2016: 1–16, http://www.simulations-plus.com/assets/GastroPlus-Brochure-Nov2016.pdf.
242. Simulations Plus Inc. Pediatric PBPK Modeling - Special Considerations in GastroPlus. In: Lukacova, V. ed., 2015: 1–16, https://www.simulations-plus.com/resource-center/?resource-category=videos.
243. Kohlmann P et al. Investigating Oral Absorption of Carbamazepine in Pediatric Populations. *AAPS J* 2017; 19(6): 1864-77.
244. Edginton AN, Ritter L. Predicting Plasma Concentrations of Bisphenol A in Children Younger Than 2 Years of Age after Typical Feeding Schedules, using a Physiologically Based Toxicokinetic Model. *Environ Health Perspect* 2009; 117(4): 645-52.
245. Jones HM et al. Physiologically based pharmacokinetic modeling in drug discovery and development: a pharmaceutical industry perspective. *Clin Pharmacol Ther* 2015; 97(3): 247-62.
246. Rasool MF et al. A Physiologically Based Pharmacokinetic Drug–Disease Model to Predict Carvedilol Exposure in Adult and Paediatric Heart Failure Patients by Incorporating Pathophysiological Changes in Hepatic and Renal Blood Flows. *Clin Pharmacokinet* 2015; 54(9): 943-62.

List of Tables

Table 1 Age groups classification according to ICH [10; 11], FDA and WHO [5; 6]. (d - days; mo - months; yr - years).

Age Groups	ICH	FDA	WHO	Body weight (kg)		Body Surface Area (m^2)	
				male	female	male	female
Newborn	0 – 27 d *(a)*	0 – 1 mo	0 - 30 d	**birth**			
				3.4	3.2	0.22	0.21
Infants	28 d – 23 mo *(b)*	1 mo – 2 yr	1 mo – 2 yr	**1 mo**			
				4.5	4.1	0.38	0.37
				6 mo			
				7.9	7.3	0.45	0.43
				1 yr			
				9.6	8.9	0.56	0.53
Children	2 – 5 yr *(c)*	2 – 12 yr	2 – 6 yr *(d)*	**2 yr**			
				13	12	0.68	0.67
				4 yr			
				16.1	15.9	0.82	0.8
	6 - 11 yr *(e)*		6– 12 yr *(f)*	**6 yr**			
				20.9	20	0.95	0.95
				8 yr			
				25.5	25.5	1.11	1.12
				10 yr			
				32	33	1.11	1.12
Adolescents	12-16 or 18 yr *(g)*	12 – 16 yr	12 – 18 yr	**12 yr**			
				40.5	41.9	1.29	1.33
				14 yr			
				51	49.5	1.52	1.49
				16 yr			
				61	54	1.72	1.56
				18 yr			
				67	56	1.81	1.59
Adults	>16-18 yr	>16 yr	>18 yr	**20 yr**			
				85.9	72.1	2.05	1.8

(a) Usually known in literature as neonates

(b) Infants and toddlers

(c) Pre-school child

(d) Young child

(e) School child

(f) Child

(g) Depending on region

2099 **Table 2** Characteristics of usual meals in paediatric subpopulations and adults. (d - days; mo - months; yr - years)

Type of food	Age	Total caloric content			Caloric density [kcal/g]	Caloric content/recommended portion [kcal]	Portion size
		Fats [%]	Carbohydrates [%]	Proteins [%]			
Human breast milk (colostrum) [12; 44]	1-3 d	30	42	15	0.5-0.6	30-35	60 mL
Human breast milk (mature milk) [12; 44; 50]	>15 d	46-54	41-46	7	0.6-0.7	54-126	90-180 mL
Infant formulae [51]	>1 d	40-55	36-54	7-10	0.6-0.7	-42-140	70-230 mL
Follow-on formulae [51]	>6 mo	35-55	36-54	7-14	0.6-0.7	160-170	230-240 mL
Fortified milk 1+ [51]	>12 mo	37-45	39-52	12-16	0.6-0.7	150-160	240 mL
Whole cow's milk	>36 mo	47-53	27-30	21	0.6-0.7	165	250 mL
Fruit puree[a]	5 mo	2-9	87-96	2-6	0.5-0.6	50-125	100-190 g
Fruit with cereal[a]	6 mo	2-7	88-91	3-8	0.6-0.9	120-160	190 g
Porridge and Creams[a]	8 mo	25-35	55-62	10-14	1.0-1.3	200-240	180-210 g
Infant Meal[a]	5 mo	26-45	44-55	12-20	0.6-0.9	110-170	190 g
	12 mo	27-39	44-60	12-19	0.7-0.8	170-200	250 g
Recommended meal [28]	>12 mo	30-40	45-65	5-20	1.0-1.1[b]	230-380[b]	220-370 g[b]
	>4 yr	25-35	45-65	10-30	0.6-1.8[c]	150-350[c]	150-350 g[c]
Recommended meal [28]	>19 yr	20-35	45-65	10-35	1.1-1.2[d]	500-760[d]	490-680 g[d]
FDA/EMA standard breakfast[e] [52; 53]	adults	50-60	25-30	15-20	1.5-1.8	800-1000	500 g

2100

2101 [a] On average basis; calculated from a search including commercially available infant meals, fruit purees and infant formula milk products

[b] Portions of the recommended foods are adjusted to the suggestions for meal distribution as recommended in [16; 28]

[c] Parameters were calculated from recommended family recipes, aimed at promoting healthy eating habits among children [54]

[d] Parameters calculated from the proposed sample meal [28]

[e] Suggested by the US FDA and EMA in the respective guidelines on investigation of food effect bioavailability and fed bioequivalence studies [52; 53]

2108 **Table 3** Fasted gastric volumes as a function of BW reported in the literature [N: sample size; SD: standard deviation; yr - years].

Age group of participants	N	Age [yr]		Weight [kg]		Volume [mL/kg]		Ref.
		Mean (SD)	Range	Mean (SD)	Weight	Mean (SD)	Range	
infants/children/adolescents	248	8.1 (5.7)	0.17-18	31.2 (32)	3.1-115	0.35 (0.45)	0-3.14	[67]
infants/children	20	3.3 (3.9)	0.5-5	14.3 (12.1)	-	0.40 (0.6)	-	[68]
infants/children/adolescents	25	6.2 (0.7)	0.5-12	24.6 (2.8)	6.8-58.1	0.49 (0.04)	0.21-1.15	[69]
infants/children/adolescents	35	4.5 (2.9)	1.2-12	17.5 (8.1)	9-43.5	0.36 (0.42)	0-1.64	[66]
infants/children/adolescents	55	6.6	1-14	26.1	10-77	0.25 (0.04)	-	[70]
infants/children/adolescents	100	-	1-14	-	-	0.56 (0.39)	0.1-2.5	[65]
infants/children/adolescents	19	5.2 (0.55)	1-14	21 (2.17)	-	0.25	0-1.1	[71]
infants/children	66	-	1-16	-	-	0.5 (0.4)	0-1.89	[72]
infants/children/adolescents	68	7.3 (4.6)	1-18	29 (17.7)	-	0.57 (0.51)	0-2.23	[73]
children/adolescents	64	5.7 (2.5)	2-12	26.1 (7.6)	5.7 (2.5)	0.39 (0.37)	0.04-1.97	[74]
children	40	7.4 (1.7)	5-10	26.1 (7.6)	-	0.43 (0.46)	0.01-1.65	[75]
children	31	7.4 (1.6)	5-10	26 (7)	7.4 (1.6)	0.45 (0.31)	0.02-1.15	[76]
adolescents	76	15 (2)	13-19	60 (16)	15 (2)	0.48 (0.40)	0.02-2.11	[77]
adults	50	38.8 (2)	18-64	68.5 (2.3)	45.5-110.0	0.37 (0.04)	0.05-1.33	[69]

2109

Table 4 Composition of adult reference biorelevant media and age-specific (grey) simulating fasted and fed state gastric and intestinal media [109].

