Title: ***Paediatric oral biopharmaceutics: key considerations and current challenges***

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Abstract

The complex process of oral drug absorption is influenced by a host of drug and formulation properties as well as their interaction with the gastrointestinal environment in terms of drug solubility, dissolution, permeability and pre-systemic metabolism. For adult dosage forms the use of biopharmaceutical tools to aid in the design and development of medicinal products is well documented. This review considers current literature evidence to guide development of bespoke paediatric biopharmaceutics tools and reviews current understanding surrounding extrapolation of adult methodology into a paediatric population. Clinical testing and the use of *in silico* models were also reviewed. The results demonstrate that further work is required to adequately characterise the paediatric gastrointestinal tract to ensure that biopharmaceutics tools are appropriate to predict performance within this population. The most vulnerable group was found to be neonates and infants up to 6 months where differences from adults were greatest.

Keywords: Solubility, dissolution, permeability, metabolism, physiologically based pharmacokinetic modelling,

1. General Introduction

Biopharmaceutical science is used widely within drug development to predict *in vivo* performance of a medicine. The use of biopharmaceutical science within paediatric formulation development is limited and has previously been highlighted as an area where additional research is required [1, 2].

Prediction of *in vivo* performance of medicines requires knowledge regarding the physiology and anatomy of the site of drug absorption. Although many differences will be highlighted within this review the differences in the gastrointestinal physiology and anatomy of paediatric populations compared to adults has been the subject of some excellent reviews and the reader is directed to these papers for a full discussion [3-5].

This review is limited to a discussion of oral biopharmaceutics and the factors that affect differences in absorption in paediatric populations compared to adults. Information is presented to best direct *in vitro* and *in silico* testing of paediatric medicines.

2. Paediatric Biopharmaceutics – Current Regulatory Guidance Status

There has been global emphasis on improving paediatric accessibility to medicines which has increased the number of drugs tested in and labelled for use in children. For example, the United States (US) Food and Drug Administration's (FDA) paediatric labelling database has 467 reports on paediatric clinical studies listed in response to paediatric legislative changes [6]. There are existing reviews detailing overarching paediatric medicines regulations in the US and Europe (eg [7, 8]).

Paediatric labelling of medicines was introduced in 1979 in the USA [8], since that date there have been significant changes in regulations regarding paediatric medicines. Very recent (2013) FDA draft guidance [9] on information to be contained in paediatric labelling does not include any details on the nature of tests to be conducted or link to any biopharmaceutics guidance. This review details

regulatory guidance relating directly to biopharmaceutics and how this is managed for paediatric populations compared to adults.

2.1. Extrapolation of adult pharmacokinetic data into paediatric populations

US regulatory guidance on exposure-response relationships [10] includes a "Pediatric Study Decision Tree" (Figure 1) which justifies extrapolation from adult data into paediatric populations in cases where the course of the disease and effect of the drug are sufficiently similar in adults and paediatric patients. However, it is important to note that this extrapolation refers only to efficacy, not to safety or dose adjustments.

Paediatric regulation within Europe has tended to follow US regulation. Similar to the US paediatric decision tree, ICH E11 guidance states that, "When a medicinal product is to be used in the pediatric population for the same indication(s) as those studied and approved in adults, the disease process is similar in adults and pediatric patients, and the outcome of therapy is likely to be comparable, extrapolation from adult efficacy data may be appropriate" [11]. The guidance also proposes extrapolation from older to younger paediatric patients where the disease process is similar.

The EMA concept paper on extrapolation goes further than existing documents driving towards a refined algorithm that considers clinical aspects where any form of extrapolation needs to be justified together with an explicit hypothesis on the expected difference in response to a medicine between the target population and the source population [12].

However, when adult data have been used to predict performance in paediatric populations there are examples of unexplained and sometimes adverse events (eg [13-15]). A commonly cited example is the administration of chloramphenicol to neonates at doses that were extrapolated from those found effective and safe in adult patients which resulted in grey baby syndrome. These children exhibited emesis, abdominal distension, abnormal respiration, cyanosis, cardiovascular collapse and

death. A difference in metabolism and clearance in neonates relating to an immature UDP glucuronosyl transferase system were subsequently demonstrated to be responsible [16].

2.2. Biopharmaceutics-specific regulatory guidance

Biopharmaceutics guidance from the US includes information on the conduct of dissolution testing for *in vitro in vivo* correlations; the application of the biopharmaceutics classification system (BCS) to justify a biowaiver, food effect studies and bioequivalence studies. European Medicines Agency (EMA) provides biopharmaceutics guidance on similar topics: bioequivalence testing; BCS based biowaivers; the role of pharmacokinetics in the development of medicinal product in the paediatric population. These documents are reviewed in terms of paediatric applicability within Table 1.

Table 1. Applicability of existing biopharmaceutics regulatory guidance to paediatric formulation development

Guidance Document/Topic	Source and Date	Current applicability to paediatric populations	Clarification required
Dissolution Testing			
Dissolution testing guidance for IR solid dosage forms	FDA 1997 [17]	No mention of "pediatric" or "child" within existing guidance.	BCS based classifications are used to determine testing schedule yet the dose:solubility ratio fundamental to BCS classification cannot be directly extrapolated into paediatric populations The volume within the dissolution test (>500mL) is unlikely to be appropriate for the full range of paediatric patients
Extended Release Oral Dosage Forms: Development, Evaluation, and Application of *In vitro* i*n vivo* correlations	FDA 1997 [18]	No mention of "pediatric" or "child" within existing guidance.	All *in vitro* data refers to correlations with adult *in vivo* performance Existing *in vitro* testing (dissolution) is based on historic relevance for adult populations (volume, pH etc)
BCS Guidance			
Waiver of *in vivo* Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System	FDA 2000 [19]	No mention of "pediatric" or "child" within existing guidance. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1-7.5. 250 ml is derived from typical BE study protocols that prescribe administration of a drug product to fasting adult volunteers with a glass of water.	Revised paediatric BCS classifications are required to account for reduced volume of liquid taken with dosage form
Proposal to waive *in vivo* bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms	WHO 2006	No mention of "paediatric" or "child" within existing guidance. BCS high solubility definition amended to dose:solubility ratio of 250mL or lower over a pH range of 1.2-6.8 Refers to the highest dose, rather than highest dose	Revised paediatric BCS classifications are required to account for reduced volume of liquid taken with dosage form

