

Citation for published version:

Guimarães, M, Stelova, M, Holm, R, Reppas, C, Symillides, M, Vertzoni, M & Fotaki, N 2019, 'Biopharmaceutical considerations in paediatrics with a view to the evaluation of orally administered drug products – a PEARRL review.', *Journal of Pharmacy and Pharmacology*, vol. 71, no. 4, pp. 603-642. <https://doi.org/10.1111/jphp.12955>

DOI:

[10.1111/jphp.12955](https://doi.org/10.1111/jphp.12955)

Publication date:

2019

Document Version

Peer reviewed version

[Link to publication](#)

This is the peer-reviewed version of the following article: Guimarães, M., Stelova, M., Holm, R., Reppas, C., Symillides, M., Vertzoni, M. and Fotaki, N. (2019), Biopharmaceutical considerations in paediatrics with a view to the evaluation of orally administered drug products – a PEARRL review. *J Pharm Pharmacol*, 71: 603-642., which has been published in final format at: <https://doi.org/10.1111/jphp.12955>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

University of Bath

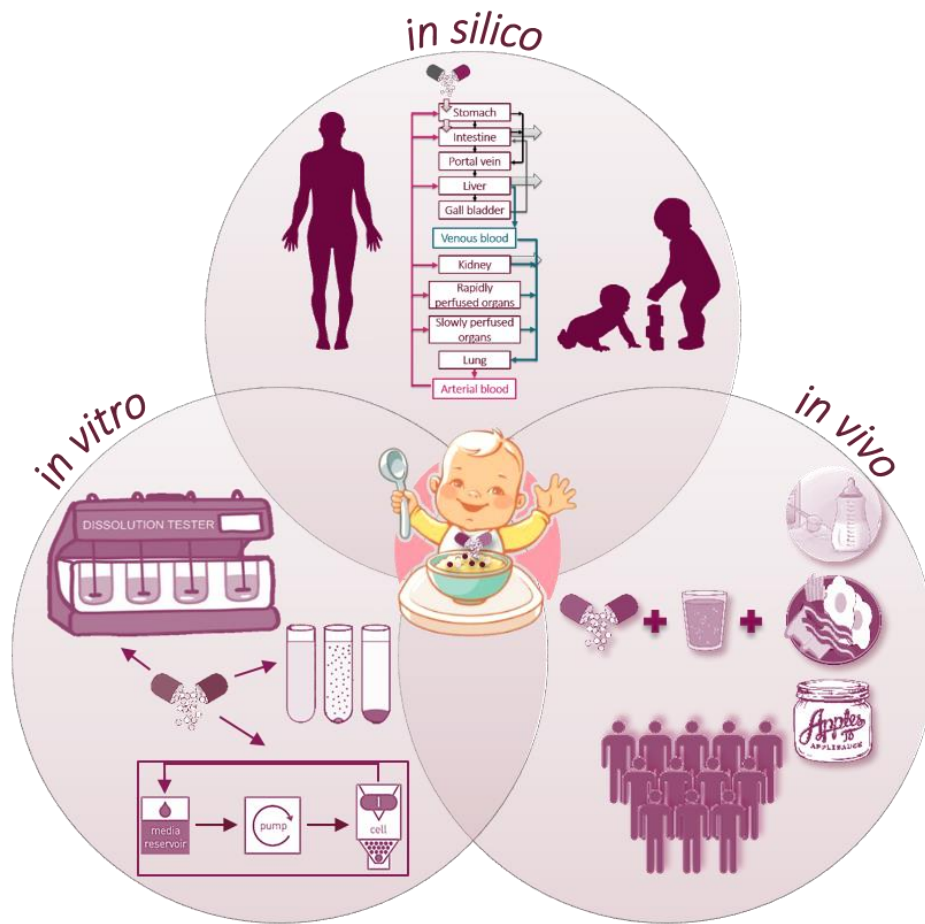
General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 Graphical Abstract



2

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

5 Mariana Guimarães^{1,¥}, Marina Statelova^{2,¥}, René Holm³, Christos Reppas², Moira Symillides²,
6 Maria Vertzoni^{2,*}, Nikoletta Fotaki^{1,*}

7

8 ¹ Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

9 ² Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

10 ³ Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg
11 30, 2340 Beerse, Belgium

12 [¥] equal contribution

13

14 * Correspondence to:

15 Dr Nikoletta Fotaki

16 Department of Pharmacy and Pharmacology, University of Bath, Claverton Down

17 Bath, BA2 7AY, United Kingdom

18 Tel. +44 1225 386728, Fax: +44 1225 386114, E-mail: n.fotaki@bath.ac.uk

19

20 Dr Maria Vertzoni

21 Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens,

22 Panepistimiopolis, 157 84 Zografou, Greece

23 Tel. +30 210 727 4035, Fax: +30 210 727 4027, E-mail: vertzoni@pharm.uoa.gr

24

25 Key words: oral absorption, paediatric, biopharmaceutics, physiology, food-effect, PBPK modeling

26 Abstract

27

28 **Objective**

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

31 **Key findings**

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

43 **Summary**

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

47

48 **Table of Contents**

49	1. Introduction	1
50	2. Paediatric nutrition	3
51	2.1. Age-dependent feeding: recommendations and practice.....	3
52	2.2. Paediatric energy needs and feeding frequency	4
53	2.3. Water and fluid intake.....	5
54	2.4. Food composition.....	7
55	2.5. Physicochemical properties of meals and beverages	8
56	3. Physiological and anatomical changes in paediatrics	9
57	3.1. Gastrointestinal volumes.....	9
58	3.2. Gastrointestinal fluid composition	11
59	3.3. Gastric emptying	16
60	3.4. Small intestinal transit times	17
61	3.5. Intestinal surface area.....	18
62	3.6. Intestinal permeability.....	19
63	3.7. Metabolism.....	21
64	4. Paediatric Biopharmaceutics Classification Systems (pBCS).....	23
65	4.1. pBCS solubility classification criteria.....	23
66	4.2. pBCS permeability classification criteria.....	26
67	4.3. Challenges for the pBCS criteria determination	27
68	5. Food effects on oral drug absorption in paediatrics.....	28
69	5.1. Regulations and current practice: administration after a meal	29
70	5.2. Regulations and current practice: co-administration of formulation with food/ drinks	31
71	5.3. Food effects and paediatric dosage forms	32
72	6. <i>In vitro</i> evaluation of drug products for paediatrics	34
73	6.1. Paediatric biorelevant media	35
74	6.2. Evaluation of drug products characteristics	36
75	7. <i>In silico</i> evaluation of drug products for paediatrics	41
76	7.1. Allometric scaling	42
77	7.2. PBPK modeling.....	42
78	7.2.1. Paediatric PBPK models: current status.....	43
79	7.2.2. Building a PBPK model	45
80	7.2.3. Examples of paediatric PBPK models: focus on oral drug absorption	49
81	7.2.4. Challenges in the paediatric oral drug absorption model	56
82	8. Conclusions	58

83

84

85	Abbreviation list
86	ADME Absorption Distribution Metabolism and Excretion
87	AUC Area under the curve
88	BCS Biopharmaceutics Classification System
89	BW Body weight
90	BSA Body surface area
91	C_{max} Maximum plasma concentration
92	CYP Cytochrome P450
93	d Days
94	EMA European Medicines Agency
95	EFSA European Food Safety Agency
96	f_a Fraction absorbed
97	FDA Food and Drug Administration
98	GE Gastric emptying
99	GI Gastrointestinal
100	GST Glutathione S-transferase
101	ICH International Conference on Harmonisation
102	K_e Rate constant of elimination
103	MMC Migrating motility complex
104	NAT N-acetyltransferases
105	mo Months
106	pBCS Paediatric Biopharmaceutics Classification System
107	PEARL Pharmaceutical Education And Research with Regulatory Links
108	PBPK Physiologically based pharmacokinetics
109	t_{1/2} Half-life time

- 110 **TIM** TNO Gastro-Intestinal Model
- 111 **t_{max}** Time at which C_{max} is reached
- 112 **PSA** Parameter sensitivity analysis
- 113 **SI** Small intestine
- 114 **SITT** Small intestinal transit times
- 115 **SULT** Sulfotransferase
- 116 **UGT** Uridine 5'-diphosphate-glucuronosyltransferase
- 117 **yr** Years
- 118 **wk** Weeks
- 119 **WHO** World Health Organization

120 **1. Introduction**

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

130

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

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

151 BW and BSA differences between paediatric age groups and adults are presented in **Table 1**. Paediatric
152 BW was retrieved from the 50th percentile boys and girls values in the Centre for Disease Control and
153 Prevention (CDC) growth charts for paediatrics; adult 50th percentile BW values were obtained from
154 clinical charts that include multiple races and a wide range of ages in U.S [8]. BSA values were
155 calculated using the Mosteller formula ($BSA = \left(\frac{Weight \times Height}{3600}\right)^{\frac{1}{2}}$) [9]. Body height used for the
156 calculations was retrieved from the same source as the respective BW. Newborns and infants are the
157 age groups that show the highest differences compared to the adult population in terms of BW and
158 BSA. The younger subpopulations show large differences in terms of physiological and anatomical
159 factors. The absorption process in the younger subpopulations is highly influenced by the type of food
160 ingested and the co-administration of medicine with food. The definition of a fasted state in newborns
161 and infants is a difficult task and should be addressed with care in the design of *in vitro* experiments.
162 In this review, the parameters concerning paediatric oral drug absorption are explored. The current
163 knowledge and considerations for the biopharmaceutical evaluation of orally administered drug
164 products for paediatrics and the *in vitro* and *in silico* tools to help guide the development of appropriate
165 paediatric medicines are discussed.

166

167 *Please place Table 1 here*

168

169

170 **2. Paediatric nutrition**

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

179

180 **2.1. Age-dependent feeding: recommendations and practice**

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

195 **2.2. Paediatric energy needs and feeding frequency**

196 Average energy requirements for healthy individuals are derived from total energy expenditure, which
197 is defined as the product of energy spent on activities and the resting energy expenditure. Equations
198 obtained from regression analysis of measured resting energy expenditure from various subject groups
199 are utilised for its prediction [25; 26]. Growth processes require additional energy for synthesis and
200 deposition of new tissues. This parameter has been shown to have the highest relative contribution to
201 total energy requirements in the first month of life (40%) and decreases to 3% during the
202 12th month [25]. The European guidelines utilise the equations for resting energy expenditure for
203 paediatrics proposed by Henry *et al.* [27]. Ultimately, different levels of physical activity are assigned
204 to the paediatric groups: light, moderate, or heavy activity. The recommended daily caloric intake for
205 European and American paediatric populations is shown in **Figure 1** [18; 26; 28; 29]. The non-linearity
206 of the energy requirements as a function of age can be explained by the BW-based nature of the
207 calculations behind them. The caloric needs of paediatric subpopulations increase with age towards
208 adult values, and factors such as gender and physical activity, become more and more relevant over
209 time [26]. According to the European Food and Safety Authority (EFSA) newborns, infants, and
210 children up to four years of age are more likely to have a sedentary level of activity (**Figure 1A**),
211 whereas older children and adolescents tend to show higher activity level (**Figure 1B**) [18]. The
212 aforementioned energy requirements are estimated for average healthy individuals [26]; various health
213 conditions, *e.g.* severe infections, fever, diarrhoea etc., would demand special treatment also with
214 regard to nutritional amount and composition [30].

215

216 *Please place Figure 1 here*

217

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

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

232 *Please place Figure 2 here*

233

234 **2.3. Water and fluid intake**

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

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

253

254 *Please place Figure 3 here*

255

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

267

268

269 2.4. Food composition

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

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

295 *Please place Table 2 here*

296

297 **2.5. Physicochemical properties of meals and beverages**

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

319 FDA and EMA with a high-calorie, high-fat standard adult breakfast as a meal for the fed state
320 investigation [52; 53]. The physicochemical properties of the FDA/EMA standard breakfast (**Table 2**)
321 [58] deviate from the physicochemical properties of the tested vehicles for paediatric use in terms of
322 pH values, viscosity, and osmolality (**Figure 4**). Although some trends can be observed from the
323 available data for the reported soft foods and drinks, e.g. fruit juices, dairy products, formula milk and
324 milk types, further investigation of the product variability between different brands with focus on their
325 physicochemical characterisation might be of interest.

326

327 *Please place Figure 4 here*

328

329 **3. Physiological and anatomical changes in paediatrics**

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

340

341 **3.1. Gastrointestinal volumes**

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

344 fluids (e.g. water, tea, clear apple juice) had been administered for at least 2 h or more, and constraint
345 of solid food/semi-solid food/other fluids lasted for a minimum of 4 h prior to the gastric volume
346 measurement. Nevertheless, studies have shown that small volumes (less than 2 mL/kg) of clear fluids
347 (such as water, tea and others) are not expected to affect measurements of gastric volume within a 2 h
348 period [65]. Literature studies have evaluated the fasted gastric volume across the paediatric
349 subpopulations, and no clear age distinction among the studied subpopulations (newborns, infants and
350 children) is reported. Maekawa *et al.* also reported that ingestion of higher volumes (10 mL/kg of BW)
351 of fluids (apple juice) ingested up to 2 h before measurements are not expected to affect gastric volume
352 [66].

353

354 *Please place Table 3 here*

355

356 In the paediatric population, it is more likely that the medication is dosed with food. Considering that
357 the youngest subpopulations are mainly in the postprandial state, due to the higher frequency of food
358 intake, food will most likely already be available in the stomach [48]. Following the ingestion of food,
359 the stomach content can increase significantly (up to 50 fold), and stomach capacity volumes can range
360 from 10 to 100 mL in newborns, 90 to 500 mL in infants, 750 to 960 mL in children, and 1500 to
361 2000 mL in adolescents and 3000 mL in adults [78]. For the youngest sub-populations, the gastric
362 volume in the fed state will be mainly represented by the volume of the food ingested [35]. Gastric
363 volume in children measured 3 h after administration of drinks (orange squash, maximum 200 mL)
364 and of drinks and biscuits (orange squash, maximum 200 mL and two plain biscuits) was 0.39 mL/kg
365 and 0.46 mL/kg, respectively (compared to 0.25 mL/kg measured after 7 h fasting) [70].

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

370 volumes were calculated by the difference between initial meal volume and gastric emptying (GE).
371 Gastric secretions were a significant contributing factor of gastric contents in the fed state: 32%, 28%,
372 and 43% v/v at 30, 60, and 90 min following feeding, respectively. A separate study showed that
373 volumes of gastric secretions corresponded to 2.0 ± 1.4 mL/kg BW in newborns (n = 8, 4 - 24 wk) in
374 the first postprandial hour [80]. Smaller contributions of gastric secretions to total gastric volume
375 (1 mL/kg in 30 min following meal intake) have also been reported in premature newborns (n = 10,
376 1 - 9 wk postnatal age) [81].

377 The gastric volume after administration of three types of food (*i.e.* human milk 18.4 ± 0.5 mL/kg;
378 SMA-SP[®] formula 17.4 ± 0.5 mL/kg; and Similac SC[®] formula 17.0 ± 0.7 mL/kg) to newborns and
379 infants (1 - 11 wk) was measured at 10, 30, and 50 min after food intake [82]. Ten minutes after feeding
380 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
381 present in the stomach [82]. Based on these studies, a mean feeding volume of newborns and young
382 infants of 23.5 ± 4.2 mL/kg has been suggested [48]. No information was found on intestinal volumes
383 across paediatric subpopulations.

384

385 **3.2. Gastrointestinal fluid composition**

386 In paediatrics, fasted gastric pH is widely described as being neutral moments after birth, ranging from
387 values of 6 to 8, mainly due to amniotic fluid ingestion [83; 84]. Contradictory information has been
388 reported with regards to the time after birth which is needed to reach acidic pH values. Nevertheless,
389 reviews of original reports show that fasted gastric acidic pH values of 1.5 to 3 are reached hours after
390 birth, up to the first two weeks of life [48; 63; 85; 86]. A summary of the pH values of GI contents of
391 paediatric population and of adults is presented in **Figure 5**.

392

393 *Please place Figure 5 here*

394

395 Newborns and young infants are mainly fed with milk, whether it is breast milk or different types of
396 formulae, which can have an impact on several characteristics, including fed gastric pH. Studies have
397 reported that pH values over 4 were detected more frequently in newborns and infants than in older
398 children [79; 106; 107], mainly due to feeding patterns in this subpopulation and the high buffer
399 capacity of breast milk and formulae [106; 108]. Comparison of two separate studies (adults vs.
400 newborns) of continuously monitoring of the fed gastric pH showed that 2 h after a meal, higher fed
401 gastric pH values (0.7 - 1.8 units) were found in newborns (2 - 15 d) [109]. The meal ingested by adults
402 consisted of a standard solid meal (1000 Kcal), opposed to newborns where formula milk was ingested
403 (14.5 - 29.0 mL/kg per feeding) [98; 99]. It should be noted that the interpretation of pH in the fed state
404 is difficult, as differences might simply arise as a function of meal composition, or the time interval
405 after intake of the meal and the measurement.

406

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

419

420 The concentration and composition of bile salts vary with age. Total duodenal bile salts concentrations
421 [48; 109] are usually reported as a small pool of bile salts in newborns and infants when compared to
422 adults, and lack in secondary bile salts [48; 111]. In the younger populations (newborns and young
423 infants), tauro-conjugation of bile acids is predominantly detected, with glycol-conjugation and
424 glycine conjugates reaching adult levels by 7 to 12 months of age [112]

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

445 (containing corn oil, glucose, polyethylene glycol-4000 and water). Fed total bile salt concentration
446 was measured in duodenal aspirates and values were ~1 mM (evaporated milk) and ~0.5 mM (modified
447 milk), and ~5.9 mM in the older group [116]. A linear trend was recently established between the
448 logarithm of age and bile salt concentration data collected from available studies of fed state duodenal
449 bile salts concentration of newborns and infants ($R^2 = 0.54$, 7 paediatric studies and 5 adult studies)
450 [109]. Based on this, mean fed intestinal bile acid concentration was found to be approximately
451 2.5 mM for newborns and 7.5 mM for infants.

452

453 The role and importance of digestive enzymes in newborns and infants has been described in a recent
454 review [48]. A summary highlighting the differences of relevant digestive enzymes between adults and
455 paediatrics will be discussed in this review. The following enzymes have been proven to be essential
456 for the digestion and lipolysis in newborns and infants: human gastric lipase, pancreatic triglyceride
457 lipase (and colipase), carboxylester hydrolase, pancreatic lipase-related protein 2, and bile salt-
458 stimulated lipase [48]. Human gastric lipase is a pre-duodenal lipase which is responsible for
459 intragastric lipolysis in newborns, its expression is fully matured at birth and its activity in the stomach
460 is similar to adults [48]. Pancreatic triglyceride lipase plays a major role in the lipid lipolysis process
461 in adults. Its activity in the fed state has been shown to be lower in newborns, possibly due to dilution
462 of enzyme levels in response to high frequent feedings in the younger subpopulations, contrary to what
463 happens in adults, where enzyme secretion is stimulated by the presence of macronutrients [48]. The
464 expression of carboxylester hydrolase and pancreatic lipase-related protein 2 is not fully developed at
465 birth [48].

466

467 Pepsin is a protease secreted by the stomach and its expression is not fully matured at birth [48]. Lower
468 pepsin secretions have been reported in younger cohorts, such as newborns and infants less than one
469 year of age, compared to older children and adults [92]. Fasted gastric pepsin concentrations in younger

470 newborns (birth and 8 d of postnatal age) appear to be approximately 15% of adult values, while older
471 newborns (10 - 32 d) and infants (67 - 110 d) express similar mean concentrations of approximately
472 41% of the adult values [109]. Similarly to pepsin, trypsin expression is not matured at birth, and lower
473 concentrations have been reported in newborns and infants when compared to children and adults [48].
474 In summary, pancreatic enzyme concentrations are lower at birth and appear to reach mature levels by
475 one year of age [63].

476

477 Limited information is available on osmolality and buffer capacity of paediatric GI fluids. A positive
478 linear correlation has been reported between the osmolality of the diet as a function of the osmolality
479 observed in the stomach and duodenum in 15 low-birth-weight newborns monitored for three hours
480 after food ingestion [117]. Maharaj *et al.* built a linear regression model for a 60 min postprandial
481 period ($R^2 = 0.95$, $n = 8$ separate feeds) to predict neonatal fed gastric osmolality based on results
482 obtained from Billeud *et al.* [109; 117]. The predictions were compared with a separate study in which
483 osmolality was measured after three separate breast milk feeds fortified with minerals/supplements
484 [118]. As an example, after a feed with an osmolality of 344 mOsmol/kg, the corresponding measured
485 fed gastric osmolality at 60 min was of 354 mOsmol/kg, and the predicted osmolality was 327
486 mOsmol/kg, with 7.6% under-prediction error. The developed model predicted fed gastric osmolality
487 within one hour after feeding, whereby the time period was selected to reflect the high frequency of
488 feeding in paediatric populations. The same approach was used to predict fed state duodenal osmolality
489 ($R^2 = 0.92$, $n = 8$ separate feeds). Due to scarcity of data in paediatrics, predictions were validated
490 against two adult studies reported by Kalantzi *et al.* and Clarysse *et al.* Measured duodenal osmolality
491 values were 405 and 392 mOsmol/kg, 60 min following administration of liquid meals characterised
492 by an osmolality of 610 and 670 mOsmol/kg, and predicted osmolality were adequate with values of
493 430 (6% over-prediction) and 454 (16% over-prediction) mOsmol/kg respectively [97; 119]. In
494 newborns and young infants, buffer capacity of the fed gastric fluids is likely to be similar to the buffer

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

498

499 **3.3. Gastric emptying**

500 Newborns and young infants have slower GE rates when compared to older children and adults [64;
501 84; 120]. In the fasted state, migrating motility complex (MMC) is responsible for the regulation of
502 the GE rate [121]. Non-nutrient liquids do not normally interfere with the MMC [122]. The gastric
503 emptying half-life ($GE_{t_{1/2}}$), is reported to be 6.9 min for a liquid non-caloric meal (5 mL/Kg) in
504 newborns (1 – 8 d), measured by epigastric impedance using four electrodes [123]. The use of other
505 techniques for the measurement of GE of liquids have shown higher values, Euler and Byrne measured
506 emptying rate of distilled water by the dilution marker technique and reported the mean $GE_{t_{1/2}}$ to be
507 15 minutes after administration of 20 mL/kg of water to infants (2 - 24 mo) [124]. Administration of
508 20 mL/kg of tap water to children (mean age 8.25 ± 2.24 yr) led to a mean $GE_{t_{1/2}}$ of 27.1 min when
509 measured by the ultrasound technique [124].