	Gastric Media						Intestinal Media						
	fasted state			fed state			fasted state			fed state			
Component	**FaSSGF**	**Pn-FaSSGF**	**Pi-FaSSGF**	**FeSSGF**	**Pnc-FeSSGF**	**Pns-FeSSGF**	**FaSSIF-V2**	**P50%-FaSSIF**	**P150%-FaSSIF**	**FeSSIF-V2**	**Pnb-FeSSIF**	**Pnc-FeSSIF**	**Pi-FeSSIF**
Sodium Taurocholate (mM)	0.08	0.02	0.060	-	-	-	3	1.5	4.5	10	2.5	2.5	7.5
Lecithin (mM)	0.02	0.005	0.015	-	-	-	0.2	0.1	0.3	2	0.5	0.5	1.5
Glyceryl Monooleate (mM)	-	-	-	-	-	-	-	-	-	5	5	6.65	5
Sodium Oleate (mM)	-	-	-	-	-	-	-	-	-	0.8	0.8	1.06	0.8
Pepsin (mg/mL)	0.1	0.015	0.025	-	-	-	-			-	-	-	-
Sodium Chloride (mM)	34.2	34.2	34.2	237.02	100.35	94.79	68.62	68.62	68.62	125.5	95	111.73	107.35
Acetic Acid (mM)	-	-	-	17.12	7.25	7.25	-	-	-	-	-	-	-
Sodium Acetate (mM)	-	-	-	29.75	64.65	64.65	-	-	-	-	-	-	-
Maleic Acid (mM)	-	-	-	-	-	-	19.12	19.12	19.12	55.02	55.02	55.02	55.02
Sodium Hydroxide (mM)	-	-	-	-	-	-	34.8	34.8	34.8	81.65	81.65	81.65	81.65
Milk:Buffer	-	-	-	1.1	1.1	1.1	-	-	-	-	-	-	-
HCl/NaOH qs	pH1.6	pH1.6	pH1.6	pH5	pH5.7	pH5.7	pH6.5	pH6.5	pH6.5	pH5.8	pH5.8	pH5.8	pH5.8
pH	1.6	1.6	1.6	5	5.7	5.7	6.5	6.5	6.5	5.8	5.8	5.8	5.8
Osmolality (mOsmol/Kg)	120.7	120.7	120.7	400	340	240	180	180	180	390	300	330	330
Buffer Capacity (mmol/L/ΔpH)	-	-	-	25	15	15	10	10	10	25	25	25	25

FaSSGF – Adult fasted-state gastric media;

Pn-FaSSGF – Paediatric fasted-state gastric media representative of newborns (0–28 days);

Pi-FaSSGF – Paediatric fasted-state gastric media representative of infants (1–12 months);

FeSSGF – Adult fed-state gastric media;

Pnc-FeSSGF – Paediatric fed-state gastric media representative of newborns (0–28 days) fed cow's milk-based formula;

Pns-FeSSGF – Paediatric fed-state gastric media representative of newborns (0–28 days) fed soy-based formula.

FaSSIF-V2 – Adult fasted-state intestinal media;

P50%-FaSSIF – Paediatric fasted-state intestinal media formulated with bile salt concentrations 50% (*i.e.* 1.5 mM) of adult levels;

P150%-FaSSIF – Paediatric fasted-state intestinal media formulated with bile salt concentrations 150% (*i.e.* 4.5 mM) of adult levels;

FeSSIF-V2 – Adult fed-state intestinal media;

Pnb-FeSSIF – Paediatric fed-state intestinal media representative of newborns (0–28 days) fed breast milk;

Pnc-FeSSIF – Paediatric fed-state intestinal media representative of newborns (0–28 days) fed cow's milk-based formula;

Pi-FeSSIF – Paediatric fed-state intestinal media representative of infants.

Figure captions

Figure 1 Average amount of energy required for paediatric populations as recommended for different physical activity levels by the EFSA (solid lines and filled symbols) and the U.S. Department of Health and Human Services and U.S. Department of Agriculture (discontinued lines and open symbols). (**A)** daily average energy requirement related to a sedentary lifestyle; (**B)** daily average energy requirement related to a moderate level of activity; Recommendations for males (blue diamonds) and females (red circles). The retrieved data for newborns and infants are independent of the physiological activity level. Data included in this figure were obtained from [18; 26; 28; 29].

Figure 2 Range of feeding volumes for formula-fed newborns and infants (**A**) and feeding intervals (**B**) for newborns and infants, receiving either infant or follow-on formula ("formula", open blocks), or being breastfed (grey-filled blocks). The feeding intervals for breastfed and formula-fed infants are the same beyond the age of two months (purple blocks) (mo: months; modified from DiMaggio and co-workers [12])

Figure 3 European recommended ranges for total water intake in paediatrics. Values include intake of water, beverages of all kind, and water from food moisture. Populations younger than 9 years: filled purple blocks; males: blocks filled in grey; females: open blocks. Recommendations for adolescents >14 years of age are also applicable for adults (d - days; mo - months; yr - years). Data used for this figure was retrieved from [36].

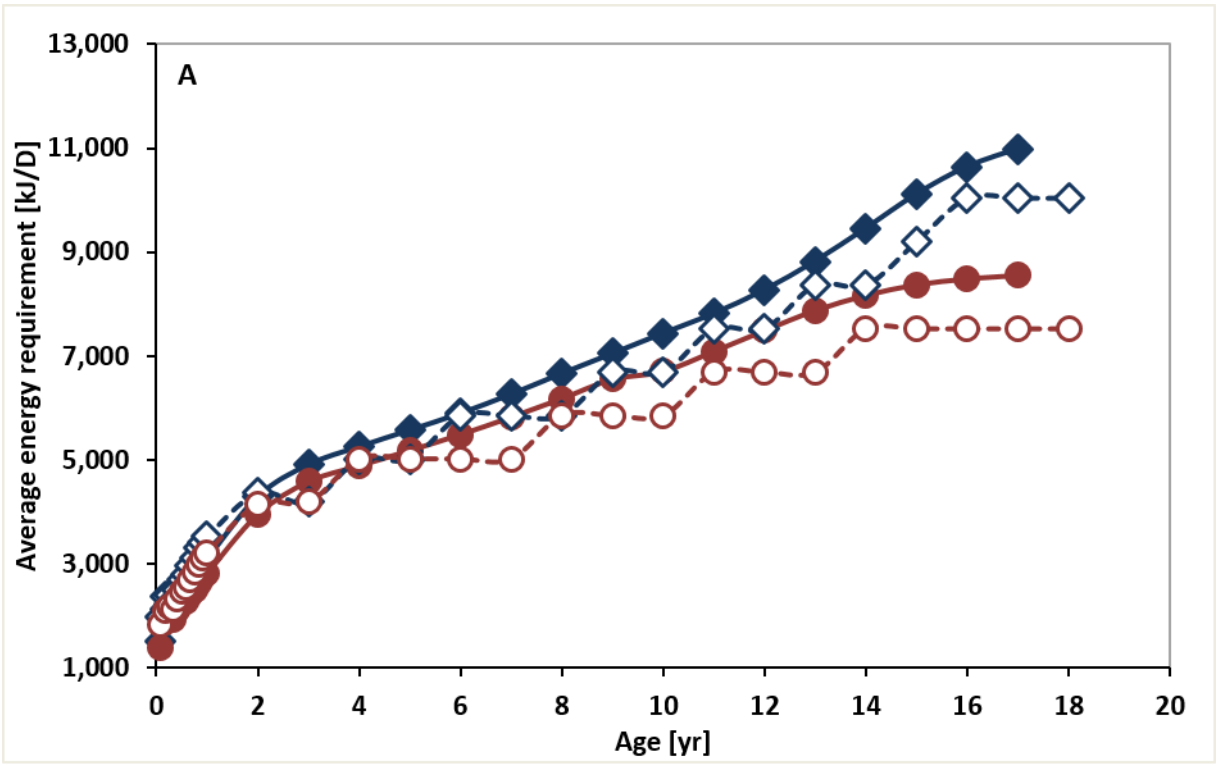
Figure 4 Physicochemical properties of various soft foods and liquids administered in paediatric populations and an adult meal used for food effect investigation of bioavailability and bioequivalence of drug products (FDA standard breakfast): (A) pH-values; (B) Buffer capacity measured with 0.1 N sodium hydroxide solution; (C) Osmolality; (D) Surface tension; (E) Viscosity; * Soft foods/foods are non-Newtonian fluids. Modified from [55; 56; 58; 59].