		strength	
Clinical Studies Guidance			
Food-Effect Bioavailability and Fed Bioequivalence Studies	FDA 2002 [20]	No mention of "pediatric" or "child" within existing guidance. Sponsors may **choose** to conduct additional studies for a better understanding of the drug product and to provide optimal labelling statements for dosage and administration (eg different meals and different times of drug intake in relation to meals). Subjects should be healthy volunteers drawn from the general population Highest dose strength should be tested Meal of 800-1000 calories to be used Medicine taken with 240mL water Sprinkle formulations should be tested using the food listed in the product labelling (eg applesauce) Special beverages as vehicles – these should also be tested as per the labelling	Appropriate standardised meals representative of paediatric populations are required to ensure testing is adequate
Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – general considerations	FDA 2003 [21]	No mention of "pediatric" or "child" within existing guidance. Clinical study should be conducted in a representative population by age, sex and race. Yet recommendation that subjects should be over 18.	Bioequivalence between formulations is currently tested in adults with extrapolation of paediatric. However, paediatric physiology may influence formulation performance thus appropriate testing should be recommended
Guideline on the Investigation of Bioequivalence	EMA 2010 [22]	Healthy volunteers stated to provide adequate for extrapolation of results into children Medicinal product to be taken with >150mL water For fed studies: Meal of 800-1000 calories to be used	
Guideline on the role of pharmacokinetics in the development of medicinal	EMA 2006 [23]	No mention of biopharmaceutics – guidance on study design rather than biopharmaceutical testing	

products in the paediatric population		strategies	
Draft guideline on pharmaceutical development of medicines for paediatric use	EMA 2013 Rev. 1 [24]	Identifies key issues in formulation design with relevance to biopharmaceutics including: managing formulation changes; the impact of manipulation with food and drink; the impact of physiology on performance of modified release dosage forms	No details are provided on the tools or appropriate testing strategies to understand issues highlighted.
Clinical Investigation of Medicinal Products in the Paediatric Population	EMA/ICH E11 2001 [11]	Relevant safety data for paediatric studies ordinarily come from adult human exposure with extrapolation for efficacy Relative bioavailability of the paediatric formulation with the adult oral formulation should typically be conducted in adults Definitive PK studies should be done in paediatric populations Knowledge of clearance pathways (renal and metabolic) and understanding age related changes of these processes aids in planning paediatric studies Specific age related changes in pre-term neonates are discussed	
Guideline on the investigation of medicinal products in the term and preterm neonate	EMA and PDCO 2009 [25]	Identifies key issues including: a need for specific formulations; understanding (from literature) of ontogeny of metabolising enzymes; changes in bioavailability due to maturational changes within the GI tract need to be considered and predicted. Relevant safety data should come from adults or older children prior to testing in neonates where possible. In vitro data that predicts the in vivo situation should be used. Population PK approaches are recommended for PK studies. Relative bioavailability studies for specific formulations should be conducted in adults or older children where	Very limited data presented on appropriate tools to use for in vitro testing to predict in vivo performance

		possible
Guideline on the investigation of drug interactions	EMA/ICH 2012 [26]	Drug-drug interactions in paediatric populations need to be considered with specific clinical studies within paediatric populations if appropriate. The use of sparse PK sampling or simulation is recommended to understand paediatric populations

A key step forwards in paediatric biopharmaceutics was the release of draft guidelines on pharmaceutical development for paediatric use released by the EMA [24]. This document highlights the need for appropriate testing to support formulation changes during development of a paediatric formulation; the importance of changes in bioavailability when extemporaneously manipulating a solid dosage form by mixing with food or drinks; the impact of physiology on the absorption of drugs administered as modified release formulations. However the guidance does not provide details on how these aspects should be measured and whether *in vitro* testing may be used to minimise the number of clinical studies conducted.

As biopharmaceutical testing is designed to mimic *in vivo* performance of medicines, it is essential that appropriate regulatory guidance is available to support the design and development of paediatric medicines to enable rational design of appropriate clinical testing.

3. **Solubility**

Intestinal absorption of drugs following oral administration is a function of drug dissolution and subsequent permeation and/or transport of the dissolved compound through the mucosa at the absorptive sites in the gastrointestinal (GI) tract. Since only dissolved drugs can be absorbed to a relevant extent, the solubility of the compound in the intraluminal fluids/contents is an essential prerequisite for its oral bioavailability.

In general, the solubility of a drug depends on the physical and chemical properties of the solute and the solvent as well as on temperature, pressure and the pH of the solution [27]. The solubility of a drug is, to a large extent, due to the polarity of the solvent, which, in GI fluids, is typically represented by an aqueous environment. Moreover, ionised compounds exhibit greater aqueous solubility than their unionised counterparts. Media pH and buffer capacity exhibit a huge impact on drug ionisation (where relevant), which , in turn, can strongly affect the aqueous solubility of such compounds [28]. As a result, the solubility of weak bases is typically lower at higher pH values where

the drug is largely present in the unionised form. Solubility of a compound in the GI contents can be affected by additional parameters, for example the fat content of the GI contents or micelle formation. For soluble drugs, most of these parameters play a subordinate role. However, they can be crucial for limiting the intraluminal solubility of poorly soluble and/or ionisable drugs. Thus, to predict the *in vivo* solubility based on *in vitro* data, it is important to adequately address the relevant parameters of intraluminal fluids within solubility measurements. In the scientific literature, various approaches are described for determining the solubility of a drug compound. However, the relevance of these approaches for estimating the solubility of a given compound in GI conditions of the paediatric population is questionable.

3.1. Compendial solubility methodology

The solubility of a drug can be expressed in several ways. European Pharmacopoeia (Ph.Eur.) [29] and United States Pharmacopoeia / National Formulary (USP/NF) [30] define the solubility as parts of solvent required for one part of solute, as listed in Table 2.

Table 2: Compendial solubility definitions [29, 30]

Descriptive Term	**Parts of solvent required for one part of solute**
Very soluble	Less than 1 part
Freely soluble	1 to 10 parts
Soluble	10 to 30 parts
Sparingly soluble	30 to 100 parts
Slightly soluble	100 to 1000 parts
Very slightly soluble	1000 to 1000 parts
Practically insoluble	More than 10000 parts

Compendial solubility experiments are typically performed at ambient temperature (15-25°C) where typical solvents comprise mainly water, different alcohols, acetone, methylene chloride and fatty oils and pH values are not addressed.

Ph. Eur. and USP/NF approaches to solubility measurements, where solubility experiments in a series of aqueous media of different pH are conducted, provide only a coarse estimate of the solubility in such conditions rather than resulting in any quantitative information on the *in vivo* solubility of the compound.

3.2. BCS solubility approach and Dose:Solubility ratio

The Biopharmaceutics Classification System (BCS) established by Amidon *et al.*. (1995) [31] is regarded as one of the most significant prognostic tools for estimating the *in vivo* bioequivalence of orally administered formulations. It is based on a scientific framework describing the major rate limiting steps in oral drug absorption. These are (i) the solubility of the active compound in GI pH conditions, (ii) the intestinal permeability characteristics of the active and (iii) its release (dissolution) from the final dosage form. This information is assumed to be a surrogate for the *in vivo* performance of oral immediate release (IR) formulations.