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

520 milk compared to infant formula in newborns and infants (4 wk - 6 mo) [127], where $GE_{t_{1/2}}$ was $48 \pm$
521 15 min, and 78 ± 14 min, respectively, indicating that infant formula empties at slower rates than breast
522 milk. The faster emptying of breast-milk was also reported by Ewer *et al.* who compared $GE_{t_{1/2}}$ of
523 breast-milk (36 min) and formula milk (72 min) in pre-term newborns (n = 14, postnatal age 4 - 26 d)
524 [128]. Staelens *et al.* compared GE in infants (n = 17, 2 d - 3 mo) fed with intact protein formula (Nan
525 1, Nestle®), a partially hydrolysed formula (Nan H.A.1, Nestle®), and an extensively hydrolysed
526 formula (experimental formula); $GE_{t_{1/2}}$ was 55, 53 and 46 min, respectively [49], confirming that faster
527 fed GE was observed following ingestion of protein hydrolysate formula, when compared with a
528 formula containing native cow's milk protein, and also that the extent of dairy protein hydrolysis may
529 affect GE. Casein-predominant feeds (typical for cow's milk products) have also been showed to
530 empty slower than feeds with a greater whey fraction, but the authors highlighted that different
531 methodology, food compositions and patient groups, limit the validity of the conclusions [129]. A
532 summary of $GE_{t_{1/2}}$ studies is presented in **Figure 6**. The use of various techniques for the $GE_{t_{1/2}}$
533 measurement may be associated with the observed variation. Increments of GE variability as a function
534 of age in **Figure 6**, can be attributed to a broader spectrum of food types ingested by the older
535 populations (*i.e.* caloric density).

536

537 *Please place Figure 6 here*

538

539 **3.4. Small intestinal transit times**

540 Analysis of available literature concerning small intestinal transit times (SITT) as a function of age,
541 indicates that there are no significant differences in SITT across ages and that the measurement
542 technique can have an impact on the estimated SITT value [134]. A limiting factor from the study
543 resides in the low number of paediatric patients included in the analysis; namely only one newborn
544 (0 - 30 d); one infant (1 mo - 2 yr); three young children (2 - 5 yr); 10 children (6 - 12 yr); and one

545 adolescent (12 - 18 yr) were present from a total of 52 subjects (16 paediatric subjects compared to 36
546 adults). Therefore, conclusions might change if data from a greater number of newborns and infants
547 was available to be included in the analysis [134].

548 The International Commission on Radiological Protection (ICRP) publication 89 also reports SITT to
549 be independent of age and type of meal ingested with a mean value of 3.9 ± 1.5 hours and recommends
550 the adoption of a reference value of 4 h for males and females of all ages. These results were obtained
551 from a meta-analysis of data derived where several techniques were used [135]. In conclusion,
552 although differences between measuring techniques have been previously reported [84; 134], SITT is
553 generally considered independent of age [48; 85].

554

555 **3.5. Intestinal surface area**

556 The intestinal surface area is related to both radius and length of the intestinal segment [84]. The length
557 of the intestine changes with growth, ranging from approximately 275 cm at birth, 380 cm at 1 year,
558 450 cm at 5 years, 500 cm at 10 years, and 575 cm at 20 years [136]. The radius of the SI also naturally
559 increases with age, and ranges from approximated values of 1.2 - 2.6 cm in newborns, compared to
560 values of 3 to 6 cm in adults [135]. Since both intestinal length and radius increase with paediatric
561 development, the functional surface area can increase significantly [137]. Furthermore, specific
562 morphological features on the luminal surface, such as folds, villi and microvilli, naturally increase the
563 surface area available for absorption [138]. SI villous patterns start developing at an early stage of
564 gestation. The growth of these features occurs by crypt hyperplasia and crypt fission (a process where
565 the crypts unzip and duplicate). Cummins *et al.* studied these mechanisms and showed that crypt
566 fission occurred predominantly during infancy, and crypt hyperplasia occurred during both infancy
567 and childhood [139; 140]. Mean crypt fission rates in newborns, infants, children and adults were
568 7.8%, 15%, 4.9%, and 1.7%, respectively. The peak of crypt fission was found to be 18% in 5 infants
569 from 6 to 12 months of age. Villus height, measured in biopsies of younger children, exhibits lower
570 values compared to healthy adults, while the crypt depth has been shown to be greater in young

571 children [63; 141]. Newborns show elongated small finger-shaped villi and small crypts, with leaf-
572 shaped villi appearing from one month after birth [140]. Feeding has been described as a modulating
573 factor of differences in villi structure between newborns and infants, where smaller crypts have been
574 described for those fed with breast milk, when compared to those fed with formula milk [140], whereas
575 other literature has described villi as single projections in children younger than three years, with
576 development of leaf or finger-shaped villi above this age [84]. Reports concerning the development of
577 these features in early childhood are conflicting and provide a rather qualitative type of
578 information [84]. Overall, comparison of newborns and infants with older children and adults, shows
579 presence of lower intestinal surface area, with differences in both structure and quantity of the villi
580 [84].

581

582 **3.6. Intestinal permeability**

583 Intestinal permeability is high at birth for preterm infants, with a decrease to adult values over the first
584 week of postnatal life [142-144]. Nevertheless, both decreases and increases in permeability during
585 the first month after birth have been reported, which might be attributed to several factors, such as
586 differences in gestational age, clinical condition, feeding regimen, and postnatal age at the time of
587 assessment [145]. It is unclear at which age full maturation of permeability processes is reached [142].
588 Children over 2 years of age present similar permeability values to adults [83; 146; 147]. Additionally,
589 processes involved in passive and active transport are fully developed in infants by ~ 4 months old
590 [137; 142]. Growth factors, hormones, breast milk and changes in the thickness and viscosity of the
591 intestinal mucus, have been described as factors underpinning the development of permeability
592 processes [145].

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

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

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

607 Contradictory literature can be found on the ontogeny of the efflux transporter P-glycoprotein (Pgp),
608 also referred to as multidrug resistance protein-1 (MDR1) [137; 142]. Mooij *et al.* studied the gene
609 expression of several hepatic and intestinal drug transporters. Intestinal mRNA expression of MDR1,
610 MRP2, and OATP2B1 was determined in surgical small bowel samples (newborns, n = 15; infants, n
611 = 3; adults, n = 14), and expression values for MDR1 and MRP2 were similar to the values in adults.
612 Intestinal OATP2B1 expression in newborns was significantly higher than in adults [152]. The
613 methodology should be considered and results should be carefully interpreted with regard to mRNA
614 data, which may not be entirely representative of transporters' protein expression or activity [153].

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

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

628

629 **3.7. Metabolism**

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

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

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

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

654 The ontogeny of hepatic metabolic enzymes has been studied more broadly than intestinal metabolism.
655 Regarding CYPs, low levels are seen in younger paediatric subpopulations. Adult values start to be
656 reported from 1 - 5 years depending on the isoform [142]. A recent examination of CYPs' hepatic
657 expression, activity and abundance as a function of age have reported greater enzyme activity and
658 abundance for enzymes of the CYP1A-3 families after birth, except for the isoform CYP3A7 [159].
659 When compared to postnatal samples, a different trend is seen, in which activity is higher than
660 abundance [159]. The evaluated samples represented the subpopulations of newborns and infants
661 (< 1 yr, n = 6), a juvenile group (1 - 18 yr, n = 10), and the adult population (>18 yr, n = 9); the lack
662 of differentiation among the juvenile group, hinders the formation of a firm conclusion on age-
663 dependent metabolic activity in this group [159]. In general, infants and juvenile groups, displayed
664 high enzymatic abundance accompanied by a lower activity, when compared to adults [159].
665 Moreover, other hepatic metabolic enzymes have shown age-dependency, such as
666 Uridine 5'-diphosphate-glucuronosyltransferase; SULT; N-acetyltransferases.

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

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

676 677 **4. Paediatric Biopharmaceutics Classification Systems (pBCS)**

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

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

694 695 **4.1. pBCS solubility classification criteria**

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

701

702 The paediatric initial gastric volumes have been calculated by a BW-extrapolation method based on
703 the initial gastric volume found in adults (250 mL, corresponding to a glass of water administered in
704 adult bioequivalence studies) and a paediatric fasted gastric fluid volume of 0.56 mL/kg [65; 146; 147;
705 163]. Slight variation of the initial gastric volume for paediatric subpopulations is observed depending
706 on the average weight reference values selected for the same paediatric age group (**Figure 7**) [146;
707 163]. The calculation of paediatric initial gastric volumes by BSA-extrapolation function based on the
708 adult initial gastric volume (*i.e.* 250 mL) and adult BSA of 1.73 m² has also been reported and results
709 in a greater volume estimated for paediatric subpopulations compared to BW-based extrapolations
710 (**Figure 7**) [164].

711 Although newborns and young infants typically receive none or only small amounts of water, the BW
712 or BSA-based extrapolations of the volumes based on adult water intake with a medicine may be
713 applicable to other typical fluids for these subpopulations, *e.g.* breast milk or formula milk. The down-
714 scaling of the recommended administered volumes in adults to children may slightly overestimate the
715 “real-life” administered volumes, as the adult value of 250 mL utilised in the extrapolation to
716 paediatrics has been reported to overestimate “real-life” administered volumes in adults [166].

717

718 *Please place Figure 7 here*

719

720 Another reasonable approach for determining the initial gastric volumes for the pBCS might be to
721 investigate the administered fluid volumes, considered representative for each paediatric sub-group,
722 and establish the limits on an empirical basis [147]. In a recent study, it was found that the majority of
723 infants and young children take no additional fluids to facilitate oral drug administration, the authors
724 explained these results with the fact that liquid formulations were commonly administered to these age
725 groups and that no additional fluid is required to facilitate drug intake [166]. In this case, the only
726 available fluid for drug dissolution would be the volume of the administered formulation, adding up to
727 5 mL for a liquid preparation [167], plus the available fluid in the fasted stomach. When fluids were
728 used to enable medication administration, water and milk were preferred for these age groups [166].
729 Liquids for drug intake by the older paediatric participants were usually reported as half a glass of
730 water, juices or soda [166]. For adults, the recommended volume to administer oral medication consists
731 of a glass of water (250 mL), whereas “real-life” studies report that only half of this volume is used
732 for medicine intake [166]. Generally, the volumes of consumed liquids increase with advancing age.
733 Evidence-based appropriate fluid volumes for drug administration throughout the paediatric subgroups
734 are insufficient to underpin a limit for the reference volume and could beneficially be investigated
735 further to provide guidelines [147]. Ultimately, it should be noted that drug administration with
736 beverages other than water has been reported to affect the drug’s bioavailability [168].
737 Further investigation is required on the need of matching dose strength to initial gastric volume for
738 each paediatric subset [142]. In the case that a default dose of the drug is not set for the subpopulation
739 of interest, an individual body-weight or BSA-based dose calculation in the phase of fast growth
740 (*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
741 the dose is doubled, while the values for solubility and initial volume remain constant [146].
742
743 For the dose/solubility-ratio, the lowest measured thermodynamic solubility of the drug in the pH range
744 1.2 - 6.8 has been proposed [160]. In the context of a pBCS, the choice of a relevant pH-range for the

745 solubility assessment requires more reliable data on paediatric GI fluid characterisation for the separate
746 paediatric subpopulations, as outlined in Section 3.2. [147]. The majority of the paediatric
747 biopharmaceutics experts surveyed by Batchelor *et al.* considered the adult pH range for solubility and
748 dissolution appropriate for the pBCS [147].

749

750 **4.2. pBCS permeability classification criteria**

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

767

768

769

770 **4.3. Challenges for the pBCS criteria determination**

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

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

794

795 **5. Food effects on oral drug absorption in paediatrics**

796 Oral delivery continues to be the route of choice for administration of most drugs both in adult and
797 paediatric populations. A review of submitted Paediatric Investigation Plans (PIPs) to the EMA in
798 2009, shows that 73% of pharmaceutical dosage forms developed for paediatric use were oral dosage
799 forms [172]. EMA defends that if possible, the formulation should be available in more than one oral
800 dosage form (solid and liquid) in order to facilitate administration and improve acceptability [10].
801 Liquid formulations are likely to be the most appropriate oral formulations from birth to 5 years due
802 to swallowability and dose flexibility. Supporting evidence shows that with support and training
803 younger children, *i.e.* below 6 years, can learn to take solid dosage forms such as tablets and capsules.
804 The definition of an ideal formulation for all paediatric age-groups is challenging due to individual
805 preferences and specific characteristics of patients [168]. An algorithm was proposed to guide the
806 development of age-appropriate medicines with a focus on acceptability in every age
807 subpopulation [173]. For newborns, liquid formulations and appropriate 2 mm mini monolithic tablets
808 were suggested. For infants, more options become available, including liquids, mini monolithic tablets,
809 multi particulates and orodispersible tablets. In children from 2 - 5 years, in addition to the
810 formulations mentioned above, chewable tablets become an option [173]. Off-label drugs are widely
811 used in paediatrics, most of the times due to lack of an appropriate paediatric oral formulation.
812 Frequently, the most commonly used formulations in adults are modified and administered to children;
813 crushing tablets or opening capsules to facilitate dosing are not uncommon practice [168]. Martir *et al.*
814 reviewed the recommendations for administration of oral drugs by the British National Formulary for
815 children and showed that the most common formulation administered to newborns are capsules, which
816 are meant to be opened, and sprinkled or mixed with food and beverages [168]. In infants, a wider
817 selection of formulations is recommended to be mixed with food, but capsules remain the most
818 frequently used formulation (30%). The following section outlines the current regulations for drug
819 administration after a whole meal or when mixed with small amounts of food or beverages and focuses

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

822

823 **5.1. Regulations and current practice: administration after a meal**

824 The EMA and FDA guidelines provide a precise framework for the conduct and evaluation of food-
825 effect bioequivalence studies in adults [52; 53]. The need of investigating drug pharmacokinetics in
826 the paediatric population has been acknowledged by regulators through the issuing of relevant
827 guidelines, while no specific regulations on food effect evaluation in paediatrics have been published
828 [5; 174; 175].

829 In order to estimate the current trends regarding bioavailability studies for paediatric formulations, a
830 search of the EU Clinical Trials Register was performed (status November 2017). The platform
831 includes 31465 clinical trials with a EudraCT protocol (16 % of which were paediatric clinical trials)
832 and additional 18700 paediatric clinical trial reports. The search yielded 32 completed and ongoing
833 bioavailability investigations, 16 of the studied formulations were intended for the oral administration
834 route. Three of the studies investigated food effects; all of them were performed in an adult study
835 population with a standardised high-caloric, high-fat breakfast. The tendency that food effects on the
836 bioavailability of paediatric drug formulations is usually investigated in adult populations has recently
837 been reported by Elder *et al.* [169]. In the context of food effect studies, age-adjusted meals were
838 sometimes taken into consideration: milk was a common meal option for formulations intended for
839 infants and younger children, whereas a breakfast was used for older children [176]. The study design
840 should aim to investigate the maximum effect, which the meal can have on the formulation of interest
841 [176].

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

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

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

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

870 5.2. Regulations and current practice: co-administration of formulation with food/ drinks

871 Small amounts of soft foods and juices are used for improving acceptability and palatability of
872 formulations in the paediatric population. Previous cases reporting significant drug bioavailability
873 alterations have raised safety concerns [59; 188-190]. As a result, vehicles (discussed in Section 2.5.)
874 which are considered safe or inappropriate to be mixed with the formulation, should be included in the
875 product information supported by relevant *in vivo* or *in vitro* studies. The amount of soft food or
876 beverage for co-administration is crucial for the study outcome, and a “small portion (*e.g.* one spoon)
877 or otherwise justified quantity of the food or drinks” is recommended by the EMA [167]. There is a
878 lack of guidance on what an exact age-appropriate amount is. EMA guideline on pharmaceutical
879 development of paediatric medicines [167], suggests an optional *in vivo* study, which can be a separate
880 bioequivalence study in adults [191], alternatively paediatric clinical trials can be conducted with the
881 vehicle(s) of choice, as reported for omeprazole and montelukast paediatric formulations [192; 193].
882 On the other hand, the sprinkling of formulations on soft food is referred in the FDA guidance on
883 Food-Effect Bioavailability and Fed Bioequivalence Studies. In the case of investigation of
884 formulations that are meant to be sprinkled on foods, a study in healthy adult volunteers is usually
885 requested by regulatory authorities [53]. Investigation of the vehicle(s), as part of the paediatric clinical
886 trial, would provide the highest reliability in terms of product safety and efficacy, although it might
887 further complicate the trial design (through introduction of additional drug administration conditions),
888 execution (*e.g.* patient recruitment difficulties), and outcome interpretation [169; 194].
889 The type and quantity of studied foods or beverages varied in adult studies investigating the
890 administration of paediatric formulations mixed with small amounts of vehicles. Quantities from one
891 tablespoon to 120 mL were reported for the commonly used soft foods and typical fluids were
892 investigated in volumes ranging from 5 to 240 mL [176; 190]. Possible food-drug interactions may
893 occur with the commonly used applesauce and apple juice, *e.g.* for fexofenadine inhibition of

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

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

906

907 **5.3. Food effects and paediatric dosage forms**

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

916 Drug absorption from innovative paediatric solid formulations, which are usually formulated into a
917 hard capsule, such as multiparticulates and mini-tablets, show less dependency on the time needed for
918 disintegration, compared to the intact formulation. Differences in the pharmacokinetic profiles have

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

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

936 Monolithic non-disintegrating formulations can usually be considered for paediatric patients older than
937 6 years of age mainly for swallowability reasons [10]. The solid monolithic formulation behaviour in
938 the presence of food is dependent on multiple factors, *e.g.* properties of the coating agent and stability
939 in different pH media, type of matrix material used, breaking force of the tablet, and general
940 formulation robustness when exposed to different GI fluids. Investigations performed in adults report
941 remarkable differences between formulations, with positive food effects (an increase of exposure
942 up to 50%) with or without absorption delay, or significantly reduced drug absorption, or no influence
943 of the prandial state [207; 208]. Formulation-related food effects for theophylline in paediatric patients

944 aged between 4 and 14 years revealed great variability after drug formulation administration after a
945 standardised breakfast, consisting of approximately 20% fats, 70% of carbohydrates, and
946 up to 13% of proteins; the total caloric count was normalised per BW 10 - 15 Kcal/kg [209]. One
947 formulation (Somophyllin[®], sustained release sprinkle product) showed no changes regarding the
948 extent of absorption, but a delayed absorption. A second sustained-release
949 formulation (Theo-Dur[®] sprinkle) showed less variable *in vivo* performance in the fasted state
950 compared to the fed state; this sprinkled formulation performed similarly in adults and paediatric
951 patients, although the negative food effect was more pronounced in the paediatric group [207; 209].
952 The exposure achieved by the monolithic theophylline formulation (Uniphyllin[®], sustained-release
953 tablet) in the fed state was doubled compared to the fasted state, due to dose dumping, which occurred
954 in 50% of the population. GI transfer delay might not only result in an unfavourable impact on the
955 timing of the drug effect when rapid drug onset is required, but it can have an impact on drug
956 bioavailability for drugs with narrow absorption windows, as observed for pregabalin controlled-
957 release tablets in adults [210]. In order to ensure that the extrapolation of food effects for non-
958 disintegrating or controlled-release formulations from adults to paediatrics is reliable, further accurate
959 knowledge about the MMC process, size of particles that can pass through the pylorus sphincter, GI
960 motility, and transit times across the GI is essential.

961

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

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

966

967

968

969 **6.1. Paediatric biorelevant media**

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

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

992

993 *Please place Table 4 here*

994 **6.2. Evaluation of drug products characteristics**

995 *In vitro* dissolution testing is a standard method used for the characterisation of drug products.
996 Questions regarding the relevance of dissolution tests within paediatrics have been raised in a recent
997 review since dissolution testing mainly aims to characterise solid oral dosage forms, and its
998 applicability to commonly used paediatric formulations as liquids, semisolids, or orally disintegrating
999 tablets is debatable [169]. Nevertheless, as mentioned in Section 4.2., paediatric solid formulations
1000 (*e.g.* chewable tablets, mini-tablets, multiparticulates, *etc.*) are gaining further popularity for low-
1001 solubility drugs [170]. The mini-paddle apparatus, that is based on the pharmacopoeia paddle apparatus
1002 (USP II apparatus with scaled down dimensions), and the flow-through cell apparatus
1003 (USP IV apparatus) have been acknowledged as superior to USP I and II apparatus, in terms of
1004 simulating paediatric conditions [169].