Figure 5 Gastric (A) and intestinal (B) pH in fasted (open symbols) and fed state (closed symbols). Paediatric and adult pH values were collected from literature and depicted as either mean (circles) or median (triangles) values. In the fed state values depicted represent values measured after ingestion of different types of food. When patients participating in the paediatric studies belonged to more than one age group, values were used as mean age, or if a specific age range was reported without denoting the groups mean age, data was depicted using the middle of the age range [65-67; 70-77; 87-105].

Figure 6 Fed Gastric Emptying half-life for newborns and young infants (0-10 wk), children and adults: values depict either mean (circle symbols) or median values (triangle symbols). Infant formula milk: yellow symbols; breast milk: blue symbols; cow's milk: green symbols; solid food: red symbols. Data was collected from

2162 different studies and milk products and solid food did not contain the same amount of calories and were
2163 administered in different volumes [49; 84; 124; 126; 130-133].
2164
2165 **Figure 7** Extrapolated initial gastric volumes during drug administration to paediatric populations based on
2166 250 mL volume of water administered to adults with solid dosage forms. Extrapolation was based on BW: grey
2167 blocks [146; 147] and white blocks [163], or based on BSA-function: black blocks [164].
2168
2169 **Figure 8** Statistics of published PBPK models, search performed on PubMed (Status August 2017; n = 93).
2170 (A) Studied paediatric subpopulations; (B) Basic model used for paediatric PBPK model development; (C) Aim
2171 of PBPK modeling; (D) Software platforms utilised for paediatric PBPK model development. (DDI – drug-drug
2172 interactions).
2173
2174 **Figure 9** BCS class distribution amongst modeled drugs, identified in the PBPK search in PubMed. Only
2175 compounds, modeled for oral absorption are considered in this figure, n = 32. The numbers above each bar refer
2176 to the number of drugs studied according to their BCS classification. ND = Not defined.
2177
2178 **Figure 10** Usual strategy for paediatric PBPK model development with a focus on oral drug absorption**. PSA**:
2179 parameter sensitivity analysis; bio-dependent drug properties: drug parameter values that depend on the drug
2180 and the adult/paediatric human physiology.
2181