Solubility determination according to the BCS is essentially different from the pharmacopoeial approach. The main principle is to determine the compounds´ solubility at 37°C over the entire GI pH range of pH 1 to 6.8 [32] or 7.5 [19], respectively. In addition, the BCS takes into account the highest dosage strength that will be administered to the patient as a single dose. Instead of indicating the solvent:solute ratio or the drug solubility in mg/mL solvent, the dose:solubility ratio (D:S) is calculated for at least three pH conditions (pH 1, 4.5, 6.8 or 7.5) taking into account the highest single dose and the experimental solubility values obtained at 37 °C in at least three media. A drug substance is classified as highly soluble when the D:S ratio is equal or less than 250 mL in all pH conditions. This amount is a conservative estimate of the fluid volume available in the GI tract under fasting-state conditions and is based on the volume usually administered with the dosage form (the so-called FDA glass of water) in a bioequivalence (BE) study [33]. However, at this point, it should be kept in mind that both the highest dosage strength assumed for the calculations, as well as the volume of 250 mL are valid for adults and cannot simply be extrapolated to the paediatric

population. Moreover, a paediatric BE standard has not been established [1], i.e. there is no standardized volume that could be referred to when calculating a D:S ratio. The potential design parameters for a paediatric BCS (PBCS) system have been discussed recently [1, 2]. However, based on the data currently available, it was not possible to decide on a volume that could be used to calculate a meaningful D:S in a paediatric population [1, 2]. However, even with these critical knowledge gaps, it is clear that a meaningful PBCS needs to be broken down into several different age groups that account for developmental changes in intestinal permeability, luminal contents, and gastrointestinal (GI) transit [1]. In contrast, it is unlikely that a simple and empiric approach translating the intraluminal fluid available into a volume assumed to be available for dissolution in the paediatric GI tract by simply using 1/10 of the volume used to simulate the volume available in the adult GI tract ($\rightarrow$ 25 mL), as tentatively proposed by Amidon [1], will provide meaningful solubility results for children of different age categories. Even with the little data available to date, as a result of dynamic developmental physiology of children, there is most likely no linear relationship between the fluid volumes available in the gastrointestinal tract. Moreover, the pH-conditions in the different GI sections can be significantly different from those in adults. This is particularly true for the first 2 to-5 years of age (Table 4) and will make a universal PBCS approach impossible.

Nevertheless, establishing a PBCS would be very helpful in the different stages of paediatric formulation development. In order to develop an *in vivo* predictive solubility test for paediatric populations, beside the pH and the volume available for drug dissolution, many additional parameters that can affect the solubility of a compound in intraluminal conditions, for example the presence of bile salts and digestive products will need to be addressed.

3.3. Biorelevant solubility approach

Several researchers have focused on developing test conditions that reflect the intraluminal conditions in the fasted and fed state upper GI tract as typically IR formulations release the drug in this area. The impact of different dosing conditions (fasted versus fed-state administration of a

dosage form) and consequences on GI physiology are also expected to occur at this site have also been considered. As a result, a series of biorelevant media to simulate conditions in the adult stomach and small intestine before and after meals have been introduced which are detailed in Table 3 [34].

Table 3: Biorelevant media used to simulate upper GI conditions in adults

Medium	GI section	Prandial status	Relevant parameters	Reference
FaSSGF pH 1.6	Stomach	Fasted	• pH • surface tension • osmolality	[35]
FeSSGF pH 5.0	Stomach	Fed	• pH • surface tension • osmolality	[36]
Milk	Stomach	Fed - immediately after a light breakfast	• pH • surface tension • fat:protein:carbohydrate ratio	[37]
Ensure Plus	Stomach	Fed - immediately after a standardized high fat breakfast (FDA Standard Breakfast)	• pH • fat:protein:carbohydrate ratio • surface tension • osmolality	[38]
FaSSIF pH 6.5	Small intestine	Fasted	• pH • surface tension • osmolality • bile salt concentration	[39]
FaSSIF-V2 pH 6.5	Small intestine	Fasted	• pH • surface tension • osmolality • bile salt concentration	[36]
FeSSIF pH 5.0	Small intestine	Fed	• pH • surface tension • osmolality • bile salt concentration	[39]
FeSSIF-V2	Small intestine	Fed	• pH	[36]

pH 5.8	• surface tension • osmolality • bile salt concentration • digestive products

Since their introduction, these biorelevant media have been successfully used in both solubility and dissolution experiments. Based on novel scientific findings in the recent past, some of these media were updated (Version 2 (V2)) to even more closely represent both the pre- and postprandial states in the upper GI tract [35, 36]. Particularly, Fed State Simulating Small Intestinal Fluid (FeSSIF) has undergone a significant revision. Besides the bile components sodium taurocholate and lecithin, the updated medium also contains digestive products, i.e. glyceryl monooleate and sodium oleate, that may contribute to the solubilisation of a drug [34]. With existing biorelevant media, it is possible to simulate different adult *in vivo* pH scenarios by selecting media appropriate to typical dosing conditions in clinical studies [20], as well as addressing various additional physicochemical parameters, for example, the osmolality, surface tension, bile salt concentration and the presence or absence of digestion products, that are expected to affect drug solubility in the GI tract of an average adult. However, as already mentioned, these media were developed on the basis of human adult physiology and do not necessarily represent the relevant conditions in the paediatric GI tract.

3.4. Biorelevant solubility approach for paediatric populations

Since the physiological conditions in the GI tract of children, associated with growth and maturation, can significantly differ from that in adults, neither compendial, BCS solubility approaches, nor the current set of biorelevant media are applicable to estimate a drugs´ *in vivo* solubility in the GI tract of a child. This will be particularly true for very young infants and children, where the developmental effects on GI physiology are reported to be most pronounced [3, 4, 40].

In paediatric drug therapy, currently many drugs are administered in the form of solutions, syrups, suspensions, thin strips or orally disintegrating granules and tablets. In contrast to most of the adult

dosage forms which are solids that are swallowed whole and where the first important site for drug dissolution is typically the stomach. Paediatric formulations are often designed to be retained in the oral cavity. Residence in the oral cavity might have an impact on the bioavailability of certain drugs and thus, the fluid composition in the oral cavity should be considered in a biorelevant paediatric solubility approach. This may be of significance for poorly soluble weakly basic drugs that are likely to precipitate in more neutral pH-conditions [41] typically found within the oral cavity. An example is the weak base itraconazole which is used in the treatment of invasive fungal infections in children and is often administered as oral solution [42]. The neutral pH conditions in the oral cavity can also result in a loss of integrity of sensitive enteric coatings of tablet or granule formulations, if the dosage form is not immediately swallowed but kept in the mouth longer than anticipated for the formulation design. Similar to the biorelevant solubility approach for adults, a truly meaningful solubility approach for paediatrics should take into account the physicochemical properties, the overall composition of oral, gastric and small intestinal fluids including pancreatic and bile secretion and the available fluid volumes in the fasted state and in response to a typical age-related meal. It clearly becomes obvious that such an approach requires a lot of detailed information on (i) the dosing conditions, including the typical maximum dose; (ii) the typical volume of fluid co-administered with the dosage form and (iii) the average size and composition of typical meals for each age category. Only with this information, is it possible to calculate a BCS-like and/or biorelevant solubility for paediatric patients that represents the oral cavity.