1005

1006 New paediatric dissolution setups have been proposed by Karkossa *et al.*, which investigated different
1007 dosing scenarios of a paediatric formulation of sodium valproate (BCS class I compound; $pK_a = 4.8$
1008 and $\log P = 2.75$) extended-release mini tablets formulation (Orfiril long[®]) [211]. Two scenarios were
1009 investigated: i) impact of gastric pH on drug release, in a new dissolution apparatus (proposed in the
1010 study as a modified USP III vessel (shortened height and glass ring in outer surface) in a water bath
1011 with stirring provided by a magnetic stirrer (550 rpm), and ii) impact of co-administration of different
1012 vehicles in a mini-paddle apparatus with a subsequent transfer to a new dissolution apparatus.
1013 Residence times for the simulation of each stage of GI tract were 30 min for the gastric compartment,
1014 240 minutes for the SI and 480 min for the proximal colon [216]. Gastric fluids were simulated by
1015 mixing 10 mL of simulated gastric fluid (pH range 1.8 - 4.0), and 50 mL of water. After 30 min,
1016 simulated gastric contents were transferred to a second vessel where 110 mL of simulated small
1017 intestinal fluid (pH 6.8 bicarbonate based simulated intestinal fluid, 50 mL) was present. Results
1018 showed that gastric pH had no impact on overall drug release. During the short-simulated fasted gastric

1019 residence time of 30 min, almost no drug was released. Approximately 50 - 60% of the dose was
1020 released during simulated small intestinal residence time, and drug release was complete at the end of
1021 the simulated passage through the SI and proximal/mid colon. The impact of co-administration of
1022 dosing vehicles on drug release was investigated with a two-stage dissolution model. Gastric residence
1023 of the administered formulation with water, apple juice or soft foods (applesauce, yoghurt, or pudding)
1024 was performed in the mini-paddle apparatus (170 mL; 30 min; 75 rpm). After the first 30 minutes,
1025 60 mL of the simulated gastric contents together with the tablets were transferred into the modified
1026 USP III vessel, with the addition of 50 mL of bicarbonate-based simulated intestinal fluid, in order to
1027 simulate the intestinal conditions. Drug release under these conditions was screened for 12 h
1028 representing residence time in the SI and proximal colon. These release studies revealed that
1029 administration of the formulation with other beverages, and soft foods should not affect bioavailability
1030 and confirmed the appropriateness of the paediatric dosing recommendation for this formulation [211].
1031 *In vitro* release profiles from experiments simulating co-administration with different soft foods
1032 (applesauce, yoghurt, and pudding) were similar to those obtained in water and apple juice, suggesting
1033 that co-administration of soft food will not affect bioavailability of the extended-release formulation.
1034 Brassine and Fotaki investigated the effect of age-related physiological parameters, the effect of dose,
1035 and the effect of hydrodynamics on the performance of carbamazepine (BCS class II; non-ionisable in
1036 the physiological pH range; $\log P = 2$) for paediatric use. Biorelevant media, with adjusted bile salt
1037 concentration, were incorporated in an *in vitro* dissolution testing to evaluate the effect of age on
1038 dissolution and release of carbamazepine pellets prepared by extrusion-spheronisation [212]. The
1039 dissolution study was conducted with the dissolution USP IV, and parameters were adjusted (flow rate
1040 and residence time) to simulate GI physiological parameters in paediatric groups (newborns, infants
1041 and children) and adults. Furthermore, the effects of the hydrodynamics on the dissolution was studied
1042 by setting the closed-loop mode (for simulation of gastric conditions) followed by the intestinal
1043 conditions simulated with the open-loop mode. Results showed a slower release of carbamazepine

1044 under all paediatric-simulated conditions when compared to the conditions used for the adults;
1045 nevertheless, no significant differences were revealed for the release of carbamazepine between the
1046 investigated paediatric groups [212].

1047 The same USP IV biorelevant set-up for the fasted state was performed to investigate age-related
1048 differences in the dissolution performance of Tegretol[®] 200 mg tablets [213]. Paediatric biorelevant
1049 media developed by Maharaj *et al.* were used. Results showed that carbamazepine was not completely
1050 dissolved in all of the tested conditions. An age-dependent dissolution profile of carbamazepine from
1051 Tegretol[®] tablet was observed in the two studied paediatric groups revealing the impact of the GI
1052 differences (fluid composition and transition times) between the age groups on dissolution.
1053 Furthermore, the use of the closed-loop mode for the simulation of dissolution in the gastric
1054 compartment resulted in a higher discrimination of the dissolution profiles between the two age groups
1055 [213].

1056
1057 Non-compendial apparatus for the evaluation of paediatric formulations have also been proposed.
1058 [169]. A TNO Gastro-Intestinal Model (TIM) paediatric setup (TIMpaediatric) has been developed,
1059 which simulates conditions in the GI tract determined by four interactive factors: i) degree of
1060 maturation of the age groups (term newborns; infant; or toddler), ii) food type, iii) health status and vi)
1061 co-medications [214]. The TIMpaediatric was applied to investigate age-related effect of
1062 co-administration of food matrices with paracetamol (BCS class I; pKa = 9.5; log P = 0.2), diclofenac
1063 (BCS class II; pKa = 4.15; log P = 4.51), and esomeprazole (BCS class II; pKa = 4.78; log P = 0.6),
1064 where bioaccessibility curves were constructed (amount of drug available when sampling). Selected
1065 dosage forms were tested in the *in vitro* TIMpaediatric by taking into consideration the simulation of
1066 daily practices used for administration of paediatric medicines, including crushing of tablets, mixing
1067 drugs with appropriate amounts of food (simulations performed for administration with formula milk
1068 vs. water), and simulation of the co-administration with proton pump inhibitor were simulated

1069 (simulations performed under high gastric pH conditions (pH 6.7 to 6.0). A validation experiment of
1070 TIMpaediatric was performed by comparing *in vitro* bioaccessibility profile with *in vivo* clinical data
1071 for Calpol® syrup suspension (containing paracetamol) mixed with food, under term-newborn, infant
1072 and toddler GI conditions, and similar bioaccessible amounts were found when compared to plasma
1073 concentration profiles, demonstrating the quality of the predictions obtained from the TIMpaediatric.
1074 Further experiments were then performed, paracetamol formulations investigated were Sinaspril®
1075 syrup, Sinaspril® tablets (crushed), and Marel® tablets (crushed, also contain caffeine) and results
1076 showed that paracetamol concentration available for intestinal absorption was independent of the
1077 different GI conditions of the age-groups, the tested dosage forms, the food matrix, and the co-
1078 administration of a proton pump inhibitor. Two brands of enteric-coated diclofenac tablets were tested
1079 (Voltaren® vs. Diclofenac Sodium Teva®), results showed that diclofenac available for absorption of
1080 is not influenced by co-administration of a proton pump inhibitor, but the administration of a crushed
1081 tablet with infant food showed a significant positive effect on diclofenac bioaccessibility. The
1082 investigated formulation of esomeprazole formulation was Nexium® enteric coated tablets (crushed),
1083 and results showed after a first dose of a crushed tablet to infants was low, but increases after repeated
1084 dosing due to a higher gastric pH by the proton pump inhibitor [214].

1085

1086 A recent literature review has been performed with the intention of developing an *in vitro* digestion
1087 model for newborns and infants (0 - 2 mo) based on a previous lipolysis model for adults [48].
1088 Considerations were taken to represent changes during the feeding cycle of newborns and infants,
1089 which is approximately 3 h. The *in vitro* digestion model was argued to be more appropriate than other
1090 *in vitro* predictive tools, due to the frequent feeding of newborns. Since newborns are mainly in the
1091 fed state, this can ultimately affect the composition of the fluids and hydrodynamics available for drug
1092 dissolution and solubilisation processes. For the design of the *in vitro* setup, several physiological
1093 factors were reviewed including GE, SITT, gastric volumes *etc*, and suggested flow rates for the

1094 transfer of GI fluids under fed state conditions. A two-step model was proposed as more appropriate,
1095 comprising a gastric phase and an intestinal phase, where the duration of each phase, and the transfer
1096 between the two phases, should be reflective of GE and SITT in newborns/young infants. The
1097 performance of Furix® 20 mg furosemide (BCS class II compound) tablets, in the newborn and infant
1098 GI tract was investigated with this set-up [215]. Fasted and fed states were simulated to represent
1099 feeding patterns in the studied population; therefore, the fasted state assumed the presence of small
1100 amounts of milk. The physiological relevant media used were composed of a chosen appropriate milk
1101 (Nan 1, Nestle®), and the inclusion of digestive enzymes (*i.e.* pancreatic triglyceride lipase and pepsin
1102 and human gastric lipase). Two *in vitro* models simulating the GI transfer were utilised. In the
1103 immediate transfer model, a concentrated intestinal medium was added in a single step at a designated
1104 time point, altering the digestion medium from gastric to intestinal medium instantaneously. In the
1105 continuous model, digestion medium was continuously pumped from a gastric to an intestinal
1106 compartment, where the concentrated medium simulating the SI fluid was present. The results
1107 suggested that the oral bioavailability of furosemide in this subpopulation increased in the presence of
1108 food [215]. In contrast, parameter manipulation, such as simulation of food digestion and crushing of
1109 the tablets seemed to cause no alterations in the oral performance of furosemide [215]. The entire
1110 furosemide dose was completely soluble in the aqueous phase of the simulated postprandial state,
1111 which led the authors to conclude a high bioavailability of the drug in the presence of food [215]. GI
1112 digestion of food ingested showed no effect on the amount of furosemide solubilised, nor did the
1113 administration of the pure powder form of furosemide, which indicates that the dosage form does not
1114 influence the oral performance of furosemide. The results suggest that presence of food in newborns
1115 and young infants is affected by the pH at fed state and volume available for drug solubilisation, which
1116 allows the that the entire dose of furosemide is solubilised in the digestion studies without being
1117 affected by excipients and digestion. On the contrary, In order to further evaluate and validate these
1118 results and usefulness of the *in vitro* models, *in vivo* data is required [215].

1119 A considerable amount of progress has been made in the development of paediatric *in vitro* dissolution
1120 tests. Compendial and non-compendial apparatus have been used, and biorelevant setups have been
1121 proposed. Nevertheless, further research is required to better characterise GI physiological and
1122 anatomical changes in paediatrics, in both the fasted and fed state, which will inevitably allow
1123 optimisation and proposal of more biorelevant models. Validation of the *in vitro* setups with clinical
1124 data would be helpful to establish confidence in these methods so that they can be used to inform the
1125 development of more complex and innovative paediatric dosage forms. Furthermore, a combination of
1126 biorelevant *in vitro* tests with paediatric PBPK models is expected to improve knowledge and
1127 understanding of oral drug absorption in paediatrics [169].

1128

1129 **7. *In silico* evaluation of drug products for paediatrics**

1130 Regulatory frameworks allow investigators to use existing adult clinical data as supporting evidence
1131 for efficacy in paediatric populations [216; 217] assuming that disease progression and exposure-
1132 response in both populations are expected to be similar. A significant number of conducted
1133 pharmacokinetic and efficacy studies in the paediatric population did not achieve labelling for various
1134 reasons, such as poor study design planning or inappropriate dose determination, indicating the need
1135 of robust and reliable approaches for interpreting and benefiting from already available clinical data
1136 [218].

1137 Predicting *in vivo* drug performance relies on the estimation of the drug's ADME properties and the
1138 understanding of the physiological processes influencing pharmacokinetic parameters. Scaling of
1139 parameters for different organisms can be facilitated by calculations using isometric or allometric
1140 functions, or be performed on a more complex level such as PBPK modeling [219].

1141

1142

1143

1144 **7.1. Allometric scaling**

1145 Paediatric parameters are calculated as a function of the normalized *BW* or *BSA* and a specific
1146 allometric coefficient [220]. For example, a fixed allometric coefficient of 0.75 is used for clearance
1147 scaling, whereas a value of one is used for the down-scaling of the volume of distribution [220].
1148 Mahmood *et al.* reported that drug clearance calculated by allometric scaling with an adjusted
1149 allometric exponent, and clearance predicted via PBPK modeling achieved similar results for
1150 newborns and infants < 3 months of age; the studied drugs were mainly cleared by
1151 glucuronidation [221]. The prediction accuracy for newborns and infants is expected to be
1152 compromised for drugs undergoing more complex metabolism, due to variable enzyme ontogeny,
1153 maturation processes, and alternative metabolic pathways. The use of fixed-coefficient allometric
1154 scaling is recommended after 2 - 5 years of age when the maturation processes can be considered
1155 completed [220; 222-225]. The method's simplicity and unproblematic utilisation contribute to its
1156 widespread application in clinical settings.

1157

1158 **7.2. PBPK modeling**

1159 While allometric functions are still useful for scaling ADME properties, PBPK modeling would be
1160 preferred, if more complex processes need to be studied [226]. PBPK modeling is an *in silico*
1161 biopharmaceutical tool describing the pharmacokinetics of a compound while taking the drug
1162 properties and drug product characteristics into consideration when introduced to a specific system
1163 (*e.g.* healthy adult body) according to a pre-defined study design (*e.g.* administered formulation). In
1164 adults, PBPK modeling is often used to predict drug product performance [227]. In paediatrics its use
1165 has increased the last decade, recognised by the EMA and FDA by publishing guiding documents on
1166 the appropriate use of previous knowledge (*e.g.* adults) in paediatric medicines development and by
1167 PBPK modeling guideline [216; 228; 229].

1168

1169 Two modeling strategies may be used to construct a PBPK model, depending on the input used for the
1170 system. The “top-down” approach is based on observed clinical data as a model for the system (human
1171 body), followed by an investigation of the components and occurring processes (*e.g.* parameter
1172 estimation from plasma drug concentration-time profiles). In contrast, a model that is based solely on
1173 a combination of physiological processes parameters and *in vitro* experiments, generating numerous
1174 connected compartments, which represent an organ or the whole body, is regarded as a “bottom-up”
1175 approach (usual PBPK model). While the latter depends on absolute knowledge of details, which
1176 contribute to drug performance in order to predict pharmacokinetics and pharmacodynamics *a priori*,
1177 the former relies completely on already obtained clinical data but may not be able to provide the
1178 necessary detail in each case. A “middle-out” concept that benefits from the combination of the two
1179 approaches might offer a sensible compromise when some parameters have not been reliably estimated
1180 yet or need refinement through already available clinical data [230; 231]. Several software platforms
1181 enable the building of PBPK models for adults (*e.g.* GI-Sim[®], PK-SIM[®], Stella[®], MATLAB[®]), while
1182 some of them do not provide an integrated detailed model of oral absorption (MATLAB[®]) [227].
1183 Additionally, commercially available software platforms, such as, GastroPlus[®] (Simulations Plus Inc.
1184 [232]), and Simcyp[®] (Simcyp Ltd., Sheffield, UK [233]), facilitate the development of whole-body
1185 PBPK models and models focused on oral drug absorption for adults and their further extrapolation to
1186 the paediatric population [234].

1187

1188 **7.2.1. Paediatric PBPK models: current status**

1189 A search in PubMed with the keywords “Paediatric PBPK” OR, “PBPK model Paediatric” AND,
1190 “infants”, “newborns”, “children”, “adolescents” OR, “PBPK paediatric modeling”, OR “mechanistic
1191 model paediatric pharmacokinetics” identified 405 relevant entries, including reviews and original
1192 articles (status August 2017). A snowball sampling of the review articles for potentially mentioned

1193 articles, complying with the focus of the search was performed and the papers, which reported a
1194 developed PBPK model for paediatric populations, were selected (n = 93; **Figure 8**).

1195

1196 *Please place Figure 8 here*

1197

1198 Pre-term and term newborns were found to be less studied (**Figure 8A**) – a trend also reported in
1199 clinical trials performed in paediatrics. Over 80% of the paediatric PBPK models were developed
1200 based on a PBPK model for adults (**Figure 8B**). Evaluation of the aims of the models developed
1201 showed numerous successful mechanistic clearance and drug-disposition models for intravenous (IV)
1202 administered drugs. Twenty nine percent of PBPK models following oral drug administration have
1203 been established until now (**Figure 8C**). A similar trend was observed for the adult PBPK models,
1204 where modeling oral drug absorption accounted for only 12% of the developed PBPK models [235].
1205 The biggest part of the PBPK models was built with the help of a commercially available software
1206 platform, whereby Simcyp[®] appeared to be the most frequently used one (**Figure 8D**). Additionally,
1207 the BCS classes of the orally administered drugs, used for modeling were analysed (**Figure 9**).
1208 A preference of PBPK model development for highly soluble drugs might be related to the fact that
1209 these would usually not introduce further solubility or dissolution complications in addition to the
1210 model uncertainties originating in the complexity of the oral drug absorption processes itself [7]. The
1211 low number of medicines modeled containing BCS IV compounds can be explained by the great
1212 number of uncertainties accompanying both permeability and solubility of these compounds in
1213 paediatric populations.

1214

1215 *Please place Figure 9 here*

1216

1217

1218 **7.2.2. Building a PBPK model**

1219 The most common approach in constructing a paediatric PBPK model is to build first the adult
1220 disposition PBPK model (**Figure 10, Step 1**), and after ensuring reliability of the intravenous model,
1221 oral administration can be incorporated (**Figure 10, Step 2**) [236].

1222 If the adult PBPK model provides an adequate prediction of the available clinical data in adults, the
1223 scaling to the paediatric population could proceed [237]. By selecting a specific paediatric population
1224 as the study population in the software platform, default age-dependent changes and parameters of
1225 physiology and anatomy are incorporated into the paediatric model.

1226

1227 *Please place Figure 10 here*

1228

1229 *Step 1: Building drug disposition PBPK model for adults*

1230 For the development of a PBPK model, system-dependent and compound-dependent parameters are
1231 needed [7; 169; 236; 238-240]. System-dependent components (*i.e.* organ sizes, blood flow, and tissue
1232 composition) are incorporated in the commercially available software platform for the species of
1233 interest (*e.g.* human, dog, mouse). Drug-dependent parameter values are derived from literature or
1234 experimental data. Parameters describing the drugs physicochemical properties (*i.e.* molecular weight,
1235 log P, pKa, compound type, and pH-dependent solubility) are used. Drug parameter values that depend
1236 on the drug and the adult human physiology (fraction unbound, permeability, plasma/blood-
1237 partitioning, intrinsic clearance) may require further investigations and adjustment for the modeled
1238 system or special population [240].

1239

1240 The human body is represented as a network of organs and tissues, linked by an arterial and venous
1241 blood, with attributed specific blood flows. The disposition model is based on differential equations
1242 that describe the distribution of the drug into the different tissue compartments and organs [7; 227;
1243 235]. A simulation takes place when the input parameters and the study design (*e.g.* selecting study

1244 population, age, sex, dose strength, dosing conditions, duration of infusion, *etc.*) have been defined. If
1245 the pharmacokinetic simulations of the model incorporating predicted values for clearance or volume
1246 of distribution mismatch the observed clinical intravenous data, model optimisation can be achieved
1247 by informing the model with clinical data (if available). Once the predictions forecast the observed
1248 data from IV administration, the modeling of oral drug absorption can be undertaken [236; 237].

1249

1250 *Step 2: Building oral absorption PBPK model for adults*

1251 The oral absorption of a drug can be modeled in detail using the relevant available commercial software
1252 oral models, such as ACAT™ model (GastroPlus®), or ADAM™ model (Simcyp®). In both models, the
1253 GI tract is divided into sequentially connected transit compartments, beginning with the stomach,
1254 which gives the input for the SI according to a specific emptying-rate. The SI is further divided into
1255 sub-compartments (representing the duodenum, upper and lower jejunum, and upper and lower ileum)
1256 and it is linked subsequently to the colon. Each compartment exhibits different surface area, luminal
1257 fluid composition and volumes, and metabolising luminal enzymes. In addition to the mass-balance
1258 differential equations, the model considers the local pH-dependent solubility by the incorporation of
1259 the Henderson-Hasselbalch equation and calculates the dissolution behaviour with *e.g.* Noyes-Whitney
1260 kinetics [227; 240]. In this step, the drug formulation, which is to be investigated, is incorporated. If
1261 relevant, available dissolution data from biorelevant *in vitro* tests can be used to inform the model
1262 [227]. Ultimately, drug dissolution, precipitation, or supersaturation are considered if relevant for the
1263 drug/drug formulation; hence the absorbed, degraded, or metabolised drug fraction are taken into
1264 account simultaneously [227].

1265 The permeability of a drug can be derived from *in vivo* or *in vitro* studies or estimated via the utilised
1266 software. In case that active transporters are involved in the drug uptake, the kinetic parameters
1267 (*i.e.* Michaelis Menten constant (K_m) and maximum rate achieved at saturating substrate concentration
1268 (V_{max})) of the substrate, the transporter availability, and activity, at the sites of interest are needed and
1269 an adequate estimation of permeability-limited transport through the cell membranes should be

1270 included [239]. If relevant information is not available in the literature or from *in vitro* studies
1271 performed, a model fitting based on *in vivo* data from oral drug administration studies can be
1272 applied [240]. The accuracy of the model's prediction needs to be confirmed and refinements should
1273 be undertaken if needed before application to other populations can proceed.

1274

1275 *Step 3: PBPK model conversion to the paediatric population*

1276 The GastroPlus® platform (PBPKPlus™ module) generates physiological parameters for the model by
1277 its feature Population Estimates for Age-Related Physiology (PEAR®). It takes the population
1278 (e.g. American/Western Japanese, and Chinese), gender (male/female) age, gestational age (including
1279 pre-mature newborns), BW, height, body-mass index, percent body fat into account and adjusts tissue
1280 volumes and perfusion rates accordingly [241]. Correspondingly, in the Simcyp population-based
1281 simulator (Simcyp®), physiological parameters are adjusted by converting to the available module
1282 Simcyp® Paediatric [237]. Age-dependent changes are introduced to the full PBPK model, e.g.
1283 adjustments of compartment volumes, blood perfusion rates, tissue compositions, specific partition
1284 coefficients for tissues. In addition to these adjustments, a model with focus on oral drug absorption
1285 in paediatrics addresses GI specific physiological parameters such as GE rates, SITT, fluid volumes
1286 throughout the GI tract, composition of the GI fluids, GI hydrodynamics, and size of the separate
1287 compartments of the GI tract; all of these parameters influence drug movement through the GI tract,
1288 drug dissolution and absorption rates, and therefore drug product performance following oral
1289 administration [125; 242].