2182 **Figure 1**



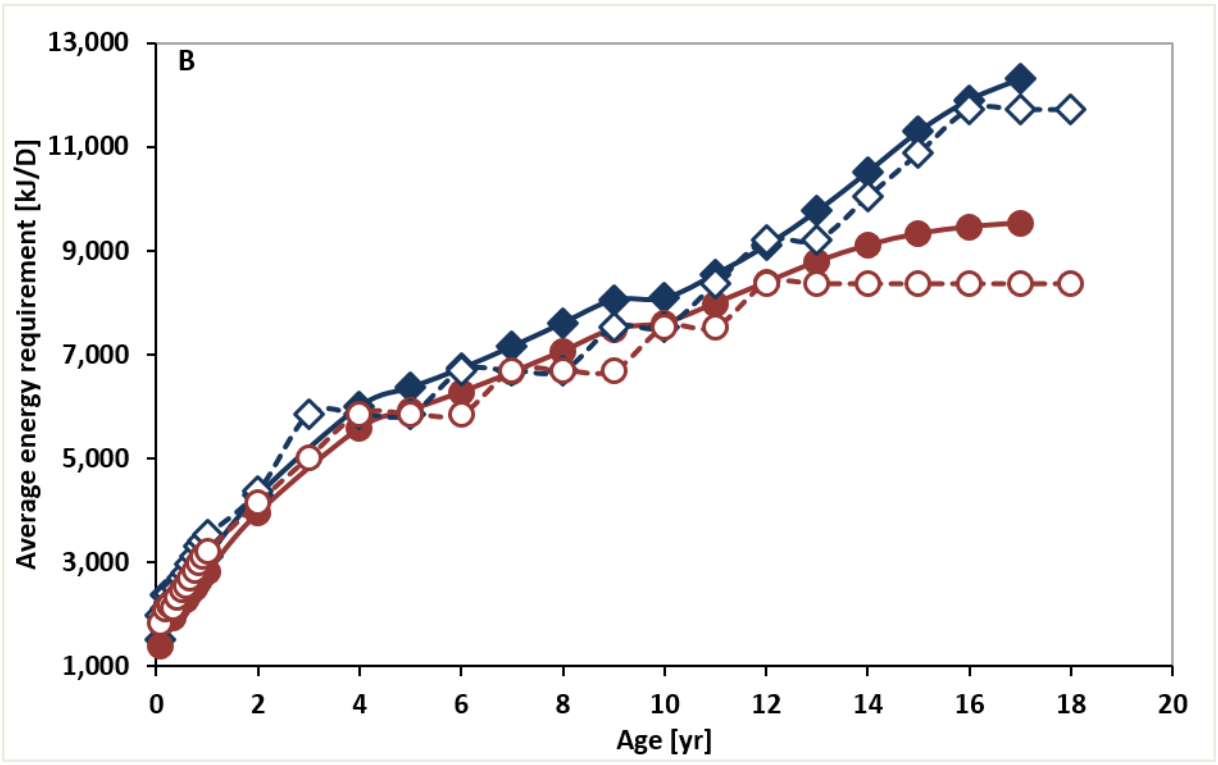


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