Based on these considerations, a universal solubility approach for the paediatric population is rather unlikely. Even, if the most significant differences in fluid composition between adults and children are likely to be seen in very young children, one needs to consider that the dimensions of the gastrointestinal tract continuously change throughout childhood and adolescence. This maturation process will affect the capacities of the oral cavity and the different GI sections and thus the volume available for dissolution, resulting from the resting volumes of fluid, the volume that can be co-administered with an oral dosage form and gastrointestinal secretions.

Even if the volume available for dissolution was available for each age group, a simple adaption of the classical BCS solubility approach to children is unlikely to provide the intended *in vivo* predictivity, since the impact of various components of the physiological fluids, for example, the presence of bile compounds and digestion products which are often essential for the solubilisation of drugs in lumen of the small intestine [34], are not taken into account. Depending on the properties of the drug, a fine-tuned, but still classical BCS-like approach is thus unlikely to result in prediction of the *in vivo* solubility in the paediatric GI tract. However, at this point, it should also be kept in mind that the BCS was never established with the objective to obtain an *in vitro* / *in vivo* correlation.

In response to the new European Union regulations on children´s medicines, recently, several attempts have been made to review literature data on the GI physiology with respect to parameters that could affect drug absorption in paediatrics and to highlight the similarities and differences between adult and paediatric populations [4]. As expected, due to ethical reasons associated with performing invasive tests in healthy children, knowledge of the physiological changes that occur along the GI tract in response to growth and maturation is incomplete [1, 4]. Several attempts have been made in reviewing and summarizing the most relevant changes in fluid composition and volumes in the upper GI tract (Table 4). However, although the intention of these reviews was to summarize and discuss the parameters of paediatric gastrointestinal physiology relevant to oral drug delivery, it is obvious that the data currently available are insufficient for designing biorelevant solubility tests for the various paediatric age groups. It is also notable that the most significant differences to adult GI physiology are found in newborns and infants, whereas as early as in pre-school children the oral, gastric and small intestinal fluid compositions seem to be very close to that of adults. However, caution is required with the data presented as the cited review articles only report average values of physiological parameters, and statistical parameters (number of subjects, minimum/maximum values, and standard deviations) are not mentioned. The typical fluid volumes available at the relevant sites in the paediatric GI tract are also not discussed. It should be further

noted, that the stated physiological values in children vary greatly within the literature [4]. Many of the reported studies were performed in a small number of subjects which were not necessarily healthy children, but often suffered from a particular disease. For example, Kaye *et al.*. (2011) reported average gastric pH values in infants that resulted from a study involving five H*elicobacter pylori*-infected and five uninfected children [43]. Many of the cited values also result from cross-referencing [3] rather than from a systematic study of the original literature. Several of the data, eg the stomach capacity [4] are reported from textbooks which are more than 15 years old. This indicates that the data might not necessarily represent what is needed for developing a biorelevant test. Overall, most of the reviewed studies did not follow the same or regulatory age classifications making comparisons difficult, as they were designed to measure particular parameters in diseased children and covered a broad range of ages. As also concluded by Abdel-Rahman *et al.* (2012) and Batchelor *et al.* (2013) [1, 2], at the moment there are significant knowledge gaps that will hinder the development of age-specific biorelevant solubility tests for children.

Table 4: Average age dependent physiological factors that may influence *in vivo* solubility, dissolution of oral dosage forms and subsequent drug absorption as reported in recent overview/review articles on paediatric physiology [1, 3, 44, 45].

Parameter	**Newborn (0-28d)**	**Infant (1m-2 years)**	**Child (2-5y)**	**Child (6-11y)**	**Adolescent (12-18y)**
Saliva pH	7 [4]		7.1 [4]	7.1 [4]	7.4 [4]
Gastric fluid pH	at birth pH is close to neutral (pH 6-8); significant acid secretion over the next 24-48 h, resulting in a pH of 1-3.5; then gastric acid secretions declines and the pH increases to near neutral for 20-30 days before its starts to decrease again [4, 44]	on a per kg basis, adult levels are approached by 2 years of age [3, 44]; from about 3 months of age [1, 4]	~ adult [44] [3, 4]	~ adult [4]	~ adult [4]
		1.4 [4]	1.5 [4]	1.5 [4]	1.5 [4]
Gastric emptying time	reduced (variable) [44] [3]	increased [3, 44]; delayed in children aged 6-8 months [3]; ~ adult after 6 months	~ adult [4]	~ adult [4]	~ adult [4]

		of age [4]			
	54-82 min [4]	12-70 min [4]	12-70 min [4]	12-70 min [4]	12-138 min [4]
Gastric acid / pepsin output	Relatively low [3, 44]	~ adult when calculated based on body weight [3, 44]	~ adult [3, 44]	~ adult [3, 44]	~ adult [3, 44]
Stomach capacity	10-100 mL [4]	90-500 mL [4]	750-960 mL [4]	750-960 mL [4]	1500 mL [4]
Small intestinal pH			~ adult [4]	~ adult [3, 4]	~ adult [3, 4]
Intestinal transit time	reduced [44]	increased [44]			
	4 h [4]	4 h [4]	3-7.5 h [4]	3-7.5 h [4]	3-7.5 h [4]
Pancreatic / biliary function	immature [3]	~ adult [3]			

4. Permeability

Intestinal absorption of macronutrients (carbohydrates, fat and proteins) as well as ions and trace elements is essential for the growth of children. Maturation of the gastrointestinal tract is reported to occur within the first 6 months; therefore drugs absorbed by transport processes used for nutrients that are essential for growth may show different absorption profiles during the first 6 months of life. Permeability of drugs within in paediatric populations is an under-researched area; this review focuses on permeability within the small intestine as this is the most important site in terms of oral biopharmaceutics. It is recognised that existing knowledge on developmental changes in the GI processes including transporter expression is very limited.

4.1. Intestinal surface area and membrane permeability

Contradictory evidence exists relating to the morphology of the small intestine during development, with reports of similar villus surface area in adults and infants [46] and villi that are narrower with smaller crypts in neonates [47]. Since the length and diameter of the small intestine increases continuously from birth till adulthood, the functional surface area of the small intestine increases more than 40-fold [48].