1290 In the ACAT Model (GastroPlus®), GI organs and their respective blood flows change dependent on
1291 age, intestinal length and radius are calculated according to intestinal growth data and are based on the
1292 assumption that proportional growth occurs throughout the SI [242]. Age-adjusted SITT values are
1293 incorporated in the model, although it should be noted that the data used for this assumption is highly
1294 dependent on the method utilised for the measurement (Section 3.4), thus introducing a level of model

1295 uncertainty [242]. Furthermore, fluid secretion volumes are scaled as a function of age for the
1296 paediatric population in the ACAT™ model (GastroPlus® version 9.0) [243]. Adult values are adopted
1297 for the gastric and intestinal pH and GE in the model. The villi structure is also reflected, as for adults,
1298 due to the qualitative nature of the information available (Section 3.5); this leads to a large uncertainty
1299 for the estimation of passive absorption of drugs, especially for the youngest populations < 3 years of
1300 age [242]. Due to the scarcity of data found for bile salt composition and site of reabsorption, adult
1301 parameter values are adopted; model inaccuracies can be expected for compounds that exhibit great
1302 solubility and permeability dependency on bile salts. Ultimately, intestinal enzyme levels for CYP3A4
1303 are implemented in the modeling platform according to age, based on paediatric *in vivo* data, but for
1304 less well-characterised intestinal enzymes and transporters adult values are utilised. Since expression
1305 density and ontogeny are expected to show differences in newborns and infants compared to adults,
1306 the user has the option to modify the default values of enzyme/transporter expression levels per
1307 intestinal compartment based on surface area, and the enzyme/transporter density in adults [242].

1308

1309 Within the Simcyp® platform, the intestinal diameter, length and surface area are scaled according to
1310 age by using BSA-based functions; here it should be noted that no correction is incorporated for the
1311 potentially additional available surface area created by villi and microvilli with increasing age [35].
1312 Fasted gastric pH for paediatrics is assigned similar values as for adults, except for the age groups of
1313 newborns and infants. For these paediatric subpopulations, higher values are considered appropriate in
1314 order to simulate the more frequently administered meals and the absence of a ‘true’ fasted state [35].
1315 Salivary secretion is described by a BW-based function and is further incorporated in the calculation
1316 of the fasted gastric volume. The fed gastric volume is calculated according to BW and is characterised
1317 for 3 age groups, based on the different daily fluid requirements and the feeding frequency [35]. Fluid
1318 secretion volumes are scaled based on BSA-functions. Intestinal pH values observed in adult
1319 populations are designated to all paediatric subpopulations [35]. GE is described as a function of meal

1320 type, the user is given a choice of simulating the effects of liquid, semi-solid or solid meal ingestion;
1321 the SITT values for paediatrics are adopted from the adult model [35]. Ultimately, the ontogeny and
1322 presence of metabolising luminal enzymes of the CYP and UGT families are calculated in the same
1323 pattern as the well-defined CYP3A4 in paediatrics. The enzyme abundance follows a BSA-dependent
1324 function, specifically assigned to the different intestinal segments. Assumptions are needed for some
1325 less investigated parameters, such as intestinal transport proteins, for which adult values are
1326 adopted [35; 244]

1327

1328 Simulation in paediatric subpopulations usually begins in the subpopulation most similar to adults,
1329 *e.g.* adolescents or children, proceeding gradually to the younger subpopulations [236]. Throughout
1330 the process, confirmation, validation, and if necessary, refinement steps are undertaken. The gradual
1331 adaption of the model facilitates easier detection of probable refinement demand [236]. Mismatches
1332 between the predicted and observed paediatric clinical data should be further investigated through
1333 parameter sensitivity analysis (PSA) [35; 125; 236]. This is also a useful approach for investigating
1334 “what-if” scenarios related to the assumptions and uncertainties which were included in the model
1335 throughout development [216].

1336

1337 **7.2.3. Examples of paediatric PBPK models: focus on oral drug absorption**

1338 Prediction of oral drug exposure to sotalol was built over the entire paediatric age range (*i.e.* newborns,
1339 infants, children and adolescents) and adults, by Khalil *et al.*, with the utilisation of two modeling
1340 software platforms, Simcyp[®] (version 12.1) and PK-SIM[®] (version 4.2.2) [238]. Sotalol is an
1341 amphoteric compound (pKa values: 8.3 and 9.7) with hydrophilic characteristics (log P of 0.37).
1342 Firstly, the adult disposition model was developed. Parameters from the model after IV administration
1343 were kept constant, and parameters relevant to oral drug absorption were adjusted. Lastly, age-specific
1344 anatomical and physiological changes, which are part of the paediatric module of the software, were

1345 taken into account. Adult values were used for several parameters, such as gastric and intestinal pH,
1346 GE, SITT, intestinal enzyme ontogeny/abundance, and intestinal transporter ontogeny/abundance.
1347 Drug-specific parameters, including solubility, remained unchanged throughout all age groups
1348 regardless of the utilised software. Information on the sotalol formulations investigated with the PBPK
1349 models, was not provided. Further complications arose from the data scarcity of neonatal and infant
1350 pharmacokinetic data, which are needed in order to validate the PBPK models. Simulations from both
1351 paediatric models (Simcyp[®] and PK-SIM[®]) were comparable and showed acceptable adequate
1352 description in adults, adolescents, children and infants, when compared with *in vivo* clinical data. For
1353 newborns, the predictions generated with the Simcyp[®] simulator successfully reflected the time at
1354 which C_{max} is reached (t_{max}), and rate of elimination (k_e) when compared with the clinical *in vivo* data,
1355 but were inadequate in the forecasting area under the curve (AUC) AUC_{last} in newborns, and maximum
1356 plasma concentration reached (C_{max}) in newborns; moreover the model tended to under-predict drug
1357 plasma levels in all paediatric subpopulations ((for AUC_{last} , C_{max} , and t_{max} for all of the paediatric
1358 populations studied: mean observed/predicted ratios >1). Results obtained with the modeling platform
1359 PK-SIM[®] successfully predicted AUC_{last} , C_{max} and k_e , although the pre-defined two-fold error range
1360 was exceeded for t_{max} in newborns and infants (<1 yr). The results from this study confirm the
1361 importance of gaining deeper insight into intestinal paracellular permeability, transporter ontogeny,
1362 intestinal fluid dynamics, and characteristics of the intestinal unstirred boundary layer in order to
1363 develop a reliable PBPK model for oral drug administration [238].

1364

1365 Paediatric PBPK models have been developed (GastroPlus[®] version not mentioned) for two highly
1366 soluble, and highly permeable compounds (sotalol and paracetamol) by Villiger *et al.* [236]. As
1367 previously described, Sotalol is an amphoteric compound, and paracetamol is a hydrophilic weak acid
1368 ($pK_a = 9.5$; $\log P = 0.2$). The same approach for model building was used as in the first example, where
1369 a drug disposition model was developed to simulate the IV profiles in adults, followed by the

1370 adjustment of parameters for oral administration in adults. Secondly, after attaining confidence in the
1371 adult models, the paediatric oral model was built in a stepwise approach. In this study, *in vitro*
1372 dissolution testing was performed for immediate-release formulations, Sotalol[®] tablets (containing
1373 sotalol) and Dafalgan[®] powder-filled sachets (containing paracetamol), in order to investigate the
1374 formulation performance and understand drug release in the GI tract [236]. For the *in vitro* tests,
1375 conditions more closely reflecting newborn physiology were simulated by adjusting GI volumes to 5
1376 mL and the use of formula milk as dissolution medium, in comparison to an adult setup, represented
1377 by 250 mL of adult biorelevant media. Results showed that the described age-adjusted conditions did
1378 not influence dissolution of both test drugs. Dissolution information was not used to inform the model
1379 building, and further information on the formulations and their incorporation into the models was not
1380 reported for the performed simulations. PSA revealed that slower mean gastric transit times led to
1381 slower absorption rate of sotalol and paracetamol in newborns and infants when compared to older
1382 children and adults [236]. Good predictions were observed after scaling age-dependent factors
1383 incorporated in the software used (Gastroplus[®]), for children 2 - 11 years, but discrepancies were
1384 again seen by Villiger *et al.* for younger populations with under-prediction of C_{max} and over-prediction
1385 of t_{max} (newborns and infants) [236]. As previously described in the first example, Khalil *et al.* also
1386 obtained good predictions for other age-groups, except for newborns [238]. Interestingly Khalil *et al.*
1387 did not conduct PSA, but Villiger *et al.* took advantage of PSA to understand the critical parameters
1388 of oral drug absorption for these compounds, and subsequent improvement of the models predictions
1389 was possible, demonstrating the importance of conducting such analysis [236]. Adjustments of mean
1390 gastric transit times (default value of 0.25 h for all age groups) was performed by incorporating
1391 prolonged times. Sotalol simulations were improved by changing mean gastric transit time from
1392 2.3 to 2.5 h in both infants and newborns, while for paracetamol, a prolonged mean gastric transit time
1393 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}
1394 and t_{max} (Observed/Predicted ratios) were seen for the simulations in newborns and infants.

1395 A mechanistic absorption model for predicting formulation performance in paediatric subjects has been
1396 described for paracetamol and theophylline (BCS class I compounds), and ketoconazole, (BCS class
1397 II compound) for the fasted and fed state using the ADAM™ module of the Simcyp® software
1398 paediatric (version 15.1) [35]. Theophylline simulations were developed for the oral administration of
1399 an oral solution to newborns, infants, and adults; the aqueous drug solubility was used for the model.
1400 Although the investigated paracetamol formulation was a suspension and required the incorporation
1401 of a dissolution model within ADAM™, no further dissolution testing was performed as previous
1402 studies have reported that drug dissolution was not the absorption rate-limiting step [35; 236]; again,
1403 the aqueous drug solubility value was incorporated in the model. Ketoconazole is a drug with a highly
1404 pH-dependent aqueous solubility; hence, reference solubility values at physiologically relevant pH
1405 range 3.3 - 7.5 were used to inform the model; dissolution data were not included as an input parameter.
1406 Additionally, the model considers further processes such as intraluminal supersaturation and
1407 precipitation and bile salt mediated solubility. Paracetamol and ketoconazole simulations were
1408 developed for the oral administration of a suspension to newborns, infants, children and young adults.
1409 Theophylline plasma profiles were predicted with good accuracy (observed/predicted ratio: 0.85 - 1.25
1410 range); the accuracy of the predictions for paracetamol and ketoconazole was evaluated as reasonable
1411 (observed/predicted ratios: 0.82 - 1.33-fold for paracetamol) [35]. The prediction for full-term
1412 newborns failed to predict the observed pharmacokinetic data for pre-term newborns. PSA revealed
1413 that extremely prolonged GE times, resulting from the absence of enteral feeding, could lead to a low
1414 systemic exposure as observed *in vivo* (*i.e.* decrease of C_{max} in the range GE 2 - 20 h), and that elevated
1415 gastric pH values (*i.e.* values higher than 4) are less likely to cause low plasma drug levels. The f_a for
1416 paracetamol and theophylline was similar in the fasted and fed state, while t_{max} was shown to be slower
1417 in the fed state. For both drugs, the slowest absorption rate among the age groups studied was the
1418 newborns. For all three compounds, t_{max} values in the fed state were greater for all ages and showed a
1419 trend towards an increase with advancing age; a slightly shorter t_{max} was demonstrated for liquid foods

1420 compared to semi-solid or solid meals. For ketoconazole, increasing age was related to a longer t_{max}
1421 and lower f_a . Higher f_a values were observed in the fed state compared to the fasted state in all ages
1422 and no difference was observed between solid and semi-solid foods [35].

1423

1424 A PBPK model was developed for montelukast (BCS class II/I; log P 8.79; pKa 2.7 and 5.8) in
1425 Simcyp® for adults and paediatric patients. Montelukast is an amphiphilic drug with a high
1426 lipophilicity [245]. The simulations were first built for adults after IV and oral administration of a
1427 solution (no information about food state), and film-coated tablets in the fasted and fed state. Following
1428 validation of the adult model, scaling was performed to simulate the administration in paediatric
1429 populations after administration of oral granules in infants, and film-coated tablets in
1430 children/adolescents, but no information was given about food state in paediatrics. The model building
1431 included the experimental *in vitro* measurements of particle size and solubility in fasted simulated
1432 gastric and intestinal fluid, and the dispersion type of the different formulations. Visually, the
1433 absorption profiles were not well described for any of the paediatric age groups and mismatches of
1434 observed vs. predicted pharmacokinetic profiles could be seen for infants after administration of
1435 granules and children. Based on the model building process where parameterisation was based on sub-
1436 models, and what information was known for each age-group, predictions of plasma concentration
1437 profiles were regarded as reasonable, which in most cases appeared to be within two-fold of the
1438 observed values (no ratios of observed/predicted were provided) [245].

1439

1440 An adult and paediatric disease PBPK model for oral administration of carvedilol, a BCS class II drug,
1441 has been developed for patients with heart failure [246]. Carvedilol is a weak base with a pKa of 7.97
1442 and log P of 4.19. The model was used to investigate the oral pharmacokinetics in infants, children,
1443 adolescents (oral suspension) and adults (capsules and oral suspension). Changes in hepatic and renal
1444 blood flows were incorporated in the model to simulate more accurately the physiology of chronic

1445 heart failure patients and the accuracy of the predicted (mean ratio observed vs. predicted)
1446 pharmacokinetic parameters were improved in adults with chronic heart failure after oral
1447 administration of a capsule or a suspension. The paediatric model for carvedilol was then constructed
1448 with the pharmacokinetic parameters of carvedilol scaled to the paediatric patients by using the
1449 paediatric module of Simcyp® (version 13.1). The predictions of the exposure of carvedilol in the
1450 paediatric patients did not show as good correlations as for adults, except for patients above 17 years
1451 of age. The limitations of the applied paediatric ADAM™ model was attributed to the lack of
1452 information on anatomical and physiological changes, such as information on gastric and intestinal
1453 pH, bile secretion, transporters, and gut fluid dynamics [246].

1454

1455 A PBPK model was developed to investigate the age dependency in oral absorption of the poorly
1456 soluble lipophilic compound, carbamazepine (non-ionisable in the physiological pH range;
1457 BCS class II; log P of 2) [243]. The model was developed to simulate administration of different
1458 formulations in the separate age groups: administration of tablets children/adolescents, suspension
1459 prepared from crushed tablets administered to newborns and infants, and administration of oral
1460 solution, suspension and Tegretol® tablets to adults. After the development of the adult model for oral
1461 administration of different formulations, doses and food status, adjustment of clearance (to take into
1462 account patient characteristics and co-medication), the model was scaled to paediatric patients using
1463 the default parameters of Gastroplus® (version 9.0) paediatric physiology adjusted module. *In vitro*
1464 experiments were conducted to investigate biorelevant solubility and dissolution (μ DISS Profiler®) in
1465 adult and paediatric biorelevant media developed by Maharaj *et al.* [109]. The dissolution experimental
1466 setups for adults and paediatrics were performed with Tegretol® tablets (or weighted fraction) added
1467 to 20 mL of the pre-heated dissolution medium (37° C). Samples were stirred at 100 rpm and the
1468 amount of dissolved drug was determined over 2 h. Dissolution experiments did not show any specific
1469 influence on carbamazepine dissolution, more than 80% dissolved in 20 min for almost all tested

1470 media, and for all tested media in 30 min. Despite this, neither dissolution experiments, nor solubility
1471 in paediatric biorelevant media were used as parameters for building the models. Simulated dissolution
1472 and f_a profiles were compared, and as expected for a BCS class II compound, permeation was not
1473 found to be a rate-limiting step for absorption. Nevertheless, aqueous solubility and solubility in adult
1474 fasted and fed intestinal simulated fluids were used in the model building process. Interestingly, PSA
1475 revealed that solubility and dose were the most sensitive parameters for carbamazepine f_a . Particle
1476 radius, SITT, fraction of small intestinal fluid volume, SI length and radius, permeability and bile salt
1477 solubilisation ratio, showed an impact at higher doses of carbamazepine, but only a minor impact at
1478 low doses. The prandial state was also shown to be critical for absorption of higher doses, where
1479 increases in the extent of absorption were observed for simulations in the fed state. With the exception
1480 of one study in paediatrics, the pharmacokinetic data used for the validation of the simulations did not
1481 specify food status of the patients. Nevertheless, both fasted and fed states were investigated.
1482 Interestingly, accuracy of the simulations in newborns was improved when assuming fed state
1483 conditions when compared to fasted state simulations, which supports the common assumption that
1484 newborns and young infants are mainly in fed state due to the high frequency of feedings. Fraction
1485 absorbed of carbamazepine was shown to be dose-dependent, at high doses f_a was sensitive to intestinal
1486 length and transit time, while simulations for lower doses of carbamazepine resulted in complete
1487 absorption, for a wide range of simulated intestinal lengths, and transit times [243]. The authors
1488 highlighted that this dose-dependency of carbamazepine is an important factor to take into account, as
1489 paediatric patients can sometimes require higher doses per BW. Finally, it was shown that age could
1490 influence both rate and extent of oral absorption. Low carbamazepine doses (children dose 9 mg/kg
1491 and newborns 5 mg/kg) was associated with complete absorption within 4 to 6 h after drug
1492 administration, in all age-groups, however a slower rate of absorption was seen for newborns in
1493 comparison with the older age-groups, moreover, high carbamazepine doses (19 and 17 mg/kg
1494 respectively) were related to incomplete absorption in children and newborns [243].

1495

1496 The examples provided above (excluding Johnson *et al.*, 2018) demonstrate the general approach
1497 followed when building the PBPK oral absorption models, as previously discussed in the Section 7.2.2.
1498 In all of the examples, knowledge gaps concerning physiological and anatomical changes in
1499 paediatrics, relevant to oral drug absorption, were pointed out as limiting factors of the models
1500 predictions. Furthermore, in most examples, several details concerning study design and formulation
1501 were lacking. The *in vitro* dissolution of the compounds was evaluated in three out of the eight
1502 examples, with two of these compounds being highly soluble ones. Moreover the dissolution data
1503 were not incorporated (as an input parameter) in the PBPK models, since no discrepancies in
1504 dissolution-adjusted conditions for paediatrics were observed for the compounds/formulations
1505 investigated so far. In future studies, it would be interesting to investigate the absorption of other
1506 classes of BCS compounds, especially poorly soluble (BCS II and IV). The prandial state in paediatric
1507 simulations has been explored in one of the examples, in most cases no information was provided for
1508 the simulations performed, which might be a result of lack of quality in clinical data for paediatrics
1509 that is used for validation of the predictions. Furthermore, the paediatric data sets used for the
1510 validation of the PBPK models, applied a sub-division of the paediatric population according to the
1511 common sub-groups. The majority of the examples were able to generate appropriate predictions for
1512 older paediatric populations (*i.e.* children) while simulations in newborns and infants were more
1513 challenging. There is still a long way to go in terms of paediatric PBPK absorption modeling, the
1514 examples of the models developed so far, are useful to generate knowledge about oral drug absorption
1515 modeling.

1516

1517 **7.2.4. Challenges in the paediatric oral drug absorption model**

1518 The determination of organ/tissue sizes (*e.g.* volume), tissue blood flow and tissue composition
1519 estimations introduce a model uncertainty. Typically, due to lack of clinical data, relevant parameters,

1520 *e.g.* length and diameter of GI tract, are extrapolated from adult data, based on BSA function for the
1521 paediatric populations and assume a proportional growth of the organs [125; 242]. The determination
1522 of GE rates and luminal composition (including the pH) in newborns and infants is challenging, due
1523 to frequent meal administration, therefore, food-related physiological responses in paediatrics is
1524 difficult to define [236]. Although biorelevant media for newborns and infants have recently been
1525 proposed [109], drug solubility estimations under conditions reflecting the luminal composition are
1526 challenging due to the limited information in the various paediatric populations and the unclear fasted
1527 vs. fed state, especially in newborns and infants. Intestinal permeability in paediatrics has been the
1528 subject of a number of studies, nevertheless, no precise values or methods have been reported; therefore
1529 the intestinal permeability for paediatric virtual populations is usually adjusted from the permeability
1530 parameter for adults (Caco-2 permeability or *in situ* permeability studies) [137; 169]. In the case of
1531 transporter involvement in the uptake or excretion of the drug, in addition to the parameters used for
1532 the adult model, the transporter availability and functionality in the paediatrics need to be confirmed
1533 and adjusted accordingly. Alternative influx and efflux routes only relevant in paediatrics populations
1534 and their contribution to the absorption process should be further investigated for the age range of
1535 interest, as shown in the process of building a PBPK model for valganciclovir, a substrate of the
1536 transporter PEPT1 [239]. In addition to the accuracy of the parameters used to describe paediatric
1537 physiology, a reasonable parameter variability value needs to be introduced in order to ensure that the
1538 generated predictions would match real-life heterogeneity among the paediatric population [227]. This
1539 can be challenging due to the nature of available paediatric data. For some of the presented examples
1540 of paediatric models in Section 7.2.3., possible formulation influence on the absorption processes was
1541 taken into consideration, although solubility and dissolution tests were not always performed, thus
1542 outlining further aspects that should be the subject of future evaluation. The established model requires
1543 validation towards clinical data acquired in the target population. Due to the lack of published high-
1544 quality clinical data in specific paediatric populations, confirmation of the developed paediatric PBPK

1545 models has not always been possible. Finally, great importance has been assigned to the comparison
1546 of the model-predicted outcomes to clinical paediatric *in vivo* data by the EMA in the “Guidelines on
1547 the qualification and reporting of PBPK modeling and simulation” and a “Reflection paper on the use
1548 of extrapolation in the development of medicines for paediatrics” [216; 229].

1549

1550 **8. Conclusions**

1551 Despite ongoing advances in the paediatric biopharmaceutics field, detailed knowledge on
1552 physiological differences among paediatric subpopulations and between adults is still lacking. While
1553 there have been many study outcomes reported on physiological parameters such as gastric fasted pH
1554 levels, GE times, and hepatic drug metabolism, other areas, such as GI fluid composition and SITT,
1555 intestinal metabolism, drug transporters and permeability, have been investigated to a very limited
1556 extent. Inconsistencies amongst meal types and frequencies throughout paediatric studies result in a
1557 complex definition of the paediatric prandial state, which further complicates the prediction of drug
1558 and formulation performance. Specific guidance by regulatory agencies on bioequivalence studies and
1559 age-specific definitions of fasted and fed state conditions for paediatrics is lacking, which make the
1560 development of solid evidence-based pBCS criteria quite challenging. Common background
1561 knowledge is needed for the development and validation of age-specific *in vitro* and *in silico*
1562 biopharmaceutics tools. A combination of both methods, *in vitro*/PBPK, can be utilised to obtain
1563 information that is able to compensate for the uncertainties of the single tool on its own.