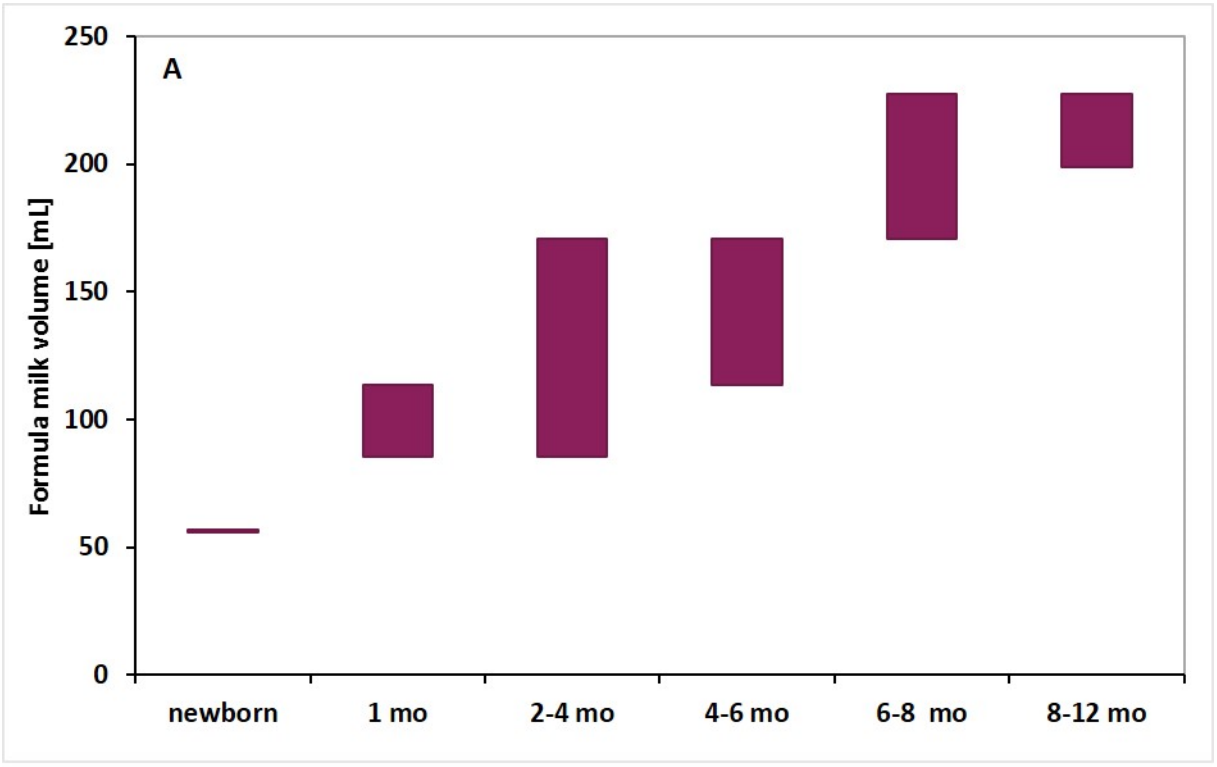
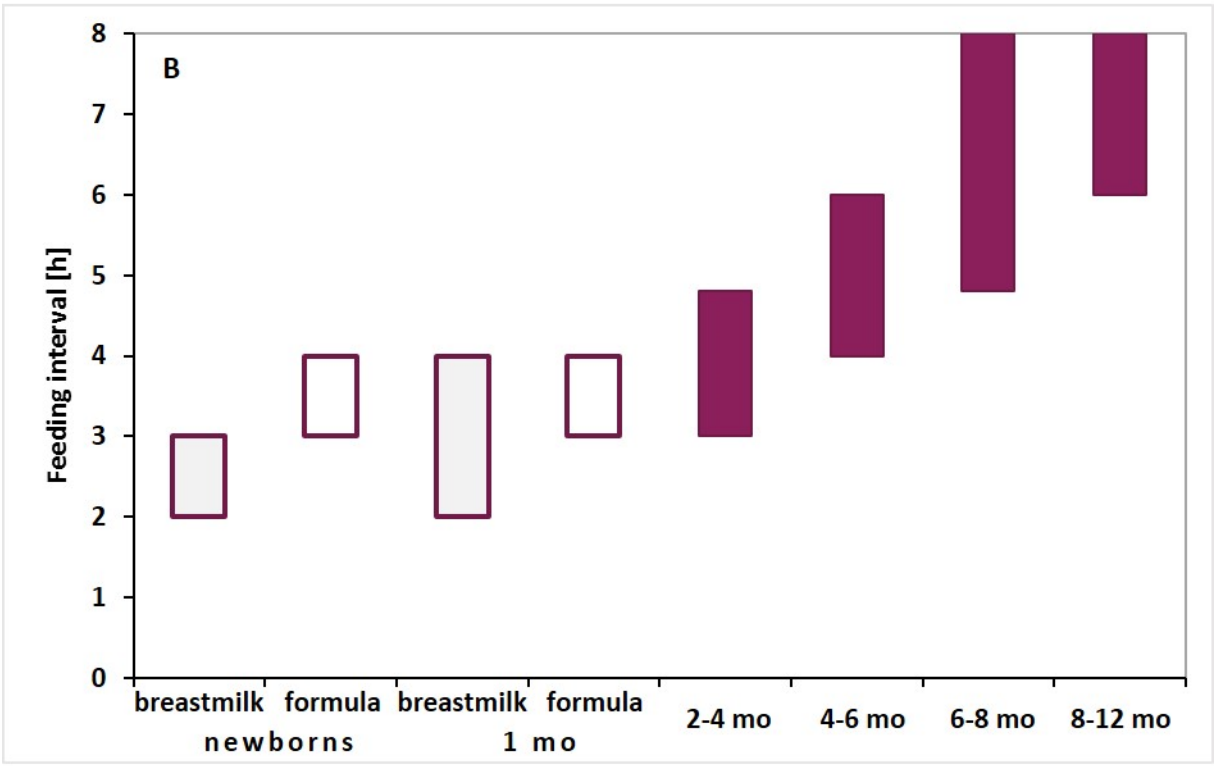
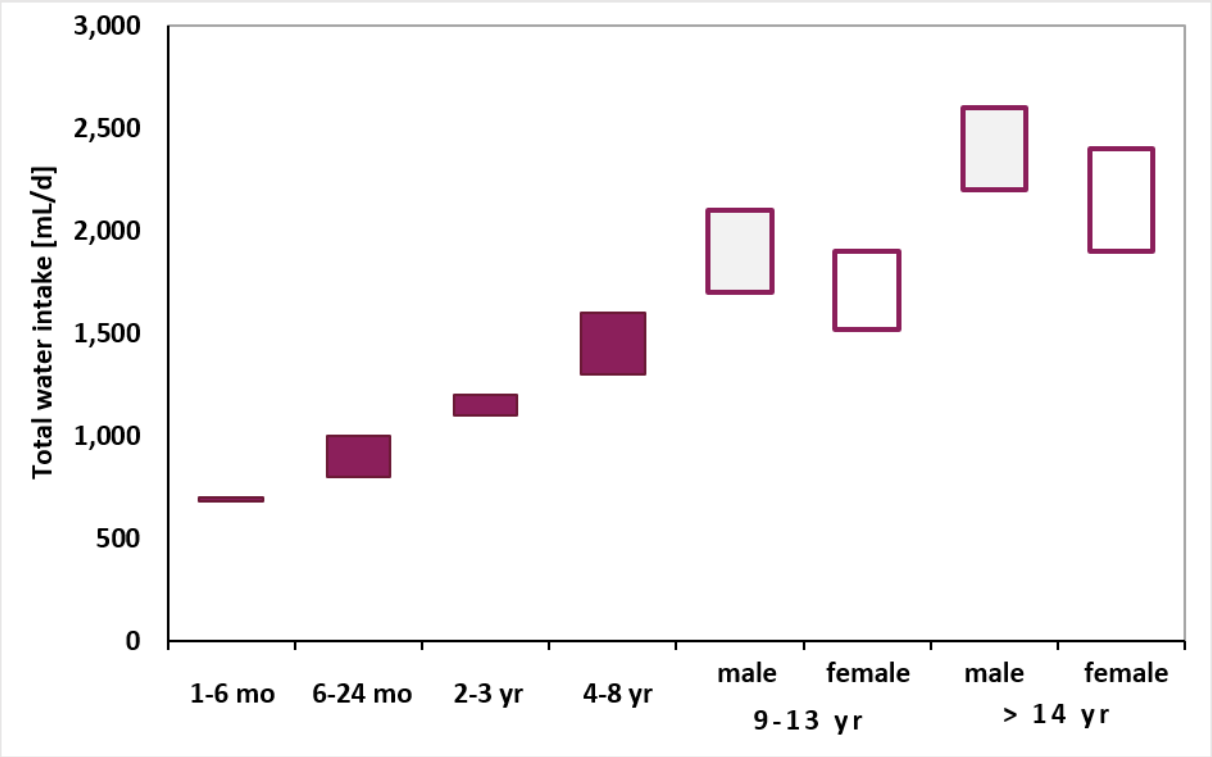
Figure 2