Intestinal permeability is reported to be high at birth and decreases progressively during the first week of life [49]. This may be related to the reduced surface area: volume ratio due to the villi being broader and providing a smaller overall surface area; this phenomenon is well documented in rats [50].

4.2. Passive permeability

The composition and fluidity of the intestinal membrane has been reported in change as a function of age; with a reduction in fluidity following the post-weaning period in rats attributed to an increase in the cholesterol:phospholipid molar ratio [51, 52].

Sugar absorption tests are typically used to assess intestinal permeability as these are minimally invasive; following enteral administration of a test solution containing lactulose and mannitol, the excretion of these sugars is measured in urine. Testing typically involves two sugars as monosaccharides (eg mannitol) are readily absorbed via the transcellular pathway but larger disaccharides (eg lactulose) are absorbed through the paracellular pathway. Therefore the ratio of lactulose:mannitol (L:M) in the urine is a measure of intestinal integrity with decreases in the L:M ratio typically attributed to a decrease in L and an increase in M recoveries. Sugar absorption studies reported from paediatric populations are detailed in Table 5.

Table 5. Sugar absorption tests conducted in paediatric populations

Age Group	Finding	Comments	Reference
Preterm infants (25-32 weeks)	L-Rhamnose used as monosaccharide showed decreased permeability with age Increased permeability of L with age Intestinal permeability (ratio L:R) increased with age		[53]
Preterm infants (26-36 weeks)	Intestinal permeability (ratio L:M) decreased with age within the first week following birth	Values within a week of birth were similar to those in term neonates	[49]
Preterm compared to term neonates	Intestinal permeability is higher in pre-term infant compared to term		[54]
Neonates within 1 week of life	Permeability of L decreased with age L:M ratio decreased with age	Permeability of L reached mature values within weeks following birth	[55]
0.5 months -14	Intestinal permeability (ratio L:M)		[56]

years	decreases with age Permeability of L does not change with age Permeability of M increases with age		
0-1 month	Intestinal permeability (ratio L:M) decreased with age	The food used in the first month of life affects the rate of decrease in paracellular permeability with breast fed infants demonstrating a more rapid decrease compared to formula fed babies	[57]
1m – 3 years	Intestinal permeability (ratio L:M) is equivalent to adult values		[58]

Abbreviations used in the table are: L = lactulose; M = mannitol and R = Rhamnose.

The conclusion from the data in Table 5 is that the permeability of the membrane decreases with age yet the age at which adult values are reached is unclear; therefore dose adjustments in the very young need to be undertaken with caution as membrane permeability may be higher than predicted based on adult values. There is controversy within the literature with contradictions in the impact of age on the permeability of monosaccharides [53, 56]. The permeability of lactulose is reported to be unchanged [56], increase [53], and decrease [55] with age highlighting the discrepancies within the literature.

4.3. Active transport permeability

Nutrients and ions are generally absorbed by active transport processes in the intestine. Expression of these transport processes is in line with the needs of the growing child. Therefore, compounds that are absorbed by transport processes that are involved in the growth of children are likely to be absorbed better in children than in adults. For example, young children (2 months to 8 years), have been shown to absorb more ingested lead than adults, 40-50 % vs 10-15 %, respectively [59]. Binding of lead to receptors in the enterocyte that serve for active transport of iron and calcium may

account for active transport of lead. But similar to the passive diffusion of calcium at higher (>2 mM) intraluminal calcium concentrations, lead can be absorbed by means of passive diffusion. It has been suggested that the higher absorption of lead in children compared to adults involves also enhanced pinocytotic activity in early life [59].

Heimann (1980) studied the enteral absorption of drugs in children with several drugs showing similar overall absorption with age yet a significant increase in the rate of absorption (Ka) with age from neonates and young babies (up to 150 days) compared to older children[60]. Drugs investigated included: D(+)xylose, which is absorbed by an active mechanism in the upper small intestine; L(+) arabinose which is absorbed by passive diffusion; sulphonamides, phenobarbitol, digoxin and β-methyldigoxin all showing similar results [60]. These findings suggest that the rate of absorption is slower in neonates and children yet the amount absorbed is similar (matched by mass). A further study was conducted to measure the effects of intestinal motility on absorption using metoclopramide; the results showed an increase in absorption rate constant, Ka, in both young and older infants yet the ratio of Ka: age remained constant [60]. These results suggest that the reduced Ka observed was not solely due to longer transit times or reduced motility but other factors are also likely to be involved.

4.4. PGP Expression in the gut wall

The efflux transporter P-glycoprotein (P-gp), also known as multidrug resistance protein-1 (MDR1), is a member of the adenosine-5'-triphosphate binding cassette family of proteins and is responsible for cellular drug efflux, transporting substances from the intracellular to the extracellular compartments. P-gp is found within the cellular membranes of the gastrointestinal tract and can markedly affect the bioavailability of certain drugs, particularly those with low solubility. Previously

it has been reported that the variability in intestinal absorption for many drugs, when used in combination, is likely to be a direct result of the variability of P-gp expression within the GI tract [61]. Many drugs that are P-gp substrates are also substrates of other efflux transporters as well as absorptive transporters (e.g. anion or cation transporters) therefore the overall transport of such drugs are most likely to be affected by both expression of P-gp and the presence of additional P-gp substrates.

Methods used to quantify expression of P-gp are complex due to the difficulties associated with isolation of integral membrane proteins. Proteomic approaches [62] were used to determine protein expression levels of trypsin-digested breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 2 (MRP2) in tissue samples [63-65], although limitations of these methods included poor reproducibility of extraction and sample digestion. Purification and reconstitution into proteoliposomes is possible (eg [66]), but expensive and technically challenging. Tucker *et al.* (2012) recently used an immunochemistry approach to generate transporter protein standards for immunoquantification [67].

Van Kalken *et al.* (1992) demonstrated expression of MDR1/P-gp mRNA in the embryonic phase of human development. However, some differences were found between foetal and adult human tissue distribution; prenatal intestine did not show staining of the epithelium, yet MDR1-mRNA expression was observed in late specimens [68]. Mahmood *et al.* (2001) studied the ontogeny of P-gp in mouse intestine, liver, and kidney. Intestinal P-gp expression was limited at birth and increased significantly with maturation. Annaert *et al.* (2010) measured drug transport in intestinal tissue to investigate P-gp modulated transport and showed that adult expression levels were reached at about 6 weeks in rats [69]. These animal models suggest that P-gp expression increased with age although there is limited information on the age at which adult expression is reached.

There is conflicting evidence regarding the ontogeny of expression of P-gp in paediatric populations. Johnson and Thomson (2008) reported that the expression of P-gp appears to increase rapidly during

the first 3-6 months of life, reaching adult levels by approximately 2 years of life [70]; whereas Fakhoury *et al.* (2005) reported that P-gp expression in the intestine was not influenced by age with mature expression in neonates and infants [71].