1564

1565 **Acknowledgement**

1566 This work has received funding from Horizon 2020 Marie Skłodowska-Curie Innovative Training
1567 Networks programme under grant agreement No. 674909

1568

References

- 1570 1. Preis M, Breitreutz J. Pediatric Drug Development and Dosage Form Design. *AAPS PharmSciTech* 2017;
1571 18(2): 239-40.
- 1572 2. European Medicines Agency (EMA). 10-year Report to the European Commission: General report on the
1573 experience acquired as a result of the application of the Paediatric Regulation. *EMA/231225/2015*.
1574 2017.
- 1575 3. Salunke S et al. European Paediatric Formulation Initiative (EuPFI) — Formulating Ideas for Better
1576 Medicines for Children. *AAPS PharmSciTech* 2017; 18(2): 257-62.
- 1577 4. Barrett JS et al. Physiologically based pharmacokinetic (PBPK) modeling in children. *Clin Pharmacol Ther*
1578 2012; 92(1): 40-9.
- 1579 5. Food and Drug Administration (FDA). General Clinical Pharmacology Considerations for Pediatric Studies
1580 for Drugs and Biological Products Guidance for Industry (draft guidance). 2014.
- 1581 6. World Health Organization (WHO). Position Paper: Paediatric Age Categories to be Used in Differentiating
1582 Between Listing on a Model Essential Medicines List for Children. World Health Organization
1583 (WHO), 2007.
- 1584 7. Maharaj AR, Edginton AN. Physiologically Based Pharmacokinetic Modeling and Simulation in Pediatric
1585 Drug Development. *CPT Pharmacometrics Syst Pharmacol* 2014; 3(11):1-13.
- 1586 8. Centers for Disease Control and Prevention (CDC). CDC Growth Charts. Atlanta, GA: Centers for Disease
1587 Control and Prevention, Department of Health and Human Services, 2000.
- 1588 9. Verbraecken J et al. Body surface area in normal-weight, overweight, and obese adults. A comparison
1589 study. *Metabolism* 2006; 55(4): 515-24.
- 1590 10. European Medicines Agency (EMA). Reflection Paper: Formulations of choice for the paediatric
1591 population *EMEA/CHMP/PEG/194810/2005*. 2006.
- 1592 11. European Medicines Agency (EMA). ICH Topic E 11 Clinical Investigation of Medicinal Products in the
1593 Paediatric Population. *PMP/ICH/2711/99*. 2001.
- 1594 12. DiMaggio DM et al. Updates in Infant Nutrition. *Pediatrics in review* 2017; 38(10): 449-62.
- 1595 13. Noimark L, Cox HE. Nutritional problems related to food allergy in childhood. *Pediatr Allergy Immunol*
1596 2008; 19(2): 188-95.
- 1597 14. Dewey KG, Brown KH. Update on technical issues concerning complementary feeding of young children
1598 in developing countries and implications for intervention programs. *Food Nutr Bull* 2003; 24(1): 5-28.
- 1599 15. Schwartz C et al. Development of healthy eating habits early in life. Review of recent evidence and
1600 selected guidelines. *Appetite* 2011; 57(3): 796-807.
- 1601 16. Gidding S et al. Dietary recommendations for children and adolescents: a guide for practitioners.
1602 *Pediatrics* 2006; 117(2): 544-559.
- 1603 17. Dewey K. Guiding principles for complementary feeding of the breastfed child. PAN American Health
1604 organization, World Health Organization. Washington D.C. 2002.
- 1605 18. European Food Safety Authority (EFSA). Scientific Opinion on nutrient requirements and dietary intakes
1606 of infants and young children in the European Union. *EFSA J* 2013; 11(10): 3408-511.
- 1607 19. Butte N et al. The Start Healthy Feeding Guidelines for Infants and Toddlers. *J Am Diet Assoc*. 2004;
1608 04(3): 442-54.
- 1609 20. Harrison M et al. A qualitative systematic review of maternal infant feeding practices in transitioning from
1610 milk feeds to family foods. *Matern Child Nutr* 2016; 13(2): e12360
- 1611 21. Schiess S et al. Introduction of complementary feeding in 5 European countries. *J Pediatr Gastroenterol*
1612 *Nutr* 2010; 50(1): 92-8
- 1613 22. Anderson AS et al. Rattling the plate-reasons and rationales for early weaning. *Health Educ Res* 2001;
1614 16(4): 471-9.
- 1615 23. Heinig MJ et al. Barriers to compliance with infant-feeding recommendations among low-income women.
1616 *J Hum Lact* 2006; 1: 27-38.

- 1617 24. Horodyski M et al. Low-income mothers' decisions regarding when and why to introduce solid foods to
1618 their infants: influencing factors. *J Community Health Nurs* 2007; 24(2): 101-18.
- 1619 25. Butte NF. Energy requirements of infants. *Public Health Nutr* 2005; 8(7A): 935-67.
- 1620 26. European Food Safety Authority (EFSA). Scientific Opinion on Dietary Reference Values for energy.
1621 *EFSA J* 2013; 11(1): 3005-115.
- 1622 27. Henry C. Basal metabolic rate studies in humans: measurement and development of new equations. *Public*
1623 *Health Nutr* 2005; 8(7a): 1133-52.
- 1624 28. U.S. Department of Agriculture, U.S. Department of Health and Human Services. Dietary Guidelines for
1625 Americans. Washington, DC: U.S. Government Writing Office, 2010.
- 1626 29. U.S. Department of Agriculture. Infant Nutrition and Feeding: A guide for use in the WIC and CSF
1627 programs. 2009: 51-96.
- 1628 30. World Health Organization (WHO). Management of the child with a serious infection or severe
1629 malnutrition : guidelines for care at the first-referral level in developing countries.
1630 *WHO/FCH/CAH/001*. Geneva, Switzerland: WHO, 2000.
- 1631 31. World Health Organization (WHO). Caring for newborns and children in the community: Caring for the
1632 newborn at home. Geneva, Switzerland: WHO, 2015.
- 1633 32. Dewey KG et al. Risk factors for suboptimal infant breastfeeding behavior, delayed onset of lactation, and
1634 excess neonatal weight loss. *Pediatrics* 2003; 112(3 Pt 1): 607-19.
- 1635 33. Bergman NJ. Neonatal stomach volume and physiology suggest feeding at 1-h intervals. *Acta paediatrica*
1636 2013; 102(8): 773-7.
- 1637 34. European Food Safety Authority (EFSA). Opinion on complementary feeding of infants. *EFSA J* 2009;
1638 7(12): 1423-61.
- 1639 35. Johnson TN et al. Development and application of a physiologically-based model of paediatric oral drug
1640 absorption. *Eur J Pharm Sci*. 2018; 115:57-67.
- 1641 36. European Food Safety Authority (EFSA). Scientific Opinion on Dietary Reference Values for water.
1642 *EFSA Journal* 2010; 3: 1459-1461.
- 1643 37. World Health Organization (WHO). Guiding principles for feeding non-breastfed children 6-24 months of
1644 age. Geneva, Switzerland: WHO, 2005.
- 1645 38. Cattaneo A et al. Infant and young child feeding: standard recommendations for the European Union In:
1646 European Network for Public Health Nutrition: Networking, M., Training, I. a. eds., 2006: 540-6.
- 1647 39. Sachdev HP et al. Water supplementation in exclusively breastfed infants during summer in the tropics.
1648 *Lancet* 1991; 337(8747): 929-33.
- 1649 40. Heyman M, Abrams S. Fruit Juice in Infants, Children, and Adolescents: Current Recommendations.
1650 *Pediatrics* 2017. 139(6): e20170967.
- 1651 41. Abrams SA, Daniels SR. Fruit Juice and Child Health. *Pediatrics* 2017; 139(4): e20170041.
- 1652 42. Bellisle F et al. A study of fluid intake from beverages in a sample of healthy French children, adolescents
1653 and adults. *Eur J Clin Nutr* 2010; 64(4): 350-55.
- 1654 43. Meyers RS. Pediatric Fluid and Electrolyte Therapy. *J Pediatr Pharmacol Ther* 2009; 14(4): 204-11.
- 1655 44. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and
1656 term breast milk. *BMC pediatrics* 2014;14(1): 216.
- 1657 45. European Food Safety Authority (EFSA). Scientific Opinion on the essential composition of infant and
1658 follow-on formulae. *EFSA Journal* 2014; 7: 3760-864.
- 1659 46. Keikha M et al. Macro- and Micronutrients of Human Milk Composition: Are They Related to Maternal
1660 Diet? A Comprehensive Systematic Review. *Breastfeed Med* 2017; 12(9): 517-27.
- 1661 47. Michaelsen KF et al. Variation in macronutrients in human bank milk: influencing factors and
1662 implications for human milk banking. *J Pediatr Gastroenterol Nutr* 1990; 11(2): 229-39.
- 1663 48. Kamstrup D et al. In Vitro Model Simulating Gastro-Intestinal Digestion in the Pediatric Population
1664 (Neonates and Young Infants). *AAPS Pharm Sci Tech* 2017; 18(2): 317-329.

- 1665 49. Staelens S et al. Gastric emptying in healthy newborns fed an intact protein formula, a partially and an
1666 extensively hydrolysed formula. *Clin Nutr* 2008; 27(2): 264-8.
- 1667 50. Michaelsen K. Feeding and nutrition of infants and young children: guidelines for the WHO European
1668 region, with emphasis on the former Soviet countries. WHO Regional Publications, European Series
1669 No. 87. 2000.
- 1670 51. European Parliament. Regulation (EU) No 609/2013 of the European Parliament and of the Council on
1671 Food Intended for Infants and Young Children, Food for Special Medical Purposes, and Total Diet
1672 Replacement for Weight Control. Official Journal of the European Union 2012, 2012: 35-56.
- 1673 52. European Medicines Agency (EMA). Guideline on the investigation of drug interactions.
1674 *EMA/CHMP/458101/2016*, 2012
- 1675 53. Food and Drug Administration (FDA). Guidance for Industry Food-Effect Bioavailability and Fed
1676 Bioequivalence Studies. 2002.
- 1677 54. U.S. Department of Health and Human Services, National Institute of Health. *Keep the Beat? Recipes:
1678 Deliciously Healthy Family Meals*, NIH Publication No. 10-7531, 2010.
- 1679 55. Kersten E et al. Physicochemical characterisation of fluids and soft foods frequently mixed with oral drug
1680 formulations prior to administration to children. *Die Pharmazie* 2016; 71(3): 122-7.
- 1681 56. Martir J et al. Characterisation of the physicochemical properties of food and drinks used for the co-
1682 administration of drugs in the paediatric populations. *APPS Published abstracts*. Available from
1683 <http://abstracts.aaps.org/published/>, AAPS annual meeting, San Diego, 2017.
- 1684 57. Neville MC, Jensen RG. E - The Physical Properties of Human and Bovine Milks. *Handbook of Milk* 57.
1685 Neville MC, Jensen RG. E - The Physical Properties of Human and Bovine Milks. *Handbook of Milk*
1686 *Composition*. San Diego: Academic Press, 1995: 81-85.
- 1687 58. Klein S et al. Media to simulate the postprandial stomach I. Matching the physicochemical characteristics
1688 of standard breakfasts. *J Pharm Pharmacol* 2004; 56(5): 605-10.
- 1689 59. Manrique YJ et al. Crushed tablets: does the administration of food vehicles and thickened fluids to aid
1690 medication swallowing alter drug release? *J Pharm Pharm Sci* 2014; 17(2): 207-19.
- 1691 60. Jong GT. Pediatric Development: Physiology, Enzymes, Drug Metabolism, Pharmacokinetics and
1692 Pharmacodynamics. In: Bar-Shalom, D., Rose, K. eds. *Pediatric Formulations: A Roadmap*. New
1693 York, NY: Springer New York, 2014: 9-23.
- 1694 61. Lu H, Rosenbaum S. Developmental Pharmacokinetics in Pediatric Populations. *J Pediatr Pharmacol*
1695 *Ther* 2014; 19(4): 262-76.
- 1696 62. Ku LC, Smith PB. Dosing in neonates: special considerations in physiology and trial design. *Pediatr Res*
1697 2015; 77(0): 2-9.
- 1698 63. Nicolas JM et al. Oral drug absorption in pediatrics: the intestinal wall, its developmental changes and
1699 current tools for predictions. *Biopharm Drug Dispos* 2016; 38(3): 209-30.
- 1700 64. Batchelor HK. Paediatric Development: Gastrointestinal. In: Bar-Shalom, D., Rose, K. eds. *Pediatric*
1701 *Formulations: A Roadmap*. New York, NY: Springer New York, 2014: 43-54.
- 1702 65. Crawford M et al. Effects of duration of fasting on gastric fluid pH and volume in healthy children. *Anesth*
1703 *Analg* 1990; 71(4): 400-3.
- 1704 66. Maekawa N et al. Effects of 2-, 4- and 12-hour fasting intervals on preoperative gastric fluid pH and
1705 volume, and plasma glucose and lipid homeostasis in children. *Acta Anaesthesiol Scand* 1993; 37(8):
1706 783-7.
- 1707 67. Schwartz DA et al. Gastric contents in children presenting for upper endoscopy. *Anesth Analg* 1998;
1708 87(4): 757-60.
- 1709 68. Nicolson SC et al. Shortened preanesthetic fasting interval in pediatric cardiac surgical patients. *Anesth*
1710 *Analg* 1992; 74(5): 694-697.
- 1711 69. Manchikanti L et al. Assessment of age-related acid aspiration risk factors in pediatric, adult, and geriatric
1712 patients. *Anesth Analg* 1985; 64(1): 11-17.

- 1713 70. Meakin G et al. Effects of fasting and oral premedication on the pH and volume of gastric aspirate in
1714 children. *Br J Anaesth* 1987; 59(6): 678-82.
- 1715 71. Sandhar BK et al. Effect of oral liquids and ranitidine on gastric fluid volume and pH in children
1716 undergoing outpatient surgery. *Anesthesiology* 1989; 71(3): 327-330.
- 1717 72. Schmidt AR et al. Gastric pH and residual volume after 1 and 2 h fasting time for clear fluids in
1718 children. *Br J Anaesth* 2015; 114(3): 477-82.
- 1719 73. Schreiner MS et al. Ingestion of liquids compared with preoperative fasting in pediatric outpatients.
1720 *Anesthesiology* 1990; 72(4): 593-7.
- 1721 74. Splinter WM et al. Clear fluids three hours before surgery do not affect the gastric fluid contents of
1722 children. *Can J Anaesth* 1990; 37(5): 498-501.
- 1723 75. Splinter WM et al. The effect of preoperative apple juice on gastric contents, thirst, and hunger in children.
1724 *Can J Anaesth* 1989; 36(1): 55-8.
- 1725 76. Splinter WM et al. Large volumes of apple juice preoperatively do not affect gastric pH and volume in
1726 children. *Can J Anaesth* 1990; 37(1): 36-9.
- 1727 77. Splinter W, Schaefer J. Ingestion of clear fluids is safe for adolescents up to 3 h before anaesthesia. *Br J*
1728 *Anaesth* 1991; 66(1): 48-52.
- 1729 78. Kaye JL. Review of paediatric gastrointestinal physiology data relevant to oral drug delivery. *Int J Clin*
1730 *Pharm* 2011; 33(1): 20-4
- 1731 79. Roman C et al. Quantitative and qualitative study of gastric lipolysis in premature infants: do MCT-
1732 enriched infant formulas improve fat digestion? *Pediatr Res* 2007; 61(1): 83-8.
- 1733 80. Cavell B. Postprandial gastric acid secretion in infants. *Acta Paediatr Scand* 1983; 72(6): 857-60.
- 1734 81. Siegel M et al. Gastric emptying in prematures of isocaloric feedings with differing osmolalities. *Pediatr*
1735 *Res* 1982; 16(2): 141-7.
- 1736 82. Armand M et al. Effect of Human Milk or Formula on Gastric Function and Fat Digestion in the
1737 Premature Infant. *Pediatr Res* 1996; 40(3): 429-37.
- 1738 83. Batchelor HK et al. Application of in vitro biopharmaceutical methods in development of immediate
1739 release oral dosage forms intended for paediatric patients. *Eur J Pharm Sci* 2013; 85(3), Part B: 833-
1740 42.
- 1741 84. Edginton AN, Fotaki N. Oral drug absorption in pediatric populations. In: Informa Healthcare USA, I. ed.
1742 *Oral Drug Absorption: Prediction and Assessment (Dressman JB and Reppas C)*. New York, 2010:
1743 108-26.
- 1744 85. Yu G et al. Similarities and Differences in Gastrointestinal Physiology Between Neonates and Adults: a
1745 Physiologically Based Pharmacokinetic Modeling Perspective. *AAPS J* 2014; 16(6): 1162-66.
- 1746 86. De Zwart LL et al. Pharmacokinetics of Ingested Xenobiotics in Children: a Comparison with Adults.
1747 RIVM Report 623860011, 2002
- 1748 87. Kelly EJ et al. The effect of intravenous ranitidine on the intragastric pH of preterm infants receiving
1749 dexamethasone. *Arch Dis Child* 1993; 69(1 Spec No): 37-9.
- 1750 88. Kelly EJ et al. Gastric acid secretion in preterm infants. *Early human development* 1993; 35(3): 215-20.
- 1751 89. Omari TI, Davidson GP. Multipoint measurement of intragastric pH in healthy preterm infants. *Arch Dis*
1752 *Child Fetal Neonatal Ed* 2003; 88(6): F517-20.
- 1753 90. Miller B et al. Gastric residual volume in infants and children following a 3-hour fast. *J Clin Anesth* 1990;
1754 2(5): 301-305.
- 1755 91. Wolman IJ. Gastric phase of milk digestion in childhood: A study of the fasting secretions and of the
1756 physiologic responses to "hard curd" (pasteurized) and "soft curd" (homogenized) milks. *Am J Dis*
1757 *Child* 1946; 71(4): 394-422.
- 1758 92. Gharpure V et al. Indicators of postpyloric feeding tube placement in children. *Crit Care Med* 2000; 28(8):
1759 2962-6.

- 1760 93. Metheny NA et al. Clinical Research: Indicators of Feeding-Tube Placement in Neonates. *Nutr Clin Pract*
1761 1999; 14(6):307-14.
- 1762 94. Fallingborg J et al. Measurement of Gastrointestinal pH and Regional Transit Times in Normal Children. *J*
1763 *Pediatr Gastroenterol Nutr* 1990; 11(2): 211-4.
- 1764 95. Westhus N. Methods to test feeding tube placement in children. *MCN Am J Matern Child Nurs* 2004;
1765 29(5): 282-7.
- 1766 96. Di Maio S, Carrier RL. Gastrointestinal contents in fasted state and post-lipid ingestion: in vivo
1767 measurements and in vitro models for studying oral drug delivery. *J Control Release* 2011; 151(2):
1768 110-22.
- 1769 97. Kalantzi L et al. Characterization of the human upper gastrointestinal contents under conditions simulating
1770 bioavailability/bioequivalence studies. *Pharm Res* 2006; 23(1): 165-76.
- 1771 98. Dressman JB et al. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm Res* 7(7):
1772 756-61.
- 1773 99. Sondheimer JM et al. Continuous gastric pH measurement in young and older healthy preterm infants
1774 receiving formula and clear liquid feedings. *J Pediatr Gastroenterol Nutr* 1985; 4(3): 352-5.
- 1775 100. Litman RS et al. Gastric volume and pH in infants fed clear liquids and breast milk prior to surgery.
1776 *Anesth Analg* 1994; 79(3): 482-5.
- 1777 101. Fuchs A, Dressman JB. Composition and physicochemical properties of fasted-state human duodenal and
1778 jejunal fluid: a critical evaluation of the available data. *J Pharm Sci* 2014; 103(11): 3398-411.
- 1779 102. Boehm G et al. Postnatal adaptation of lipase- and trypsin-activities in duodenal juice of premature
1780 infants appropriate for gestational age. *Biomed Biochimic Acta* 1990; 49(5): 369-73.
- 1781 103. Fredrikzon B, Olivecrona T. Decrease of lipase and esterase activities in intestinal contents of newborn
1782 infants during test meals. *Pediatr Res* 1978; 12(5): 631-4.
- 1783 104. Rune SJ, Viskum K. Duodenal pH values in normal controls and in patients with duodenal ulcer. *Gut*
1784 1969; 10(7): 569-71.
- 1785 105. Robinson PJ et al. Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci*
1786 1990; 35(10): 1299-304.
- 1787 106. Mooij MG et al. Ontogeny of oral drug absorption processes in children. *Expert Opin Drug Metab*
1788 *Toxicol* 2012; 8(10): 1293-303.
- 1789 107. Mason S. Some Aspects of Gastric Function in the Newborn. *Arch Dis Child* 1962; 37(194): 387-391.
- 1790 108. De Koning BAE et al. Developmental Changes in the Processes Governing Oral Drug Absorption. In:
1791 Bar-Shalom, D., Rose, K. eds. *Pediatric Formulations: A Roadmap*. New York, NY: Springer New
1792 York, 2014: 25-42.
- 1793 109. Maharaj AR et al. Assessment of Age-Related Changes in Pediatric Gastrointestinal Solubility. *Pharm*
1794 *Res* 2016; 33(1): 52-71.
- 1795 110. Barbero GJ et al. Investigations on the bacterial flora, pH, and sugar content in the intestinal tract of
1796 infants. *J Pediatr* 1952; 40(2): 152-63.
- 1797 111. Abrahamse E et al. Development of the Digestive System— Experimental Challenges and Approaches of
1798 Infant Lipid Digestion. *Food Dig* 2012; 3(1-3): 63-77.
- 1799 112. Bourlieu C et al. Specificity of infant digestive conditions: some clues for developing relevant in vitro
1800 models. *Crit Rev Food Sci Nutr* 2014; 54(11): 1427-57.
- 1801 113. Jarvenpaa AL et al. Feeding the low-birth-weight infant. III. Diet influences bile acid metabolism.
1802 *Pediatrics* 1983; 72(5): 677-83.
- 1803 114. Signer E et al. Role of bile salts in fat malabsorption of premature infants. *Arch Dis Child* 1974; 49(3):
1804 174-80.
- 1805 115. Harries JT et al. Intestinal bile salts in cystic fibrosis: studies in the patient and experimental animal.
1806 *Arch Dis Child* 1979; 54(1): 19-24.
- 1807 116. Glasgow JF et al. A comprehensive study of duodenal bile salts in newborn infants and their relationship
1808 to fat absorption. *Ir J Med Sci* 1980; 149(9):346-56.