A
Formula milk volume [mL]
250
200
150
100
50
0
newborn
1 mo
2-4 mo
4-6 mo
6-8 mo
8-12 mo


B
Feeding interval [h]
8
7
6
5
4
3
2
1
0
breastmilk
formula
breastmilk
formula
newborns
1 mo
2-4 mo
4-6 mo
6-8 mo
8-12 mo

2187 **Figure 3**



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2189

Figure 4

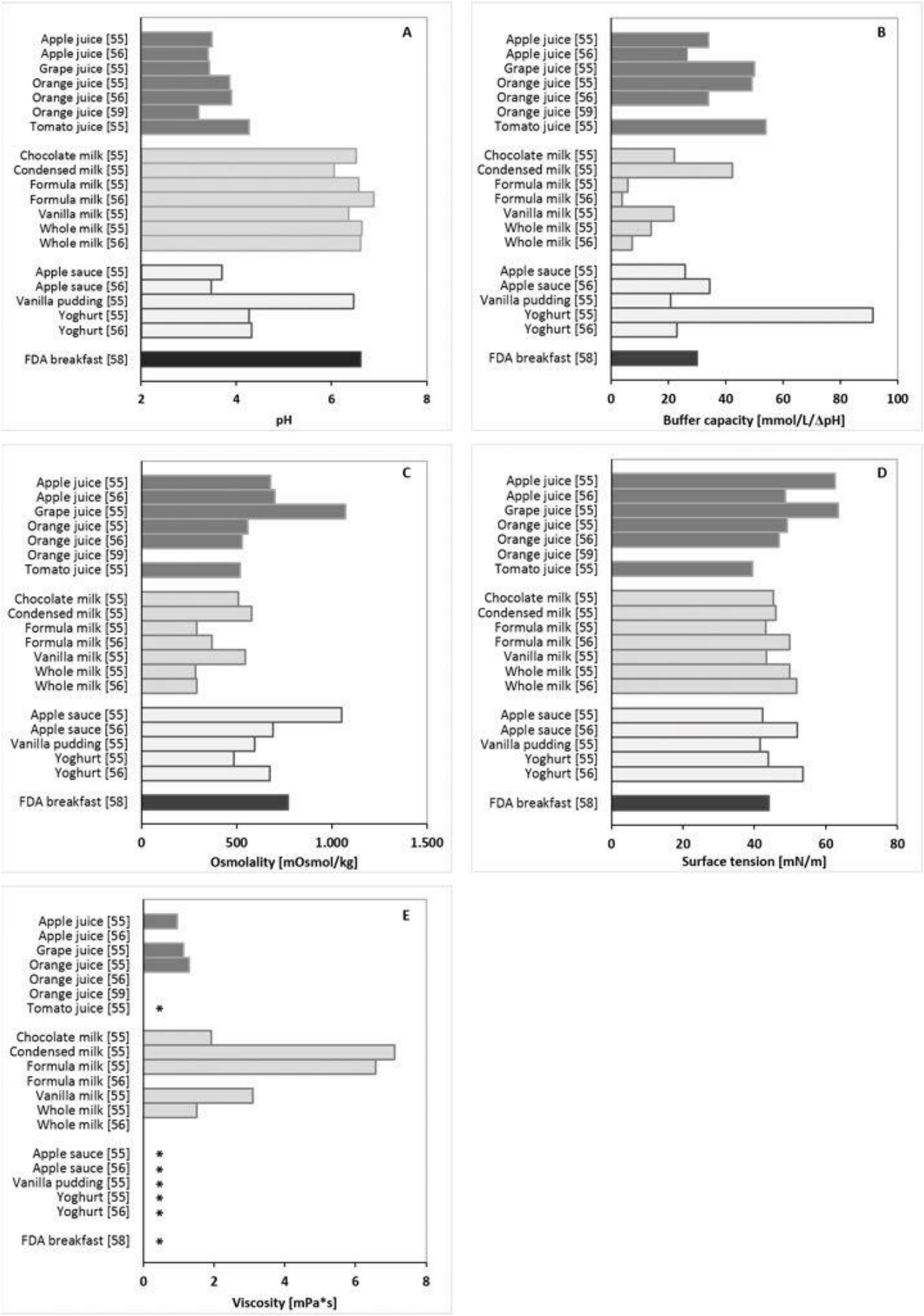
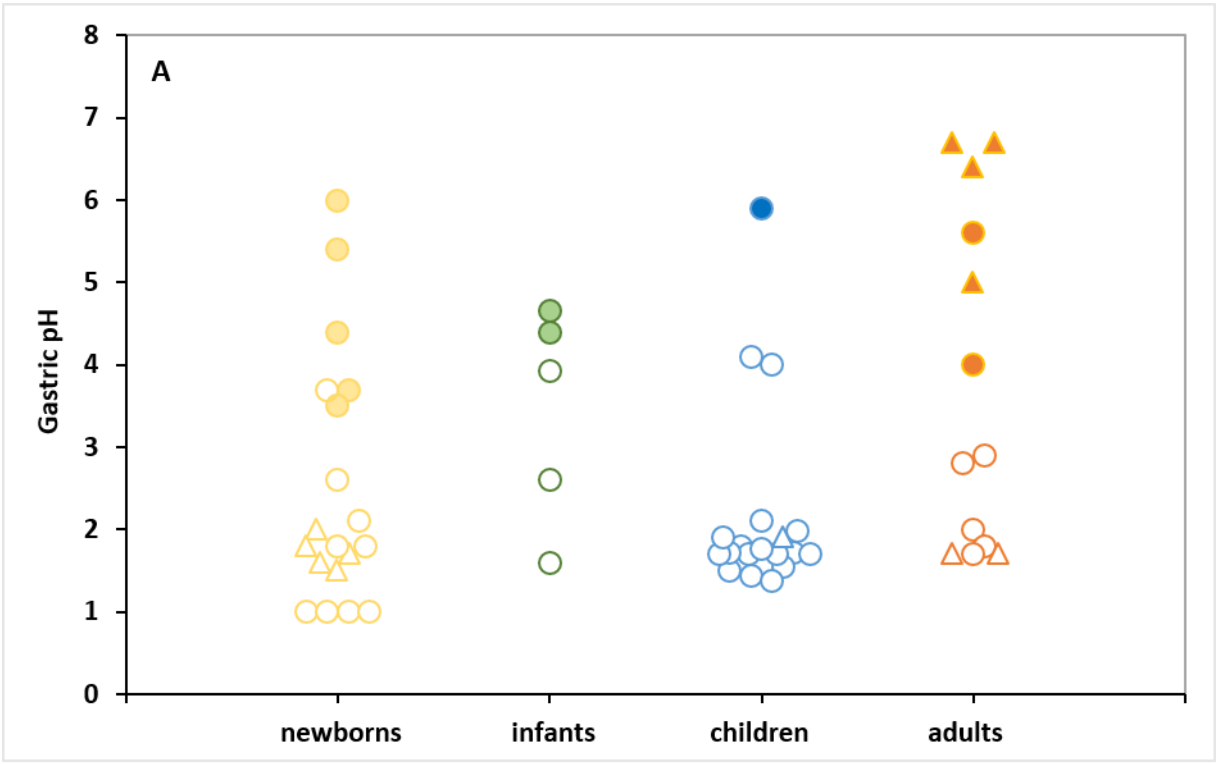