4.5. Techniques to measure permeability and the impact of age

Permeability may be assessed by a variety of techniques including those based on *in silico* drug properties such as logD or hydrogen binding potential; *in vitro* passive diffusion across a membrane or cultured cell line or excised human or animal tissue; or in situ perfusion techniques. Typically simpler methods are used in the first instance with cut offs in terms of high and low permeability measured by comparison to drugs where *in vivo* fraction absorbed in known. There is specific guidance for formal BCS classification of high permeability [19]. All human perfusion data have been conducted in adult populations [72] and to date there have been no attempts to correlate these values of paediatric measures of permeability.

Caco-2 cell lines are the most commonly used cell lines to measure permeability as they are high throughput and acceptable methods for BCS classification. However, these cell lines are known to underestimate true permeability due to (i) under-/over-expression of Pgp efflux pumps; (ii) reduced paracellular transport due to the absence of lipid pores; (iii) non-specific binding to components within the apparatus and (iv) high laboratory variability in the characteristics of Caco-2 cell cultures used, depending on passage used and growth media [73]. MDCK cells (of canine origin) are sometimes used as an alternative to Caco-2 cell lines as they form a monolayer in a shorter time period [74]; however the expression of various efflux pumps are low in this cell line. Double transfected MDCK cells where the canine transporters are replaced by human transporters are often used to better understand the influence of transporters on drug permeability (e.g. [75]).

Due to very limited availability of human intestinal tissue for investigating intestinal drug transport, *in vitro* and *ex vivo* mechanistic studies on intestinal drug permeability have typically been

conducted in intestinal tissue obtained from animals. In rats microvascular permeability of a dye was reported to increase more than twofold from weaned to adult animals with a further increase observed in aged rats [76].

Annaert *et al.* (2010) measured the permeability of several drugs across excised rat intestinal tissue from 6 week old (young) animals compared to adult rats (14 weeks) using an Ussing chamber [69]. Metoprolol was used to assess transcellular transport with values similar in the young and adult rats; atenolol was used to assess paracellular transport and showed a decrease in permeability in adults compared to young rats; talinolol was used to assess P-gp modulated transcellular transport also showed a decrease in permeability in adult rats compared to young animals [69]. Additional studies were undertaken to better understand the impact of age on P-gp transport using verapamil in addition to talinolol; in adult rats the presence of verapamil significantly decreased the permeability of talinolol whereas the permeability was unaffected in young rats. This finding indicates that P-gp has a very limited role in the absorption of talinolol in the young animals [69].

Said *et al.* (1990) measured the transport of biotin across intestinal tissue in rats comparing suckling, weaning and adult rats with significant decreased in permeability associated with age grouping; this finding was attributed to a decrease in the number of biotin transport carriers with age [77].

Garcia-Miranda *et al.* (2005) measured L-carnitine uptake across intestinal tissue from foetal, newborn, weaning, suckling and adult rats; high uptake was observed in foetal and newborn rats with a decrease during the suckling period to unmeasurable levels following weaning [78]. Carnitine/organic cation transporter (OCTN2) is the carrier system involved in apical L-carnitine uptake in the gut; Garcia-Miranda *et al.* (2005) measured OCTN2 mRNA from rat intestinal samples and the results matched those observed for transport with expression peaking in foetuses and newborn rats with a subsequent decrease with age [78].

Folate absorption from the small intestine is mediated via the reduced-folate carrier (RFC). Balamurugan and Said (2003) measured folic acid uptake across brush border membrane vesicles isolated from suckling, weanling and adult rats; they reported a significant decrease in uptake with age [79]. Quantification of RFC protein and mRNA levels demonstrated significantly higher levels in suckling compared with weanling rats, which were in turn significantly higher than that in adult rats.

4.6. Permeability within the oral cavity

As stated in section 3.4 (Biorelevant solubility approach for paediatric populations) formulations used in paediatric populations are often retained within the oral cavity for longer than comparative adult preparations. Absorption within the oral cavity offers a means to bypass first-pass metabolism and avoid of pre-systemic elimination within the GI tract; however the relatively small surface area of the oral mucosa and the significant loss of drug due to uncontrolled swallowing and salivary flow are the main limitations of this route. There is no literature evidence that details differences in permeability with age in buccal tissues. However, clinical data is available to demonstrate absorption of tacrolimus and midazolam via the oral cavity in paediatric patients [80, 81].

4.7. Developing paediatric permeability methods

The lack of high quality paediatric pharmacokinetic studies reported limits knowledge regarding permeability mechanisms within this population. The few bioavailability studies that have examined the absorption of drugs (eg, phenobarbital, sulfonamides, and digoxin) and nutrient macromolecules (eg, arabinose and xylose) suggest that the processes of both passive and active transport are fully mature in infants by approximately four months of age. However, most studies conducted revealed that absorption in neonates and infants is slower than that of children and adults [82]. Furthermore, the relative smaller absorptive surface area in infants and neonates, and thus fewer receptors and transport proteins per square unit intestine, is also likely result in a slower absorption of compounds. Current evidence regarding P-glycoprotein expression and other absorptive and efflux

transporters is contradictory therefore there may be differences in absorption of drugs in the very young.

Additional research is required to better understand and characterise absorptive sites within the paediatric GI tract to enable representative models to be developed.

5. Dissolution testing and *in vitro in vivo* correlation (IVIVC)

Drug dissolution in the physiological environment of the GI tract is often a rate limiting step in the oral drug absorption process. Only dissolved drug can permeate the mucosa at the absorptive sites in the GI tract [83]. Hence, both solubility of the drug and its dissolution rate are crucial for the *in vivo* behaviour. To some extent these properties are determined by the physicochemical characteristics of the drug itself and in addition, they can be strongly affected by the physiological conditions in the gastrointestinal tract [84]. Thus, when the aim is to estimate the *in vivo* dissolution process of a given formulation, it is essential to adequately address the relevant *in vivo* parameters within an *in vitro* setup.

5.1. Compendial dissolution approach

In 1970, the rotating basket system (apparatus 1) became the first apparatus to be incorporated into the United States Pharmacopoeia (USP) [85] as a dissolution device for solid oral dosage forms. Shortly thereafter the paddle method (apparatus 2) [86] was accepted as the second official dissolution method. During the following years, the field of dissolution grew and a number of pharmacopoeias adopted additional apparatus and refinements thereof. Currently, dissolution test devices for testing solid oral dosage forms are described in various pharmacopoeia, such as the US (USP), the European (PhEur), the British (BP) and the Japanese Pharmacopoeia (JP). The largest number of official dissolution methods can be found in the USP, which currently describes seven different dissolution devices.