- 1809 117. Billeaud C et al. Gastric emptying in infants with or without gastro-oesophageal reflux according to the
1810 type of milk. *Eur J Clin Nutr* 1990; 44(8): 577-83.
- 1811 118. Tharimontrichai A, Janjindamai W. Postprandial osmolality of gastric contents in very low-birth-weight
1812 infants fed expressed breast milk with additives. *Southeast Asian J Trop Med Public Health* 2009;
1813 40(5): 1080-6.
- 1814 119. Clarysse S et al. Postprandial evolution in composition and characteristics of human duodenal fluids in
1815 different nutritional states. *J Pharm Sci* 2009; 98(3): 1177-92.
- 1816 120. Bowles A et al. Specific aspects of gastro-intestinal transit in children for drug delivery design. *Int J*
1817 *Pharm* 2010 16;395(1-2):37-43.
- 1818 121. Bonner JJ et al. Does age affect gastric emptying time? A model-based meta-analysis of data from
1819 premature neonates through to adults. *Biopharm Drug Dispos* 2015; 36(4): 245-57
- 1820 122. Mudie DM et al. Physiological Parameters for Oral Delivery and In vitro Testing. *Mol Pharm*
1821 2010; 7(5): 1388-405.
- 1822 123. Lange A et al. Gastric emptying patterns of a liquid meal in newborn infants measured by epigastric
1823 impedance. *Neurogastroenterol Motil* 1997; 9(2): 55-62.
- 1824 124. Hauser B et al. Gastric Emptying of Liquids in Children. *J Pediatr Gastroenterol Nutr* 2016; 62(3): 403-
1825 8.
- 1826 125. Cristofolletti R et al. Exploratory Investigation of the Limiting Steps of Oral Absorption of Fluconazole
1827 and Ketoconazole in Children Using an In Silico Pediatric Absorption Model. *J Pharm Sci* 2016;
1828 105(9): 2794-803.
- 1829 126. Van Den Driessche M, Veereman-Wauters G. Gastric emptying in infants and children. *Acta*
1830 *Gastroenterol Belg* 2003; 66(4): 274-82.
- 1831 127. Cavell B. Gastric emptying in infants fed human milk or infant formula. *Acta Paediatr Scand* 1981;
1832 70(5):639-41.
- 1833 128. Ewer AK et al. Gastric emptying in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* 1994; 71(1):F24-
1834 7.
- 1835 129. Meyer R et al. Systematic review of the impact of feed protein type and degree of hydrolysis on gastric
1836 emptying in children. *BMC gastroenterology* 2015; 137.
- 1837 130. Hauser B et al. Gastric emptying of solids in children: reference values for the (13) C-octanoic acid
1838 breath test. *Neurogastroenterol Motil* 2016; 28(10): 1480-7.
- 1839 131. Dressman JB et al. Dissolution testing as a prognostic tool for oral drug absorption: immediate release
1840 dosage forms. *Pharm Res* 1998; 15(1): 11-22.
- 1841 132. Malik R et al. Assessment of gastric emptying in children: Establishment of control values utilizing a
1842 standardized vegetarian meal. *J Gastroenterol Hepatol* 2016; 31(2): 319-25.
- 1843 133. Hardoff R et al. Gastric emptying time and gastric motility in patients with Parkinson's disease. *Mov*
1844 *Disord* 2001; 16(6): 1041-7
- 1845 134. Maharaj AR, Edginton AN. Examining Small Intestinal Transit Time as a Function of Age: Is There
1846 Evidence to Support Age-Dependent Differences among Children? *Drug Metab Dispos* 2016; 44(7):
1847 1080-9.
- 1848 135. The International Commission on Radiological Protection (ICRP). Basic anatomical and physiological
1849 data for use in radiological protection: reference values: ICRP Publication 89. *Annals of the ICRP*
1850 2002; 32(3-4): 1-277.
- 1851 136. Weaver L et al. Small intestinal length: a factor essential for gut adaptation. *Gut* 1991; 32(11): 1321-23.
- 1852 137. Batchelor HK, Marriott JF. Paediatric pharmacokinetics: key considerations. *Br J Clin Pharmacol* 2015;
1853 79(3): 395-404.
- 1854 138. Bai JPF et al. Literature Review of Gastrointestinal Physiology in the Elderly, in Pediatric Patients, and
1855 in Patients with Gastrointestinal Diseases. *J Pharm Sci* 2016; 105(2): 476-83.
- 1856 139. Cummins AG et al. Crypt fission peaks early during infancy and crypt hyperplasia broadly peaks during
1857 infancy and childhood in the small intestine of humans. *J Pediatr Gastroenterol Nutr* 2008; 47(2):
1858 153-7.

- 1859 140. Cummins AG, Thompson FM. Effect of breast milk and weaning on epithelial growth of the small
1860 intestine in humans. *Gut* 2002; 51(5): 748-541.
- 1861 141. Penna FJ et al. Jejunal mucosal morphometry in children with and without gut symptoms and in normal
1862 adults. *J Clin Pathol* 1981; 34(4): 386-92.
- 1863 142. Batchelor HK et al. Paediatric oral biopharmaceutics: key considerations and current challenges. *Adv*
1864 *Drug Deliv Rev* 2014;73: 102-26.
- 1865 143. Riezzo G et al. Maturation of gastric electrical activity, gastric emptying and intestinal permeability in
1866 preterm newborns during the first month of life. *Ital J Pediatr* 2009; 35(1)-6.
- 1867 144. Van Elburg RM et al. Intestinal permeability in relation to birth weight and gestational and postnatal age.
1868 *Arch Dis Child Fetal Neonatal Ed* 2003; 88(1): F52-5.
- 1869 145. Kerr CA et al. Early life events influence whole-of-life metabolic health via gut microflora and gut
1870 permeability. *Crit Rev Microbiol* 2015; 41(3): 326-40.
- 1871 146. Batchelor HK. Paediatric biopharmaceutics classification system: Current status and future decisions. *Int*
1872 *J Pharm* 2014; 469(2): 251-3.
- 1873 147. Batchelor HK et al. Towards the development of a paediatric biopharmaceutics classification system:
1874 Results of a survey of experts. *Int J Pharm* 2016; 469(2): 1151-7.
- 1875 148. Colome G et al. Intestinal permeability in different feedings in infancy. *Acta paediatrica* 2007; 96(1):
1876 69-72.
- 1877 149. Akram G, Mullen AB. Paediatric nurses' knowledge and practice of mixing medication into foodstuff. *Int*
1878 *J Pharm Pract* 2012; 20(3): 191-8.
- 1879 150. Stratiki Z et al. The effect of a bifidobacter supplemented bovine milk on intestinal permeability of
1880 preterm infants. *Early Hum Dev* 2007; 83(9): 575-9.
- 1881 151. Mooij MG et al. Human Intestinal PEPT1 Transporter Expression and Localization in Preterm and Term
1882 Infants. *Drug Metab Dispos* 2016; 44(7): 1014-9.
- 1883 152. Mooij MG et al. Ontogeny of human hepatic and intestinal transporter gene expression during childhood:
1884 age matters. *Drug Metab Dispos* 2014; 42(8): 1268-74.
- 1885 153. Prasad B et al. The Promises of Quantitative Proteomics in Precision Medicine. *J Pharm Sci* 2017;
1886 106(3): 738-44.
- 1887 154. Brouwer KL et al. Human Ontogeny of Drug Transporters: Review and Recommendations of the
1888 Pediatric Transporter Working Group. *Clin Pharmacol Ther* 2015; 98(3): 266-87.
- 1889 155. Elmorsi Y et al. Ontogeny of Hepatic Drug Transporters and Relevance to Drugs Used in Pediatrics.
1890 *Drug Metab Dispos* 2016; 44(7): 992-8.
- 1891 156. Abdel-Rahman SM et al. Summary of the National Institute of Child Health and Human Development–
1892 Best Pharmaceuticals for Children Act Pediatric Formulation Initiatives Workshop–Pediatric
1893 Biopharmaceutics Classification System Working Group. *Clin Ther* 2012; 34(11): S11-24.
- 1894 157. Quigley EMM. Microflora Modulation of Motility. *Neurogastroenterol Motil* 2011; 17(2): 140-7.
- 1895 158. Merchant HA et al. Age-mediated changes in the gastrointestinal tract. *Int J Pharm* 2016; 512(2): 382-95.
- 1896 159. Sadler NC et al. Hepatic Cytochrome P450 Activity, Abundance, and Expression Throughout Human
1897 Development. *Drug Metab Dispos* 2016; 44(7): 984-91.
- 1898 160. Amidon G et al. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro
1899 drug product dissolution and in vivo bioavailability. *Pharm Res* 1995; 3: 413-20.
- 1900 161. Lennernäs H, Abrahamsson B. The use of biopharmaceutic classification of drugs in drug discovery and
1901 development: current status and future extension. *J Pharm Pharmacol* 2005; 57(3): 273-85.
- 1902 162. Ku M. Use of the Biopharmaceutical Classification System in Early Drug Development. *AAPS J* 2008;
1903 10(1): 208-12.
- 1904 163. Shawahna R. Pediatric Biopharmaceutical Classification System: Using Age-Appropriate Initial Gastric
1905 Volume. *AAPS J* 2016; 18(3): 728-36.
- 1906 164. Gandhi SV et al. Considerations for a Pediatric Biopharmaceutics Classification System (BCS):
1907 Application to Five Drugs. *AAPS PharmSciTech* 2014; 15(3): 601-11.

- 1908 165. Mahmood I. Dosing in children: a critical review of the pharmacokinetic allometric scaling and
 1909 modelling approaches in paediatric drug development and clinical settings. *Clin Pharmacokinet* 2014;
 1910 53(4): 327-46.
- 1911 166. Hens B et al. Evaluation of real-life dosing of oral medicines with respect to fluid and food intake in a
 1912 Dutch-speaking population. *J Clin Pharm Ther* 2017; 42(4): 467-74.
- 1913 167. European Medicines Agency (EMA). Guideline on pharmaceutical development of medicines for
 1914 paediatric use. *EMA/CHMP/QWP/805880/2012, Rev 2*. 2013.
- 1915 168. Martir J et al. Recommended strategies for the oral administration of paediatric medicines with food and
 1916 drinks in the context of their biopharmaceutical properties: a review. *J Pharm Pharmacol* 2016; 69(4):
 1917 384-97
- 1918 169. Elder DP et al. Medicines for Pediatric Patients—Biopharmaceutical, Developmental, and Regulatory
 1919 Considerations. *J Pharm Sci* 2017; 106(4): 950-60.
- 1920 170. Boateng J. Drug Delivery Innovations to Address Global Health Challenges for Pediatric and Geriatric
 1921 Populations (Through Improvements in Patient Compliance). *J Pharm Sci* 2017; 106(11): 3188-98.
- 1922 171. Purohit V. Biopharmaceutic Planning in Pediatric Drug Development. *AAPS J* 2012; 14(3): 519-22.
- 1923 172. Quijano Ruiz B et al. Pediatric formulation issues identified in Paediatric Investigation Plans. *Expert Rev*
 1924 *Clin Pharmacol* 2014; 7(1): 25-30.
- 1925 173. Mistry P et al. Evidence of acceptability of oral paediatric medicines: a review. *J Pharm Pharmacol*
 1926 2017; 69(4): 361-76.
- 1927 174. European Medicines Agency (EMA). Guideline on the role of pharmacokinetics in the development of
 1928 medicinal products in the paediatric population. *EMEA/CHMP/EWP/147013/2004*, 2008
- 1929 175. Food and Drug Administration (FDA). Guidance for Industry General Considerations for Pediatric
 1930 Pharmacokinetic Studies for Drugs and Biological Products. 1998.
- 1931 176. Batchelor H. Influence of Food on Paediatric Gastrointestinal Drug Absorption Following Oral
 1932 Administration: A Review. *Children* 2015; 2(2): 244-71.
- 1933 177. Lancaster DL et al. 6-Thioguanine in children with acute lymphoblastic leukaemia: influence of food on
 1934 parent drug pharmacokinetics and 6-thioguanine nucleotide concentrations. *Br J Clin Pharmacol*
 1935 2001; 51(6): 531-9.
- 1936 178. Borrmann S et al. The effect of food consumption on lumefantrine bioavailability in African children
 1937 receiving artemether-lumefantrine crushed or dispersible tablets (Coartem) for acute uncomplicated
 1938 *Plasmodium falciparum* malaria. *Trop Med Int Health* 2010; 15(4): 434-41.
- 1939 179. Ginsburg CM et al. Effect of feeding on bioavailability of griseofulvin in children. *J Pediatr* 1983;
 1940 102(2): 309-11.
- 1941 180. De Guchtenaere A et al. Pharmacokinetic data on oral desmopressin reducing dosage by changing to a
 1942 new oral lyophilisate (melt) formulation. *J Urol* 2012; 187(4) Suppl: e301.
- 1943 181. Lonnerholm G et al. Oral mercaptopurine in childhood leukemia: influence of food intake on
 1944 bioavailability. *Pediatr Hematol Oncol* 1989; 6(2): 105-12.
- 1945 182. Mccracken GH, Jr. et al. Pharmacologic evaluation of orally administered antibiotics in infants and
 1946 children: effect of feeding on bioavailability. *Pediatrics* 1978; 62(5): 738-43.
- 1947 183. Riccardi R et al. Influence of food intake on bioavailability of oral 6-mercaptopurine in children with
 1948 acute lymphoblastic leukemia. *Pediatr Hematol Oncol* 1986; 3(4): 319-24.
- 1949 184. Sofianou-Katsoulis A et al. Reduction in bioavailability of 6-mercaptopurine on simultaneous
 1950 administration with cow's milk. *Pediatr Hematol Oncol* 2006; 23(6): 485-7.
- 1951 185. Stevens RC et al. Effect of food and pharmacokinetic variability on didanosine systemic exposure in
 1952 HIV-infected children. Pediatric AIDS Clinical Trials Group Protocol 144 Study Team. *AIDS Res*
 1953 *Hum Retroviruses* 2000; 16(5): 415-21.
- 1954 186. De Bruyne P et al. Pharmacokinetics of desmopressin administered as tablet and oral lyophilisate
 1955 formulation in children with monosymptomatic nocturnal enuresis. *Eur J Pediatr* 2014; 173(2): 223-8.

- 1956 187. Ginsburg CM et al. Comparative Pharmacokinetics of Amoxicillin and Ampicillin in Infants and
1957 Children. *Pediatrics* 1979; 64(5): 627-31.
- 1958 188. Fleisher D et al. Drug, meal and formulation interactions influencing drug absorption after oral
1959 administration. Clinical implications. *Clin Pharmacokinet* 1999; 36(3): 233-54.
- 1960 189. Jann MW et al. Interaction of dietary pudding with phenytoin. *Pediatrics* 1986; 78(5): 952-3.
- 1961 190. Notterman DA et al. Effect of dose formulation on isoniazid absorption in two young children. *Pediatrics*
1962 1986; 77(6): 850-2.
- 1963 191. Tuleu C, Breitzkreutz J. Educational paper: formulation-related issues in pediatric clinical pharmacology.
1964 *Eur J Pediatr* 2013; 172(6): 717-20.
- 1965 192. Knorr B et al. Pharmacokinetics and safety of montelukast in children aged 3 to 6 months. *J Clin*
1966 *Pharmacol* 2006; 46(6): 620-7.
- 1967 193. Andersson T et al. Pharmacokinetics of orally administered omeprazole in children. International
1968 Pediatric Omeprazole Pharmacokinetic Group. *Am J Gastroenterol* 2000; 95(11): 3101-6.
- 1969 194. Turner MA et al. Paediatric drug development: the impact of evolving regulations. *Adv Drug Deliv Rev*
1970 2014; 73(2-13).
- 1971 195. Dresser GK et al. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to
1972 decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* 2002; 71(1): 11-20.
- 1973 196. Batchelor HK et al. Food effects in paediatric medicines development for products Co-administered with
1974 food. *Int J Pharm* 2017; 536(2): 530-5.
- 1975 197. Strickley RG et al. Pediatric drugs-a review of commercially available oral formulations. *J Pharm Sci*
1976 2008; 97(5): 1731-74.
- 1977 198. European Commission. Medicinal Products for Paediatric use. *Regulation (EC) No 1901/2006*. Official
1978 Journal of the European Union, 2006.
- 1979 199. Toothaker RD, Welling PG. The effect of food on drug bioavailability. *Annu Rev Pharmacol Toxicol*
1980 1980; 20: 173-99.
- 1981 200. Kakuda TN et al. Pharmacokinetics of darunavir after administration of an oral suspension with low-dose
1982 ritonavir and with or without food. *Clin Pharmacol Drug Dev* 2014; 3(5): 346-52.
- 1983 201. Salem AH et al. A novel ritonavir paediatric powder formulation is bioequivalent to ritonavir oral
1984 solution with a similar food effect. *Antivir Ther* 2015; 20(4): 425-32.
- 1985 202. Stampfuss J et al. The effect of food on the absorption and pharmacokinetics of rivaroxaban. *Int J Clin*
1986 *Pharmacol Ther* 2013; 51(7): 549-61.
- 1987 203. Brouwers J et al. Parallel monitoring of plasma and intraluminal drug concentrations in man after oral
1988 administration of fosamprenavir in the fasted and fed state. *Pharm Res* 2007; 24(10): 1862-9.
- 1989 204. Dohil R, Rioux P. Pharmacokinetic Studies of Cysteamine Bitartrate Delayed-Release. *Clin Pharmacol*
1990 *Drug Dev* 2013; 2(2): 178-85.
- 1991 205. Mclean A et al. The influence of food on the bioavailability of a twice-daily controlled release
1992 carbamazepine formulation. *J Clin Pharmacol* 2001; 41(2): 183-6.
- 1993 206. Cassilly D et al. Gastric emptying of a non-digestible solid: assessment with simultaneous SmartPill pH
1994 and pressure capsule, antroduodenal manometry, gastric emptying scintigraphy. *Neurogastroenterol*
1995 *Motil* 2008; 20(4): 311-9.
- 1996 207. Karim A. Karim A. Effects of food on the bioavailability of theophylline from controlled-release
1997 products in adults. *J Allergy Clin Immunol* 1986; 78(4 Pt 2): 695-703.
- 1998 208. Sips AP et al. Food does not effect in bioavailability of theophylline from Theolin Retard. *Eur J Clin*
1999 *Pharmacol* 1984; 26(3): 405-7.
- 2000 209. Pedersen S. Effects of food on the absorption of theophylline in children. *J Allergy Clin Immunol* 1986;
2001 78(4 Pt 2): 704-9.
- 2002 210. Chew ML et al. Pharmacokinetics of pregabalin controlled-release in healthy volunteers: effect of food in
2003 five single-dose, randomized, clinical pharmacology studies. *Clin Drug Investig* 2014; 34(9): 617-26.

- 2004 211. Karkossa F et al. Simulating Different Dosing Scenarios for a Child-Appropriate Valproate ER
 2005 Formulation in a New Pediatric Two-Stage Dissolution Model. *AAPS PharmSciTech* 2017; 18(2):
 2006 309-316.
- 2007 212. Fotaki N, Brassine C. Age related biorelevant dissolution testing for pediatric pellet formulations *APPS*
 2008 *Published abstracts*. Available from <http://abstracts.aaps.org/published/>, AAPS annual meeting, San
 2009 Antonio, 2013
- 2010 213. Mencarelli G et al. Age related biorelevant dissolution testing for paediatric formulations *Int J Pharm*
 2011 2018; 536: 490-522.
- 2012 214. Havenaar R et al. In vitro gastrointestinal model (TIM) with predictive power, even for infants and
 2013 children? *Int J Pharm* 2013; 457(1): 327-32.
- 2014 215. Klitgaard M et al. Studying furosemide solubilization using an in vitro model simulating gastrointestinal
 2015 digestion and drug solubilization in neonates and young infants. *Eur J Pharm Sci.*2017; 109: 191-99.
- 2016 216. European Medicines Agency (EMA). Draft reflection paper on the use of extrapolation in the
 2017 development of medicines for paediatrics. *EMA/199678/2016*, 2017.
- 2018 217. Food and Drug Administration (FDA). Leveraging Existing Clinical Data for Extrapolation to Pediatric
 2019 Uses of Medical Devices: Guidance for Industry and Food and Drug Administration Staff. 2016
- 2020 218. Dunne J et al. Extrapolation of adult data and other data in pediatric drug-development programs.
 2021 *Pediatrics* 2011; 128(5): e1242-9.
- 2022 219. Sharma V, Mcneill J. To scale or not to scale: the principles of dose extrapolation. *Br J Pharmacol* 2009;
 2023 157(6): 907-21.
- 2024 220. Samant TS et al. Quantitative clinical pharmacology for size and age scaling in pediatric drug
 2025 development: A systematic review. *J Clin Pharmacol* 2015; 55(11): 1207-17.
- 2026 221. Mahmood I et al. Prediction of Clearance in Neonates and Infants (≤ 3 Months of Age) for Drugs That
 2027 Are Glucuronidated: A Comparative Study Between Allometric Scaling and Physiologically Based
 2028 Pharmacokinetic Modeling. *J Clin Pharmacol* 2017; 57(4): 476-83.
- 2029 222. Bjorkman S. Prediction of cytochrome p450-mediated hepatic drug clearance in neonates, infants and
 2030 children : how accurate are available scaling methods? *Clin Pharmacokinet* 2006; 45(1): 1-11.
- 2031 223. Calvier EA et al. Allometric Scaling of Clearance in Paediatric Patients: When Does the Magic of 0.75
 2032 Fade? *Clin Pharmacokinet* 2017; 56(3): 273-85.
- 2033 224. Edginton AN et al. A mechanistic approach for the scaling of clearance in children. *Clin Pharmacokinet*
 2034 2006; 45(7): 683-704.
- 2035 225. Jones H et al. A novel strategy for physiologically based predictions of human pharmacokinetics. *Clin*
 2036 *Pharmacok* 2006; 45(5): 511-42.
- 2037 226. Zhuang X, Lu C. PBPK modeling and simulation in drug research and development. *Acta Pharmaceutica*
 2038 *Sinica B* 2016; 6(5): 430-40.
- 2039 227. Kostewicz ES et al. PBPK models for the prediction of in vivo performance of oral dosage forms. *Eur J*
 2040 *Pharm Sci* 2014; 57: 300-21.
- 2041 228. Food and Drug Administration (FDA). Physiologically Based Pharmacokinetic Analyses — Format and
 2042 Content Guidance for Industry (draft guidance). 2016.
- 2043 229. European Medicines Agency (EMA). Guideline on the qualification and reporting of 4 physiologically
 2044 based pharmacokinetic (PBPK) modelling 5 and simulation. *EMA/CHMP/458101/2016*, 2016.
- 2045 230. Upton RN et al. An introduction to physiologically-based pharmacokinetic models. *Paediatr Anaesth*
 2046 2016; 26(11): 1036-46.
- 2047 231. Tsamandouras N et al. Combining the ‘bottom up’ and ‘top down’ approaches in pharmacokinetic
 2048 modelling: fitting PBPK models to observed clinical data. *Br J Clin Pharmacol* 2015; 79(1): 48-55.
- 2049 232. Simulations Plus Inc., 2017. <https://www.simulations-plus.com/>.
- 2050 233. Certara USA I 2011-2018. <https://www.certara.com/>.
- 2051 234. Bouzom F et al. Physiologically based pharmacokinetic (PBPK) modelling tools: how to fit with our
 2052 needs? *Biopharm Drug Dispos* 2012; 33(2): 55-71.