A
Apple juice [55]
Apple juice [56]
Grape juice [55]
Orange juice [55]
Orange juice [56]
Orange juice [59]
Tomato juice [55]
Chocolate milk [55]
Condensed milk [55]
Formula milk [55]
Formula milk [56]
Vanilla milk [55]
Whole milk [55]
Whole milk [56]
Apple sauce [55]
Apple sauce [56]
Vanilla pudding [55]
Yoghurt [55]
Yoghurt [56]
FDA breakfast [58]
2
4
6
8
pH
B
Apple juice [55]
Apple juice [56]
Grape juice [55]
Orange juice [55]
Orange juice [56]
Orange juice [59]
Tomato juice [55]
Chocolate milk [55]
Condensed milk [55]
Formula milk [55]
Formula milk [56]
Vanilla milk [55]
Whole milk [55]
Whole milk [56]
Apple sauce [55]
Apple sauce [56]
Vanilla pudding [55]
Yoghurt [55]
Yoghurt [56]
FDA breakfast [58]
0
20
40
60
80
100
Buffer capacity [mmol/L/ΔpH]
C
Apple juice [55]
Apple juice [56]
Grape juice [55]
Orange juice [55]
Orange juice [56]
Orange juice [59]
Tomato juice [55]
Chocolate milk [55]
Condensed milk [55]
Formula milk [55]
Formula milk [56]
Vanilla milk [55]
Whole milk [55]
Whole milk [56]
Apple sauce [55]
Apple sauce [56]
Vanilla pudding [55]
Yoghurt [55]
Yoghurt [56]
FDA breakfast [58]
0
500
1.000
1.500
Osmolality [mOsmol/kg]
D
Apple juice [55]
Apple juice [56]
Grape juice [55]
Orange juice [55]
Orange juice [56]
Orange juice [59]
Tomato juice [55]
Chocolate milk [55]
Condensed milk [55]
Formula milk [55]
Formula milk [56]
Vanilla milk [55]
Whole milk [55]
Whole milk [56]
Apple sauce [55]
Apple sauce [56]
Vanilla pudding [55]
Yoghurt [55]
Yoghurt [56]
FDA breakfast [58]
0
20
40
60
80
Surface tension [mN/m]
E
Apple juice [55]
Apple juice [56]
Grape juice [55]
Orange juice [55]
Orange juice [56]
Orange juice [59]
Tomato juice [55]
Chocolate milk [55]
Condensed milk [55]
Formula milk [55]
Formula milk [56]
Vanilla milk [55]
Whole milk [55]
Whole milk [56]
Apple sauce [55]
Apple sauce [56]
Vanilla pudding [55]
Yoghurt [55]
Yoghurt [56]
FDA breakfast [58]
0
2
4
6
8
Viscosity [mPa*s]

Figure 5


A
Gastric pH
8
7
6
5
4
3
2
1
0
newborns
infants
children
adults

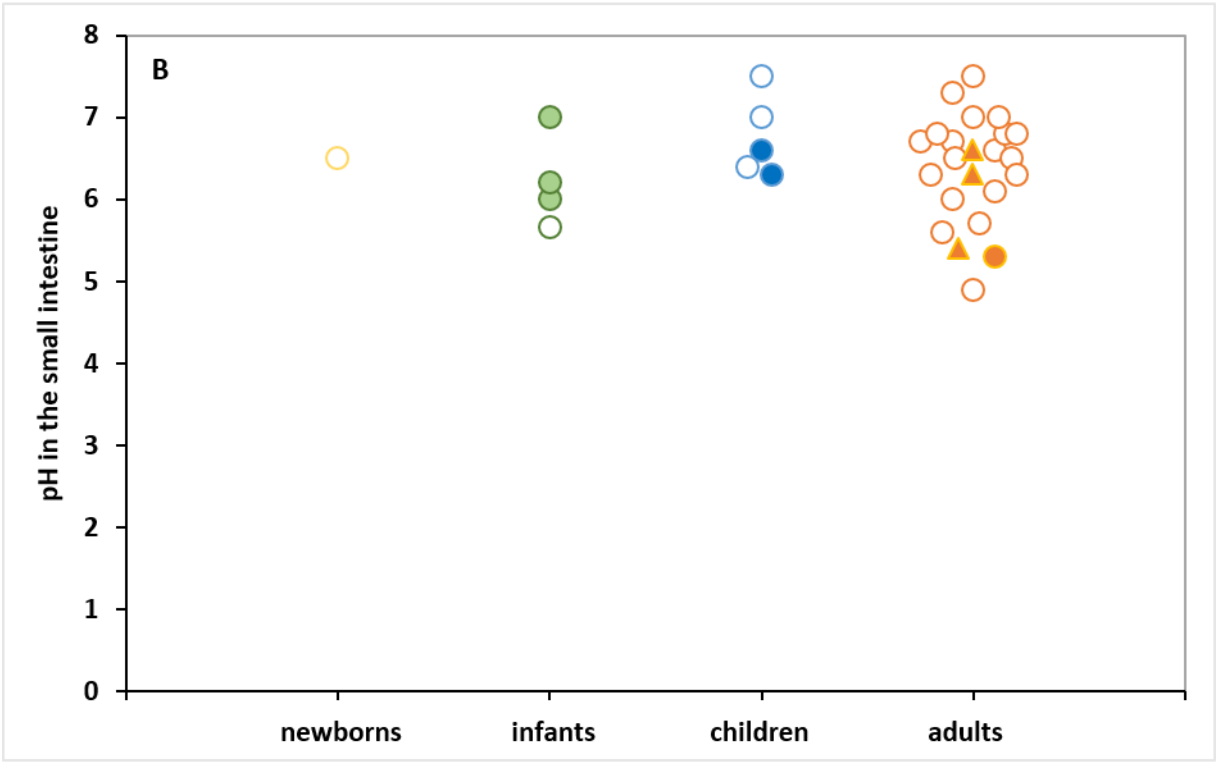
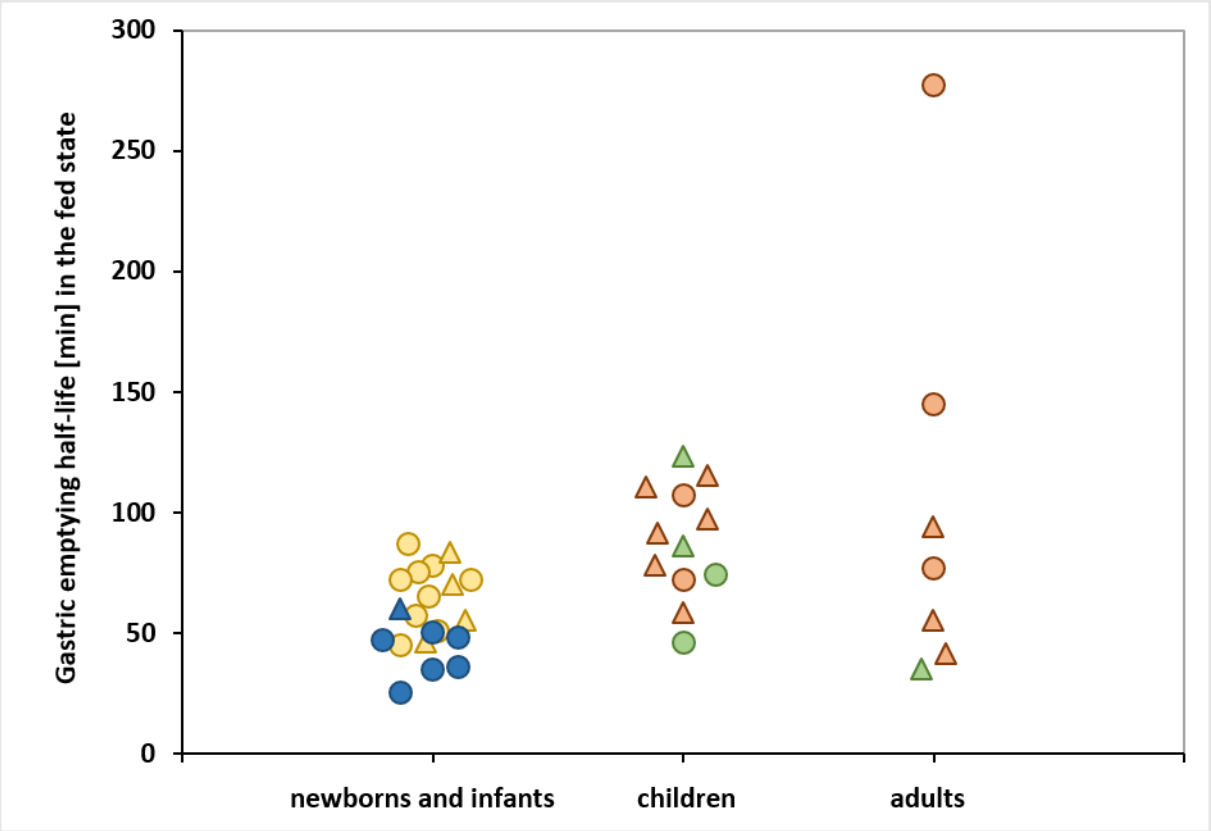
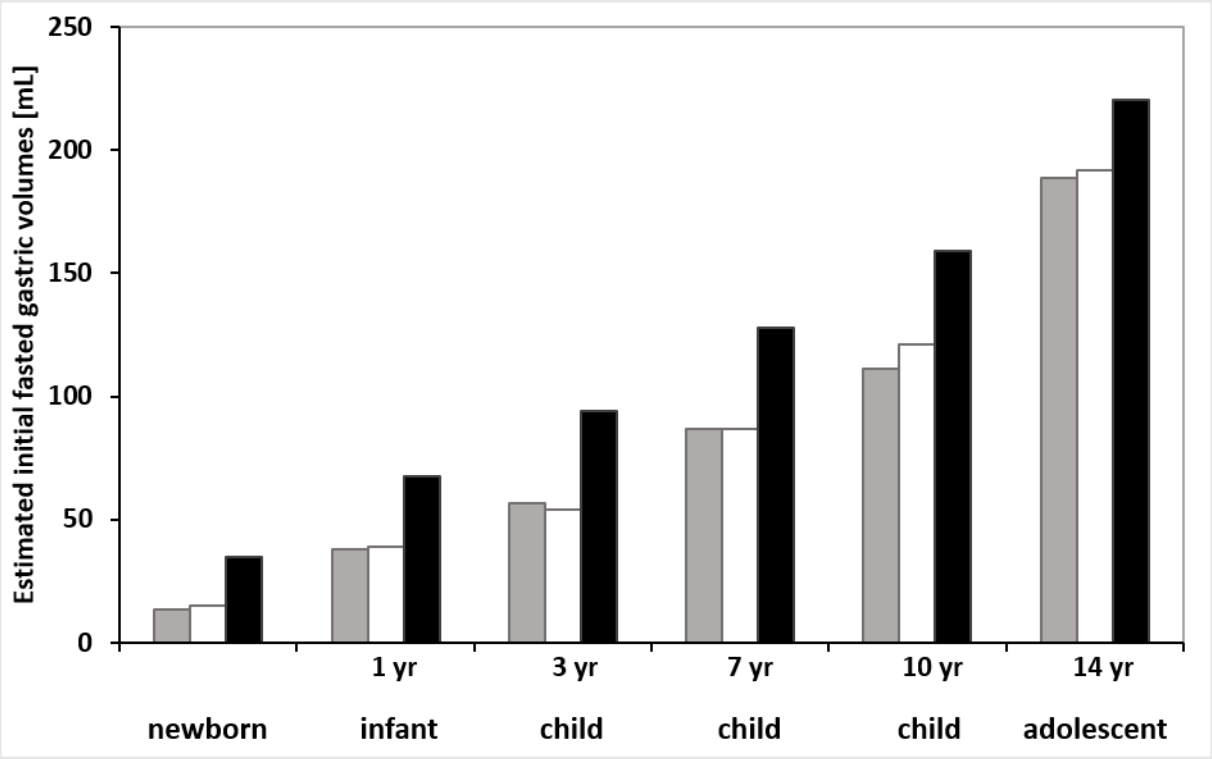