During the last decades, dissolution testing has evolved as a highly valuable *in vitro* test to characterize drug delivery performance. For a long time, it has been mainly used as a tool for quality control and regulatory purposes. Many monographs specifying dissolution conditions for quality control of specific drug/formulation combinations can be found in international pharmacopoeia. Because of the importance dissolution testing has assumed in the last few decades, various official regulations have been published worldwide. For instance, the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) have published several guidance documents that provide information and recommendations on the development of dissolution test methodology, set dissolution specifications and describe the regulatory applications of dissolution testing.

Current compendial methodologies used to characterize drug release from oral dosage forms are mostly still based on the paddle and basket apparatus in combination with compendial media, i.e. simple aqueous buffers. The typical media volume is in the range of 500-1000 mL and where the volume is not sufficient for complete drug dissolution, surfactants, such as sodium lauryl sulphate (SLS) or Tween 80, are added to provide sink conditions. While such test methods are generally useful for quality control, they do not reflect complex human gastrointestinal physiology and, particularly for formulations containing poorly soluble drugs, are of limited use when the objective is to predict the *in vivo* performance of a dosage form or to achieve an IVIVC for both adult and paediatric formulations. In recent years however, there has been a strong push to identify bioavailability problems of a drug formulation based on the results of appropriately designed dissolution experiments.

5.2. BCS dissolution approach

As indicated in the previous section, the BCS [31] was established as a prognostic tool for estimating the *in vivo* bioequivalence of orally administered formulations. The original purpose of developing such a scientific framework was to reduce regulatory burden without compromising the quality of drug products. According to official FDA and EMA guidelines [19] [22] *in vivo* bioequivalence studies

may be exempted if an assumption of equivalence of *in vivo* performance can be justified by satisfactory *in vitro* data. Currently, a BCS-based biowaiver is restricted to highly soluble drug substances with known human absorption formulated in solid oral IR formulations. BCS dissolution experiments are designed to screen for similar *in vitro* dissolution of two IR products under physiologically relevant experimental pH conditions. *In vitro* dissolution has to be investigated using the basket or the paddle apparatus at 100 or 50 rpm in up to 900 mL of medium within the pH-range of 1 – 6.8 (at least pH 1.2, 4.5, and 6.8) [19, 22]. In contrast to the compendial test design, the use of surfactants is not allowed. A rapid or very rapid drug release (> 85% within 30 or 15 min) in all three pH conditions indicates that no bioavailability issues with the IR formulation should be experienced. Nowadays, the BCS dissolution approach is not only used in the regulatory bioequivalence assessment but also as a scientific and mechanistic classification tool at almost every stage of drug discovery and development [87].

The main principle of the BCS *in vitro* dissolution experiments is to determine drug release over the entire GI pH range. However, as already discussed for the BCS solubility approach, no additional parameters that can affect intraluminal solubility and dissolution of a compound are addressed in this *in vitro* test design. Beyond that, the media volume is high and thus often even not representative for the fluid volumes available in the upper GI tract of adults. This is particularly true for the fasted state. The dissolution profiles obtained in such conditions hardly can be a surrogate for the *in vivo* performance of these formulations since neither the pH conditions, nor the volume or any additional media parameters seem to be appropriate to simulate the intraluminal contents in the upper GI tract of children. It is also questionable, if the basket and the paddle run at 100 or 50 rpm, respectively, can reflect the hydrodynamics, i.e. GI motility and fluid / chyme movement in the paediatric GI tract in a meaningful manner.

5.3. Biorelevant dissolution approach

The biorelevant dissolution media shown in Table 4 were developed to reflect the physicochemical parameters of intraluminal fluids in the fasted and fed state upper GI tract of an average adult. These media were successfully used in establishing *in vivo in vitro* correlations for oral immediate- and modified release (MR) formulations, using simple compendial dissolution equipment, such as the paddle, the flow-through cell or the reciprocating cylinder apparatus [83, 88-93]. The application of biorelevant media was also successful when the aim was to predict *in vivo* precipitation of weakly basic drugs after gastric dissolution and subsequent emptying into the small intestine [94, 95] using a non-compendial transfer model [41, 96]. The latter results indicate that not only in the case of MR formulations which typically release the drug along various segments of the GI tract, but also for several IR formulations adequate prediction of the *in vivo* performance is only possible with a sophisticated test setups. Particularly for dosage forms containing poorly soluble weak acids and bases that may precipitate in the GI lumen, a multi-segment approach as used in the transfer experiments can better address the changing GI environment to which drug and dosage form are exposed prior to reaching the site of absorption.

Through the use of modern techniques in monitoring the gastrointestinal transit of oral dosage forms, it is known that (i) independent of the nature of the formulation gastrointestinal transport is a discontinuous rather than a continuous process; (ii) there is also not necessarily a continuous phase of free fluid available in the gut lumen; and (iii) the intraluminal conditions can be strongly affected by food intake [97]. Based on these findings, in the last decade, a variety of biorelevant dissolution approaches, addressing not only the (changing) media composition and digestion (see Table 4), but also the relevant residence times and motility patterns in the human gastrointestinal tract, were introduced. Approaches that address conditions in the upper GI tract include for instance a biorelevant stress tester to simulate the impact of physiologically-based mechanical stress that may occur during the GI passage of a dosage form and also to simulate an intermittent contact of

the dosage form with the dissolution medium [98, 99], the dynamic gastric model (DGM) to simulate mechanical and enzymatic gastric processing of foods and the impact of digestive processes on the dosage form performance [100] and the TIM-1 artificial digestive system, representing a multi-compartmental, dynamic, computer-controlled gastrointestinal model intended to simulate a range of physiological parameters including the secretion of saliva, gastric- and pancreatic juice along with digestive enzymes, bile flow, peristalsis for mixing and transport, the changing gastric and intestinal pH conditions and the continuous removal of digested lipophilic and hydrophilic compounds [101]. With these models, the complex influences of physiological parameters on *in vivo* dissolution is likely to be even better understood and application of such models often provide a good *in vivo* predictivity for the average adult subject.

5.4. Biorelevant dissolution approach for paediatrics

The dissolution methods cited in previous sections (5.1-5.3) were developed to better understand the impact of different physiological parameters on *in vivo* drug release [41, 83, 88-96, 98-101]. In many cases, application of such physiological based models enabled an IVIVC to be achieved and thus, they have established themselves as valuable tools in formulation development. However, these approaches address adult physiology rather than simulating physiological conditions in the GI tract of children. Therefore, without modification, they will not be applicable to simulate the physiological parameters relevant to drug release and absorption in the paediatric population.