- 2053 235. Jamei M. Recent advances in development and application of physiologically-based pharmacokinetic
2054 (PBPK) models: a transition from academic curiosity to regulatory acceptance. *Pharmacol Rep* 2016;
2055 2(3): 161-69.
- 2056 236. Villiger A et al. Using Physiologically Based Pharmacokinetic (PBPK) Modelling to Gain Insights into
2057 the Effect of Physiological Factors on Oral Absorption in Paediatric Populations. *AAPS J* 2016;18(4):
2058 933-47.
- 2059 237. Maharaj AR et al. A Workflow Example of PBPK Modeling to Support Pediatric Research and
2060 Development: Case Study with Lorazepam. *AAPS J* 2013; 15(2): 455-64.
- 2061 238. Khalil F, Läer S. Physiologically Based Pharmacokinetic Models in the Prediction of Oral Drug
2062 Exposure Over the Entire Pediatric Age Range—Sotalol as a Model Drug. *AAPS J* 2014; 16(2): 226-
2063 39.
- 2064 239. Lukacova V et al. A Physiologically Based Pharmacokinetic Model for Ganciclovir and Its Prodrug
2065 Valganciclovir in Adults and Children. *AAPS J* 2016; 18(6): 1453-63.
- 2066 240. Kuepfer L et al. Applied Concepts in PBPK Modeling: How to Build a PBPK/PD Model. *CPT*
2067 *Pharmacometrics Syst Pharmacol* 2016; 5(10): 516-31.
- 2068 241. Simulations Plus Inc. Gastroplus PBPK Modeling Software... from Discovery through Development.
2069 2016: 1–16, <http://www.simulations-plus.com/assets/GastroPlus-Brochure-Nov2016.pdf>.
- 2070 242. Simulations Plus Inc. Pediatric PBPK Modeling - Special Considerations in GastroPlus. In: Lukacova, V.
2071 ed., 2015: 1–16, <https://www.simulations-plus.com/resource-center/?resource-category=videos>.
- 2072 243. Kohlmann P et al. Investigating Oral Absorption of Carbamazepine in Pediatric Populations. *AAPS J*
2073 2017; 19(6): 1864-77.
- 2074 244. Edginton AN, Ritter L. Predicting Plasma Concentrations of Bisphenol A in Children Younger Than 2
2075 Years of Age after Typical Feeding Schedules, using a Physiologically Based Toxicokinetic Model.
2076 *Environ Health Perspect* 2009; 117(4): 645-52.
- 2077 245. Jones HM et al. Physiologically based pharmacokinetic modeling in drug discovery and development: a
2078 pharmaceutical industry perspective. *Clin Pharmacol Ther* 2015; 97(3): 247-62.
- 2079 246. Rasool MF et al. A Physiologically Based Pharmacokinetic Drug–Disease Model to Predict Carvedilol
2080 Exposure in Adult and Paediatric Heart Failure Patients by Incorporating Pathophysiological Changes
2081 in Hepatic and Renal Blood Flows. *Clin Pharmacokinet* 2015; 54(9): 943-62.
- 2082
2083
2084
2085
2086
2087

2088 **List of Tables**

2089

2090 **Table 1** Age groups classification according to ICH [10; 11], FDA and WHO [5; 6]. (d - days; mo - months; yr

2091 - years).

2092

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			
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 yr			
	6 - 11 yr ^(e)	2 – 12 yr	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			
Adolescents	12-16 or 18 yr ^(g)	12 – 16 yr	12 – 18 yr	10 yr			
				32	33	1.11	1.12
				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				
Adults	>16-18 yr	>16 yr	>18 yr	18 yr			
				67	56	1.81	1.59
				20 yr			
				85.9	72.1	2.05	1.8

2093

^(a) Usually known in literature as neonates

2094

^(b) Infants and toddlers

2095

^(c) Pre-school child

2096

^(d) Young child

2097

^(e) School child

2098

^(f) Child

2099

^(g) Depending on region

2100

2101 **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

2102

2103 ^a On average basis; calculated from a search including commercially available infant meals, fruit purees and infant formula milk products

2104 ^b Portions of the recommended foods are adjusted to the suggestions for meal distribution as recommended in [16; 28]

2105 ^c Parameters were calculated from recommended family recipes, aimed at promoting healthy eating habits among children [54]

2106 ^d Parameters calculated from the proposed sample meal [28]

2107 ^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

2109

2110 **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]

2111

2112 **Table 4** Composition of adult reference biorelevant media and age-specific (grey) simulating fasted and fed state gastric and intestinal media
 2113 [109].

Component	Gastric Media						Intestinal Media						
	fasted state			fed state			fasted state			fed state			
	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
Glycerol 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

2114 FaSSGF – Adult fasted-state gastric media;
 2115 Pn-FaSSGF – Paediatric fasted-state gastric media representative of newborns (0–28 days);

2116 Pi-FaSSGF – Paediatric fasted-state gastric media representative of infants (1–12 months);

2117 FeSSGF – Adult fed-state gastric media;

2118 Pnc-FeSSGF – Paediatric fed-state gastric media representative of newborns (0–28 days);

- 2119 **Pnc-FeSSGF** – Paediatric fed-state gastric media representative of newborns (0–28 days) fed cow’s milk-based formula;
- 2120 **Pns-FeSSGF** – Paediatric fed-state gastric media representative of newborns (0–28 days) fed soy-based formula.
- 2121 **FaSSIF-V2** – Adult fasted-state intestinal media;
- 2122 **P50%-FaSSIF** – Paediatric fasted-state intestinal media formulated with bile salt concentrations 50% (*i.e.* 1.5 mM) of adult levels;
- 2123 **P150%-FaSSIF** – Paediatric fasted-state intestinal media formulated with bile salt concentrations 150% (*i.e.* 4.5 mM) of adult levels;
- 2124 **FeSSIF-V2** – Adult fed-state intestinal media;
- 2125 **Pnb-FeSSIF** – Paediatric fed-state intestinal media representative of newborns (0–28 days) fed breast milk;
- 2126 **Pnc-FeSSIF** – Paediatric fed-state intestinal media representative of newborns (0–28 days) fed cow’s milk-based formula;
- 2127 **Pi-FeSSIF** – Paediatric fed-state intestinal media representative of infants.

2128 **Figure captions**

2129

2130 **Figure 1** Average amount of energy required for paediatric populations as recommended for different physical
2131 activity levels by the EFSA (solid lines and filled symbols) and the U.S. Department of Health and Human
2132 Services and U.S. Department of Agriculture (discontinued lines and open symbols). **(A)** daily average energy
2133 requirement related to a sedentary lifestyle; **(B)** daily average energy requirement related to a moderate level of
2134 activity; Recommendations for males (blue diamonds) and females (red circles). The retrieved data for newborns
2135 and infants are independent of the physiological activity level. Data included in this figure were obtained from
2136 [18; 26; 28; 29].

2137

2138 **Figure 2** Range of feeding volumes for formula-fed newborns and infants **(A)** and feeding intervals **(B)** for
2139 newborns and infants, receiving either infant or follow-on formula (“formula”, open blocks), or being breastfed
2140 (grey-filled blocks). The feeding intervals for breastfed and formula-fed infants are the same beyond the age of
2141 two months (purple blocks) (mo: months; modified from DiMaggio and co-workers [12])

2142

2143 **Figure 3** European recommended ranges for total water intake in paediatrics. Values include intake of water,
2144 beverages of all kind, and water from food moisture. Populations younger than 9 years: filled purple blocks;
2145 males: blocks filled in grey; females: open blocks. Recommendations for adolescents >14 years of age are also
2146 applicable for adults (d - days; mo - months; yr - years). Data used for this figure was retrieved from [36].

2147

2148 **Figure 4** Physicochemical properties of various soft foods and liquids administered in paediatric populations
2149 and an adult meal used for food effect investigation of bioavailability and bioequivalence of drug products (FDA
2150 standard breakfast): **(A)** pH-values; **(B)** Buffer capacity measured with 0.1 N sodium hydroxide solution; **(C)**
2151 Osmolality; **(D)** Surface tension; **(E)** Viscosity; * Soft foods/foods are non-Newtonian fluids. Modified from
2152 [55; 56; 58; 59].

2153

2154 **Figure 5** Gastric **(A)** and intestinal **(B)** pH in fasted (open symbols) and fed state (closed symbols). Paediatric
2155 and adult pH values were collected from literature and depicted as either mean (circles) or median (triangles)
2156 values. In the fed state values depicted represent values measured after ingestion of different types of food.
2157 When patients participating in the paediatric studies belonged to more than one age group, values were used as
2158 mean age, or if a specific age range was reported without denoting the groups mean age, data was depicted using
2159 the middle of the age range [65-67; 70-77; 87-105].

2160

2161 **Figure 6** Fed Gastric Emptying half-life for newborns and young infants (0-10 wk), children and adults: values
2162 depict either mean (circle symbols) or median values (triangle symbols). Infant formula milk: yellow symbols;
2163 breast milk: blue symbols; cow’s milk: green symbols; solid food: red symbols. Data was collected from

2164 different studies and milk products and solid food did not contain the same amount of calories and were
2165 administered in different volumes [49; 84; 124; 126; 130-133].

2166

2167 **Figure 7** Extrapolated initial gastric volumes during drug administration to paediatric populations based on 250
2168 mL volume of water administered to adults with solid dosage forms. Extrapolation was based on BW: grey
2169 blocks [146; 147] and white blocks [163], or based on BSA-function: black blocks [164].

2170

2171 **Figure 8** Statistics of published PBPK models, search performed on PubMed (Status August 2017; n = 93). (A)
2172 Studied paediatric subpopulations; (B) Basic model used for paediatric PBPK model development; (C) Aim of
2173 PBPK modeling; (D) Software platforms utilised for paediatric PBPK model development. (DDI – drug-drug
2174 interactions).

2175

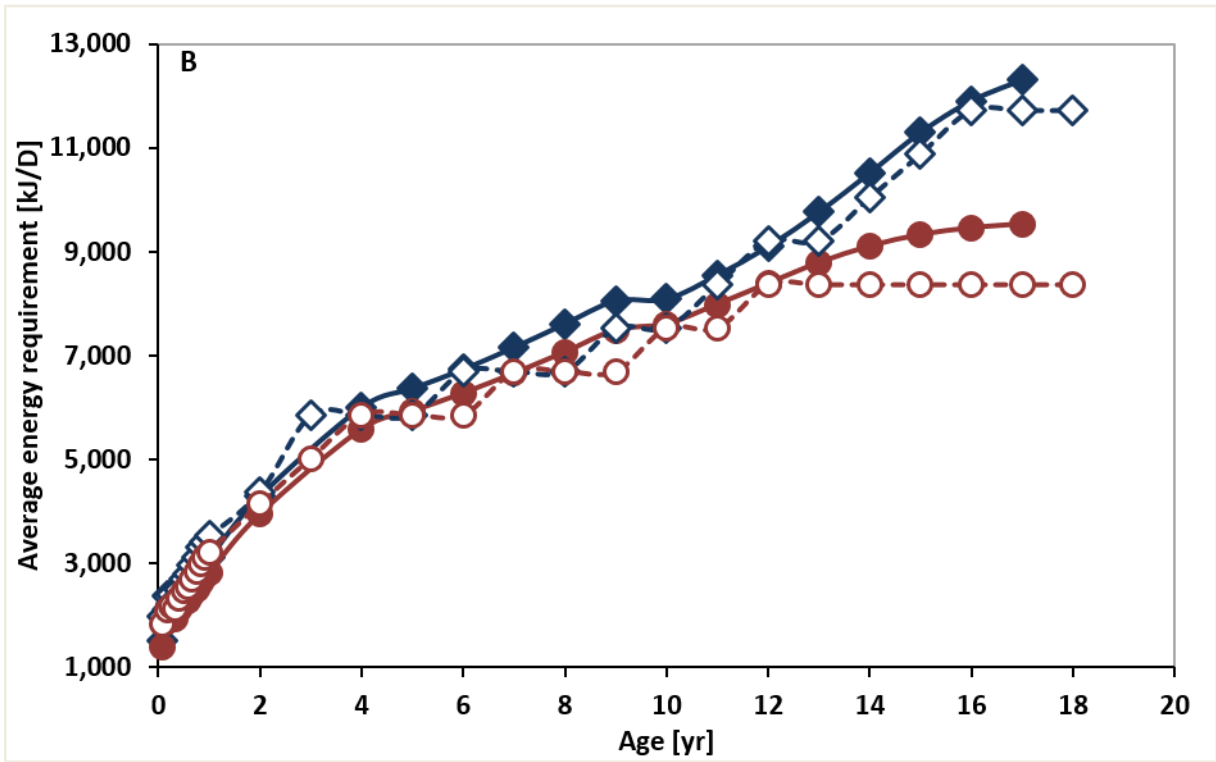
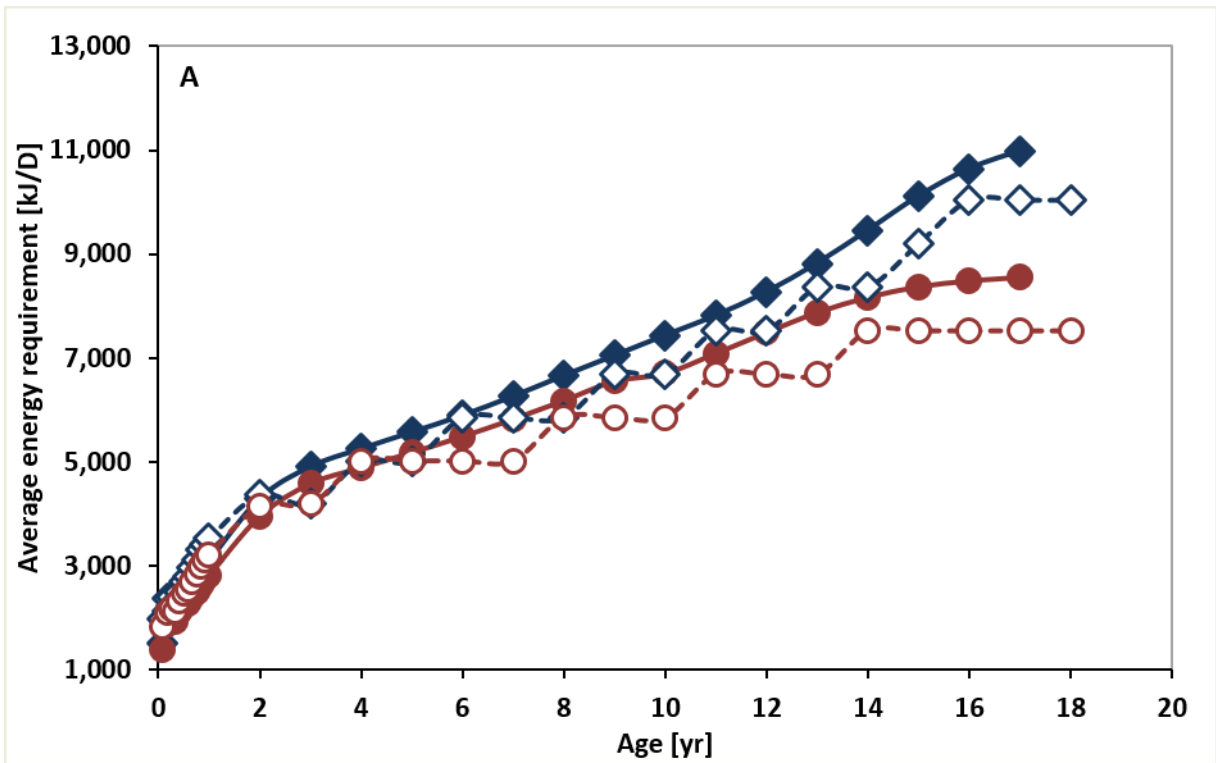
2176 **Figure 9** BCS class distribution amongst modeled drugs, identified in the PBPK search in PubMed. Only
2177 compounds, modeled for oral absorption are considered in this figure, n = 32. The numbers above each bar refer
2178 to the number of drugs studied according to their BCS classification. ND = Not defined.

2179

2180 **Figure 10** Usual strategy for paediatric PBPK model development with a focus on oral drug absorption. **PSA:**
2181 parameter sensitivity analysis; bio-dependent drug properties: drug parameter values that depend on the drug
2182 and the adult/paediatric human physiology.

2183

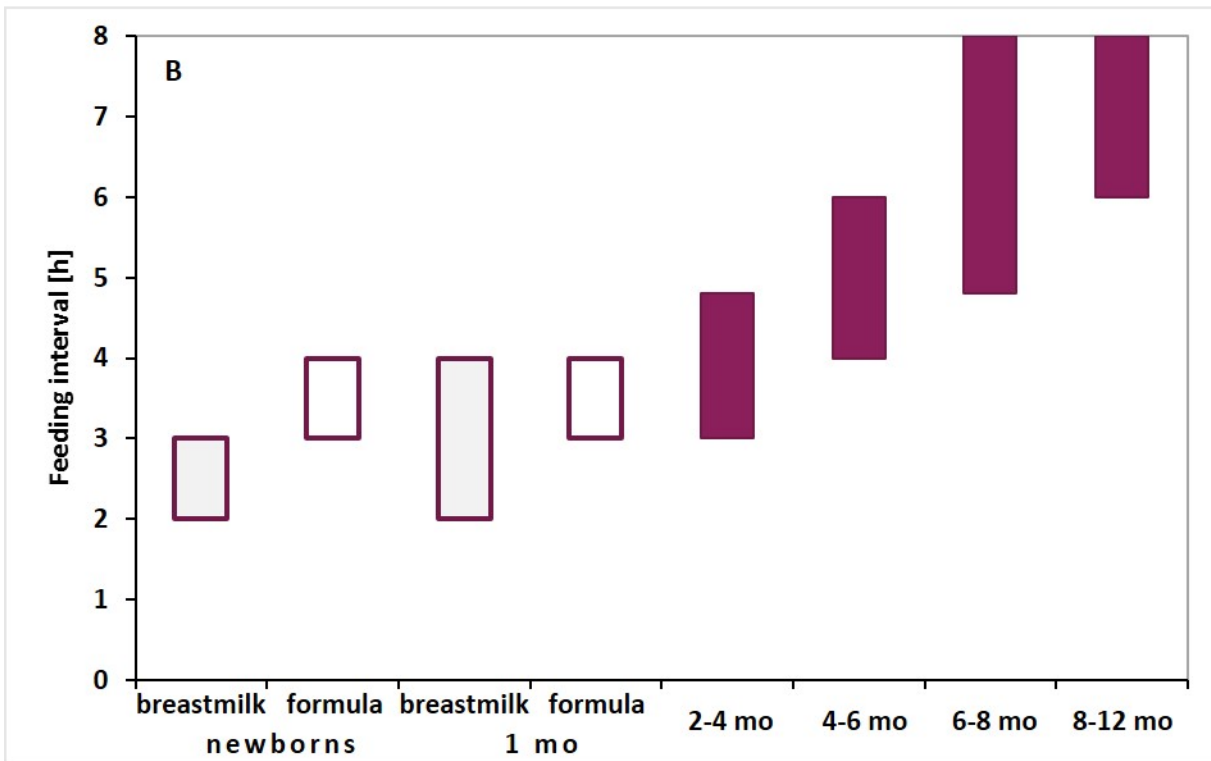
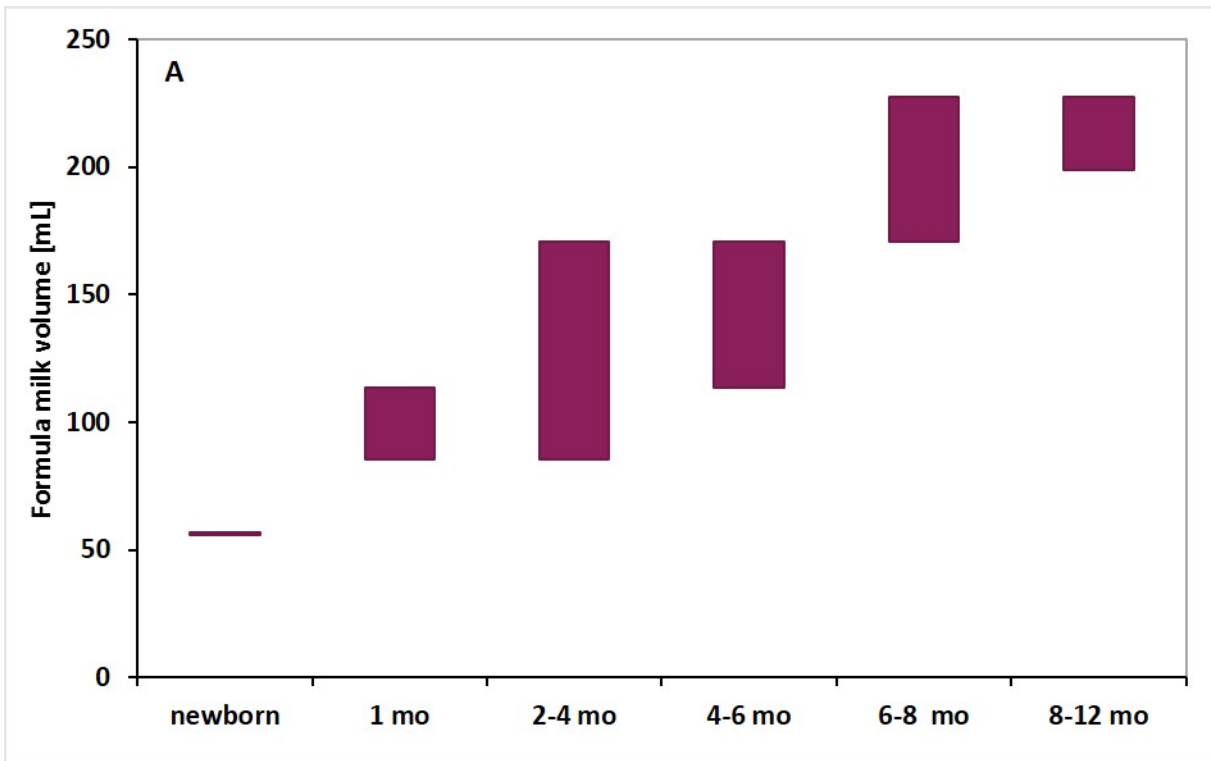
2184 Figure 1