B
pH in the small intestine
8
7
6
5
4
3
2
1
0
newborns
infants
children
adults

Figure 6


Gastric emptying half-life [min] in the fed state
300
250
200
150
100
50
0
newborns and infants
children
adults

2197 **Figure 7**



2198

Figure 8

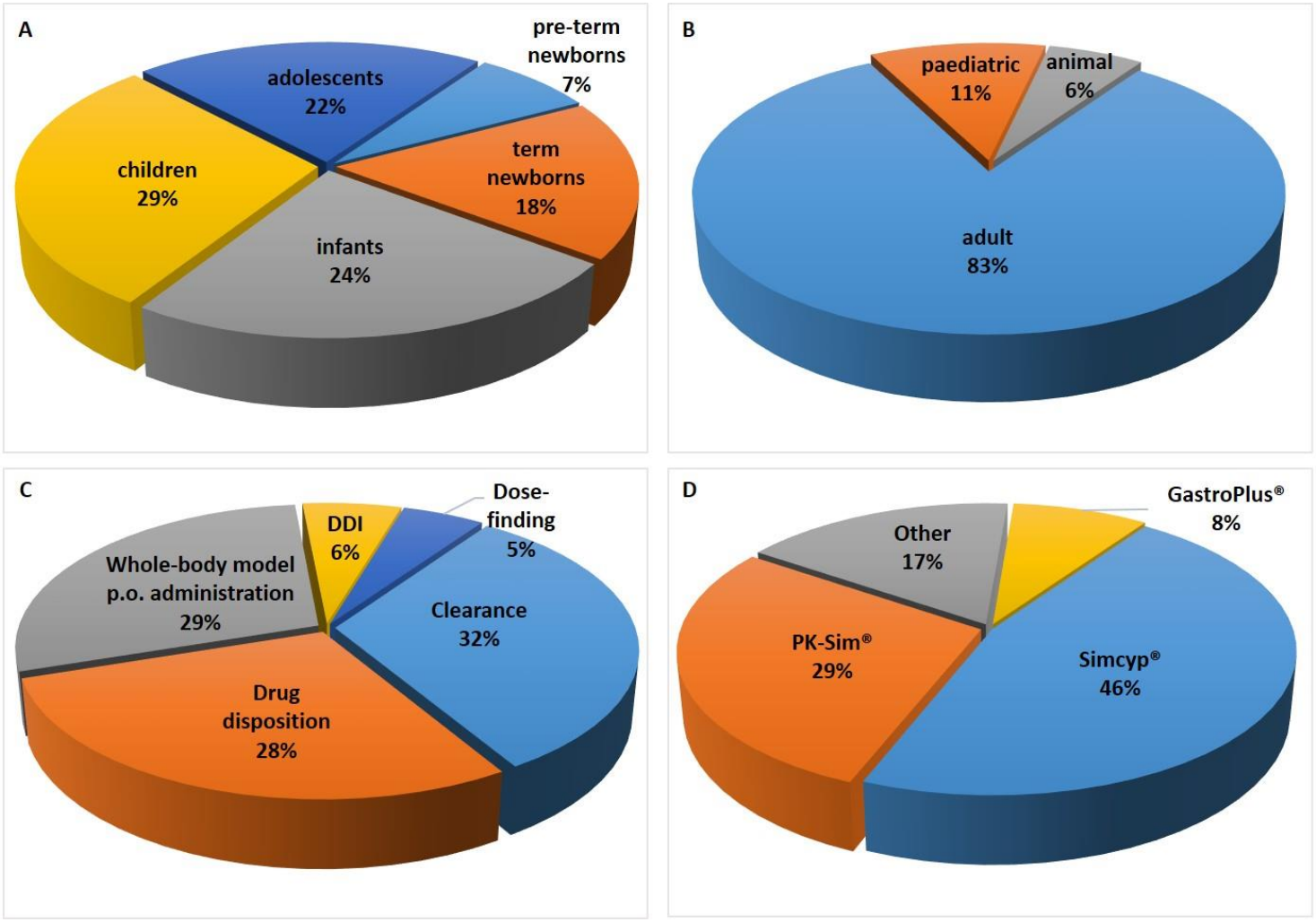
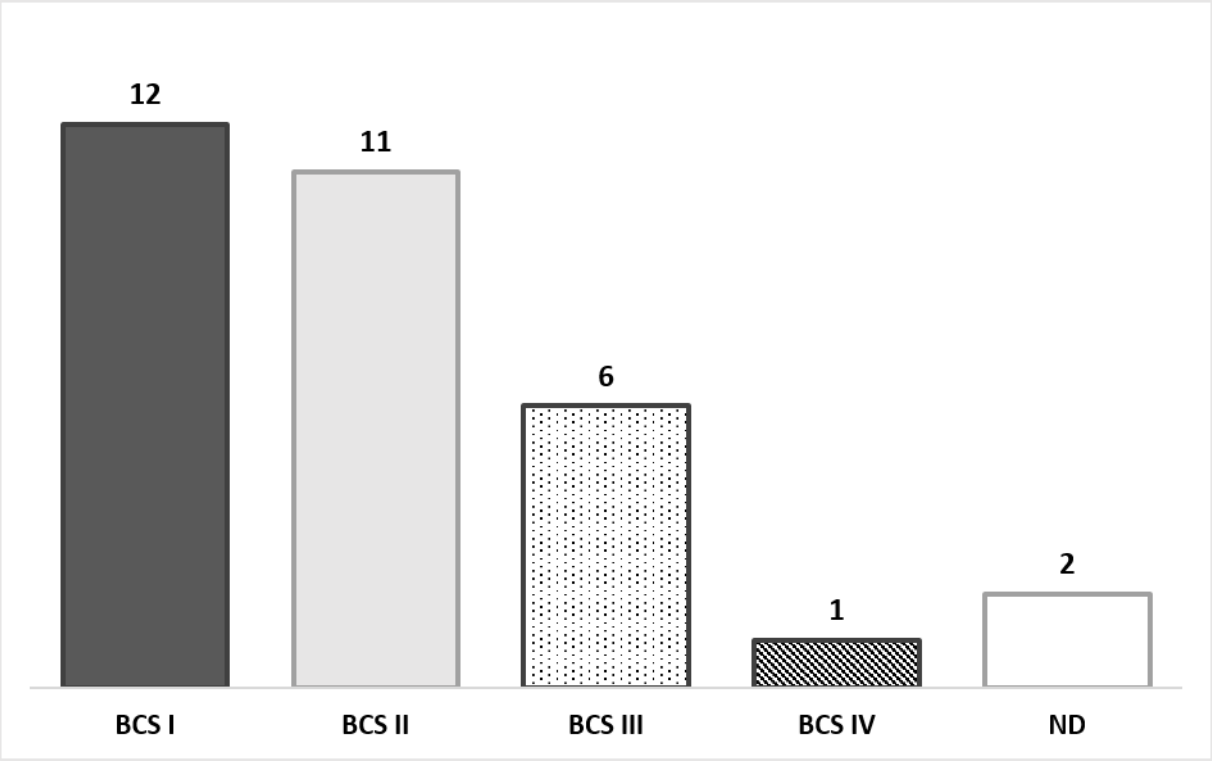
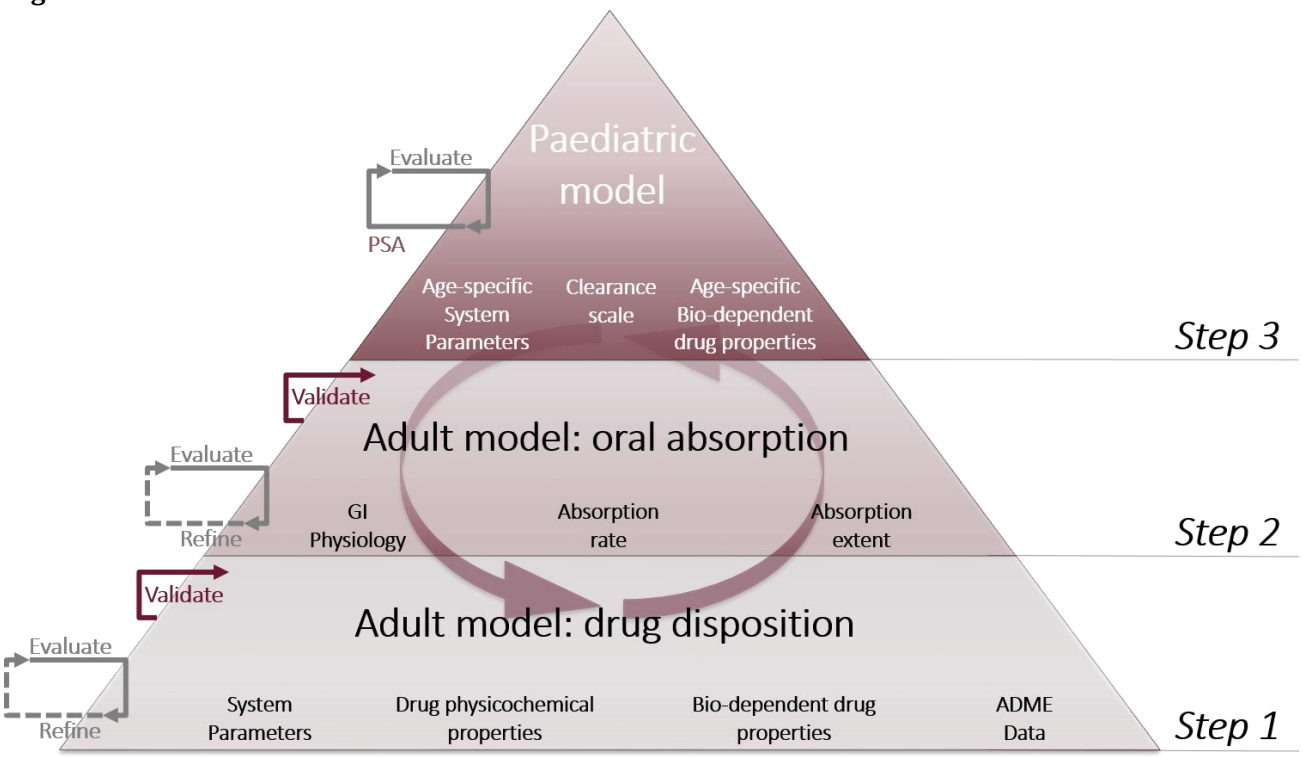

A
pre-term newborns 7%
adolescents 22%
children 29%
term newborns 18%
infants 24%
B
paediatric 11%
animal 6%
adult 83%
C
Dose-finding 5%
DDI 6%
Whole-body model p.o. administration 29%
Clearance 32%
Drug disposition 28%
D
GastroPlus® 8%
Other 17%
PK-Sim® 29%
Simcyp® 46%

Figure 9



2204 **Figure 10**



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2206

Graphical Abstract