2185

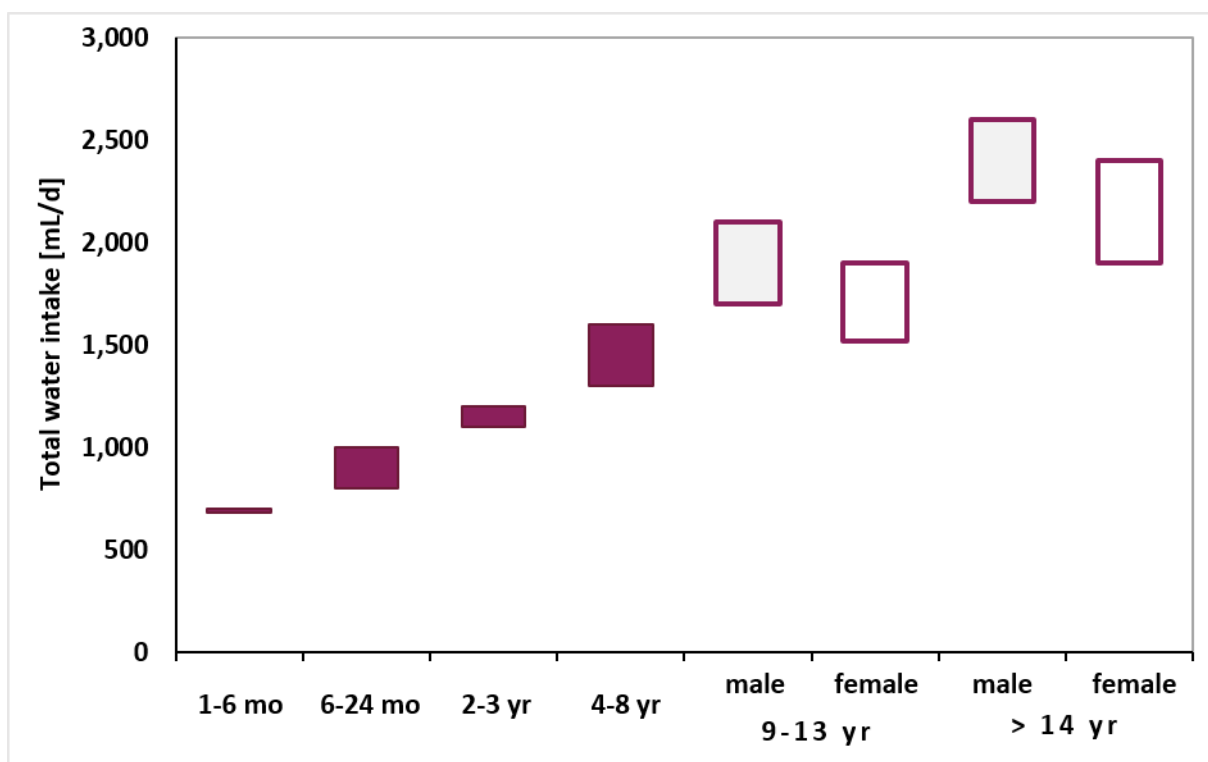
2186

2187 **Figure 2**



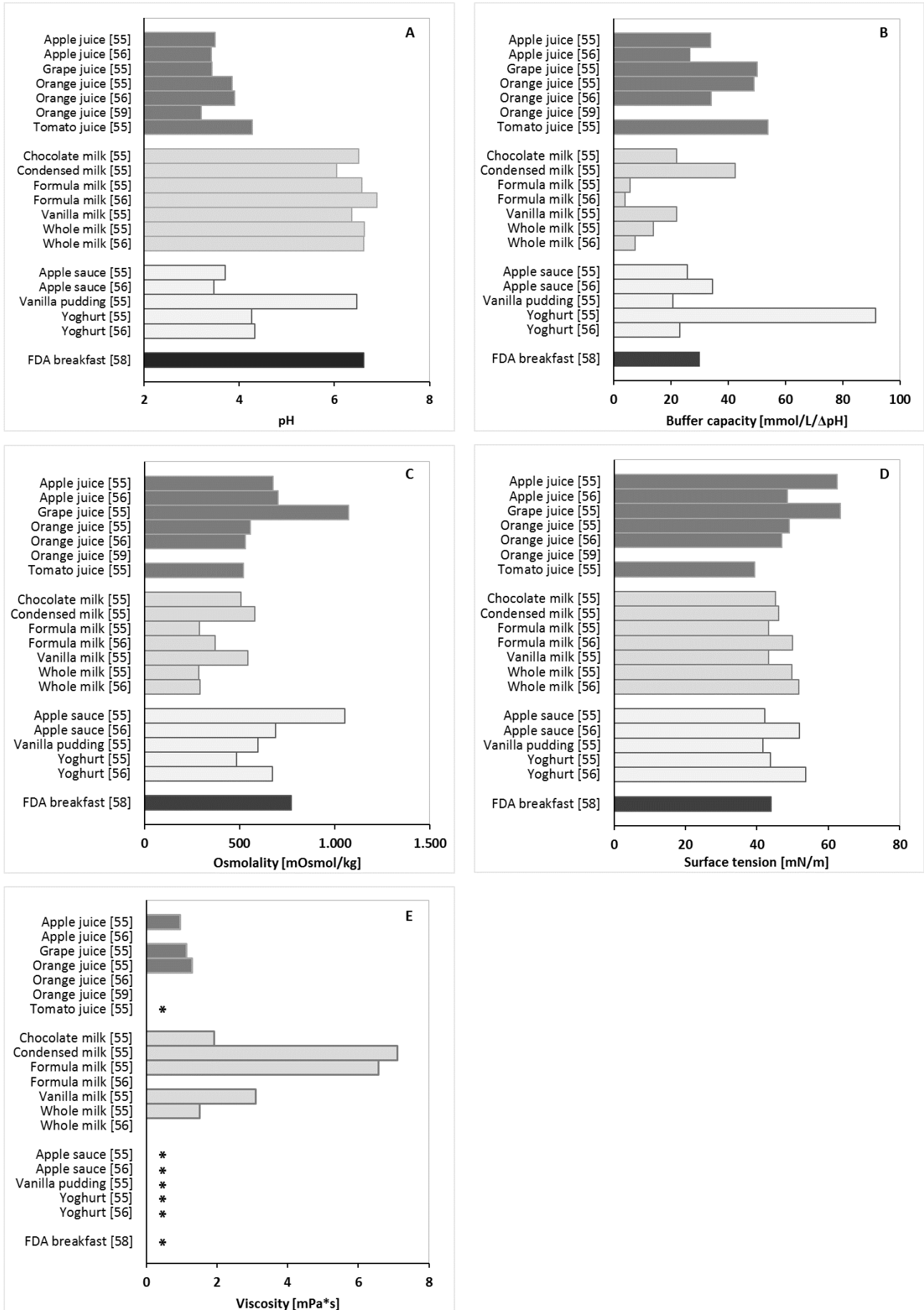
2188

2189 **Figure 3**

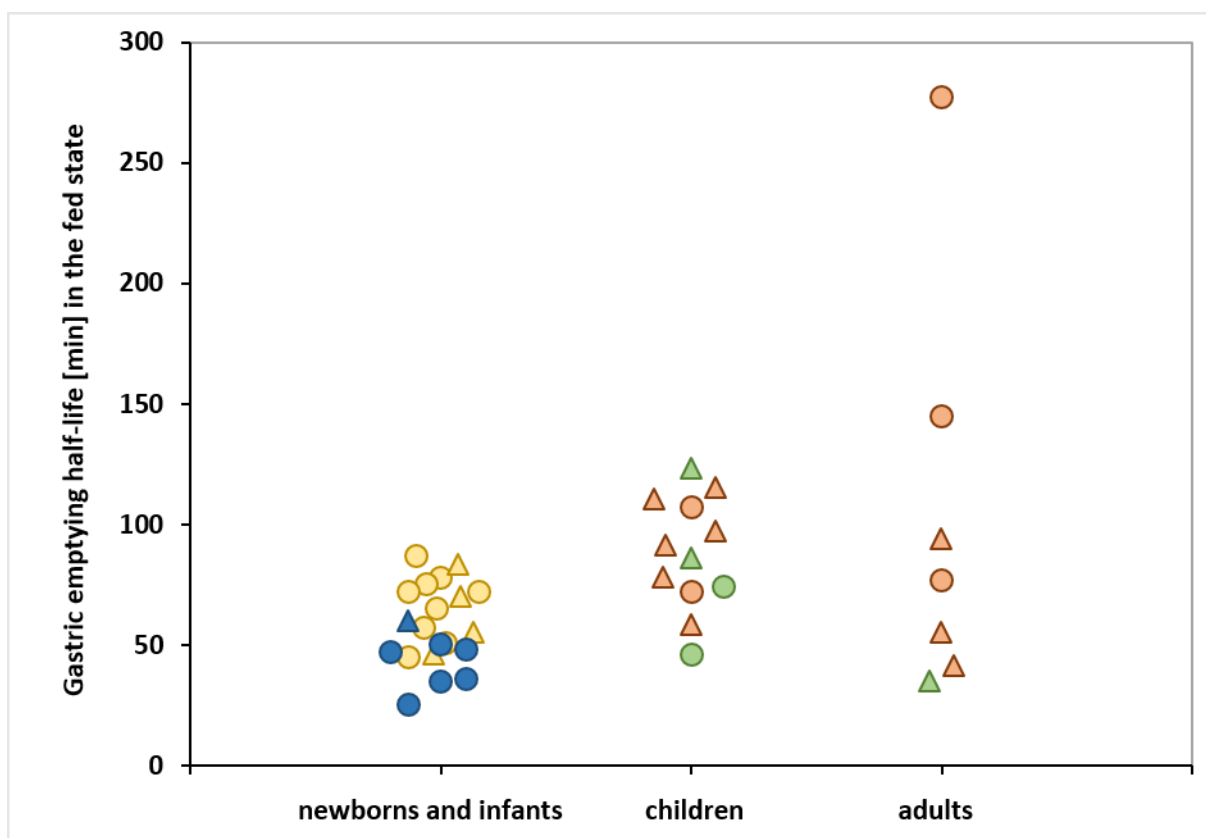


2190
2191

Figure 4



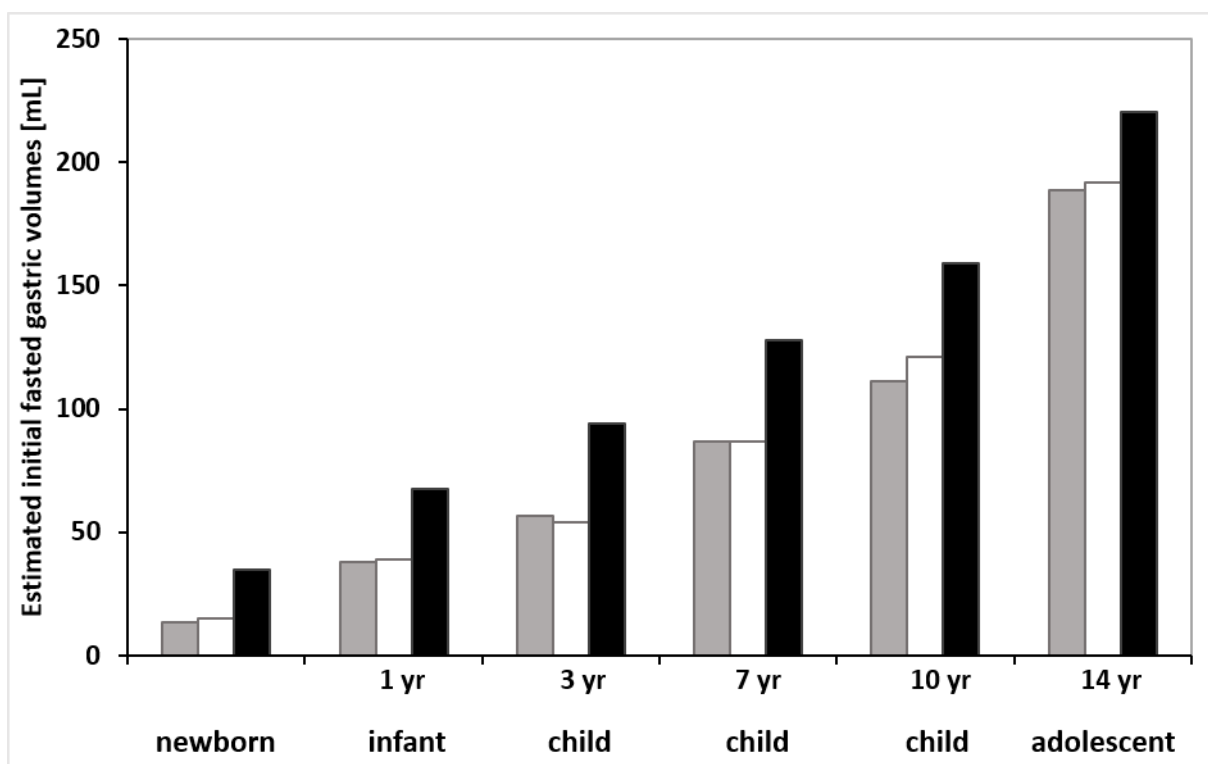
2196 **Figure 6**



2197

2198

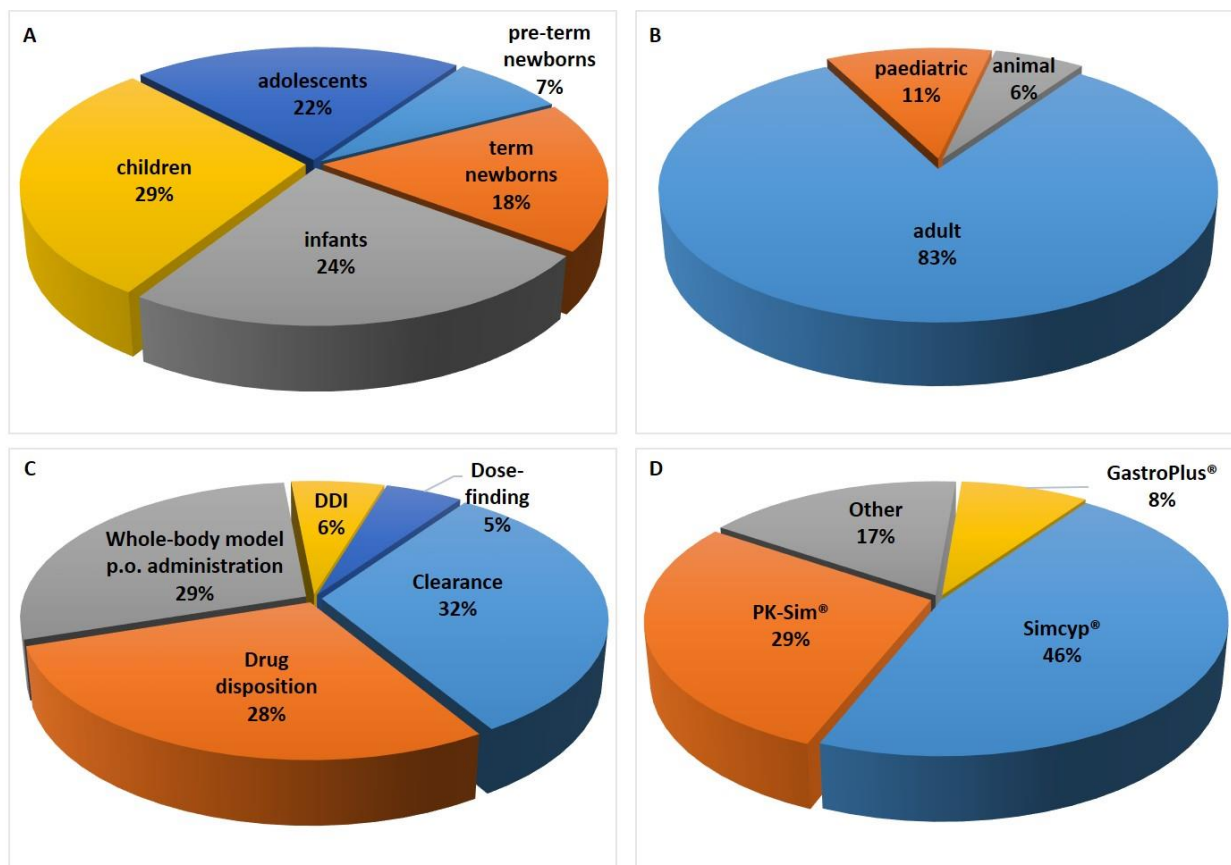
2199 **Figure 7**



2200

2201

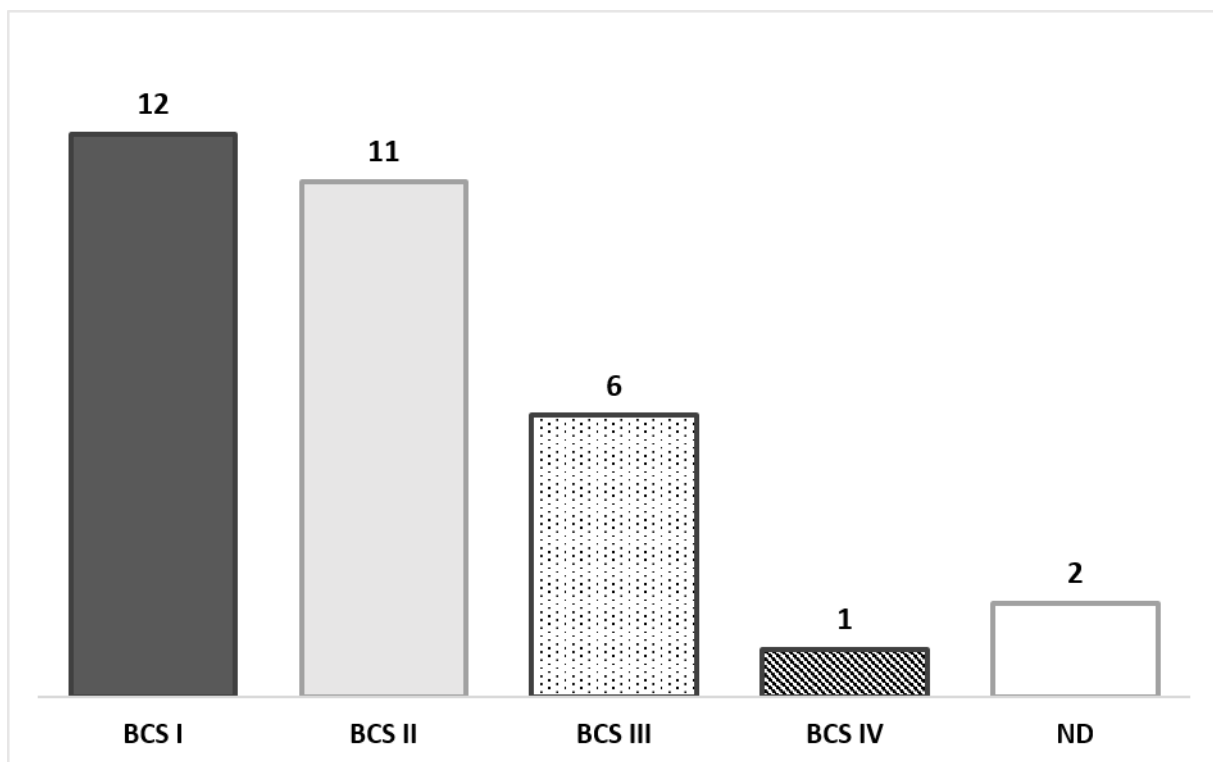
2202 **Figure 8**



2203

2204

2205 **Figure 9**



2206
2207