In addition, whilst these biorelevant approaches for the adult population address many details of human GI physiology, they are not necessarily applicable to simulate conditions in the oral cavity. Since current paediatric drug therapy is dominated by oral liquids, multi particulate dosage forms or formulations intended to disintegrate in the oral cavity [102-104], this site is of particular importance in paediatric drug therapy. For the cited, but also for any other oral dosage form, contact with saliva can have a huge impact on the overall *in vivo* performance since it might help to

dissolve the drug, yet can also cause precipitation or degradation of the drug or result in unforeseen drug release from enteric coated dosage resulting in exposure to the taste buds. To date, a biorelevant model simulating all aspects relevant to dissolution in the oral cavity has neither been established for adults, nor for children. For assessing the *in vitro* behaviour of chewable tablets and medicated chewing gums in quality control, a mastication apparatus is described in the PhEur [29]. For oral lyophilisates and orodispersible tablets, a disintegration test performed in water is regarded as an adequate quality control method [29]. For orodispersible films and compressed lozenges the Ph.Eur. requests a suitable test to be carried out to demonstrate the appropriate release of the active substance(s), but details on how such a test should be performed are not given [29]. In both USP [105] and the FDA Dissolution Methods Database [106] dissolution methods for orally disintegrating tablets are specified [107]. However, these methods are based on either the paddle or the basket apparatus and experiments have to be performed in 500 – 1000 mL of water or simple buffer media. Thus, the methods might be useful for QC, but are not suitable for any biorelevant approach.

Over the last years, several alternative methods for screening drug release of orally disintegrating / dissolving formulations have been proposed. These range from modified paddle and setups through several non-compendial disintegration testers and the texture analyzer through several other non-compendial devices [107-110]. These methods also use different endpoints such as drug dissolution, wetting time, dispersion or disintegration [109]. However, these methods were mainly developed for quality control and only partly address the conditions relevant to dissolution in the oral cavity. This is particularly true for the media applied in these experiments. In most of the approaches water and simple buffer media, sometimes not even representing the physiological pH-range in the oral cavity, were utilized. However, a biorelevant test setup would require using a physiologically relevant medium based on the physicochemical characteristics of human saliva. Several simulated salivary fluids have been proposed in the last decades [111-116] and were recently reviewed by Marques *et*

al. (2011) [117]. The simulated salivas differ in composition but all fluids contain a variety of electrolytes and some of them also several of the numerous additional constituents of human saliva, mainly represented by enzymes and mucosal glycoproteins. Nevertheless, similar to the all other biorelevant fluids discussed in this review, the simulated salivary fluids reported in literature address the average saliva composition of adults rather than considering the saliva composition of children of different age groups.

It is clear that a universal approach to dissolution testing to predict *in vivo* drug release in the paediatric GI tract is extremely complex as the physiological aspects relevant to age, dosage form and dosing conditions have to be addressed in the test design.

To date, no concepts for biorelevant dissolution approaches for paediatrics have been presented. However, as already discussed in previous sections, there is a clear need in establishing such test methods. A BCS-like but biorelevant solubility test approach would represent an adequate base for such a dissolution test design, i.e. establishing a set of biorelevant media simulating the physicochemical conditions following typical dosing conditions in the upper fasted and fed state GI tract of the paediatric population is essential. Moreover, the age-dependent fluid volumes available in the GI segments relevant to drug release as well as the residence times in these segments would need to be addressed. As indicated before, in recent review articles [3, 4, 44] typical fluid volumes available at the relevant sites in the paediatric GI tract were not discussed. Detailed information on gastric emptying and intestinal transit times are also lacking and residence times in the oral cavity are not discussed. However, even with the little information presented in the cited articles [3, 44], it is obvious that gastric emptying and small intestinal transit in newborns and infants are essentially different from children, adolescents and adults. As gastric emptying determines the rate at which a drug appears at the main site of absorption, this should be one of the key parameters in the biorelevant dissolution test design.

However, even following characterization of the paediatric GI tract and establishing a set of biorelevant media for the different age groups, there are several additional parameters such as residence time in the oral cavity, passage times through the different segments in the GI tract and GI motility will also need to be addressed to develop a relevant model. Compendial paddle and basket equipment are unlikely to be appropriate for this purpose since they cannot be run with the small media volume that might particularly be required for simulating GI conditions in the very young children. Therefore, the flow through cell is considered to be the best current compendial option. In the last few decades, various miniaturized dissolution setups have been introduced and the mini paddle [118, 119] also might represent be an alternative to the compendial paddle apparatus.

Whenever the intention is not to simply screen the impact of media volume and composition but also that of additional physiological parameters on drug release, non-compendial (-based) setups will be required. In cases where *in vivo* drug release from oral IR dosage forms in adults is affected by several physiological factors, *in vitro* results obtained with physiologically based dissolution methods such as the transfer model, biorelevant stress test apparatus, the Dynamic Gastric Model (DGM), or the TIM-1 apparatus typically provide a much higher *in vivo* predictivity compared to results obtained with simple (compendial) test setups. Therefore, physiologically based dissolution models should be developed for different paediatric age groups.

Several formulations like oral films, orally disintegrating tablets or granules, but also oral liquid formulations will require a test setup simulating conditions in the oral cavity. As discussed before. compendial apparatus are not suitable for this purpose and also the alternative approaches presented in the literature cannot be regarded as biorelevant. Therefore, there is a strong need to establish adequate dissolution tests addressing these needs. In addition, establishing an orogastric transfer model to screen for the impact of residence in the oral cavity on the overall *in vivo* performance of the formulation should be considered. As already discussed for a biorelevant paediatric solubility approach, a longer residence in the oral cavity could result in: precipitation of

poorly soluble weakly basic drugs; impaired stability of a compound; loss of integrity of enteric coatings and consequently affect the bioavailability of a number of compounds and formulations.

5.5. Developing paediatric biorelevant dissolution testing methods/apparatus

Currently, biorelevant dissolution tests for the paediatric population are not available. However, such test methods represent an essential prerequisite for increasing the safety and efficacy of oral paediatric drug formulations. Biorelevant dissolution methods should enable to establish IVIVCs, that is, predict the *in vivo* performance of an oral dosage form based on results obtained in an *in vitro* setup addressing the relevant physiological parameters of children of the different age groups. In designing such biorelevant and *in vivo* predictive dissolution tests for the paediatric population, as well as oral and gastrointestinal solubility of orally administered drugs essential information is required on the following: (i) the residence times in the oral cavity and the different GI sections, (ii) the fluid volumes available at these sites and (iii) the motility pattern and the passage times in the paediatric GI tract. Unfortunately, most of these data cannot be obtained from the current literature. However, the few physiological data available to date clearly indicate that particularly in the very young age groups (newborns, infants and pre-school children) big differences to the adult physiology has been reported. As already mentioned for the biorelevant solubility approach, there is a strong need to adequately simulate the composition of salivary, gastric and small intestinal fluid in both the fasted and the fed state. Thus, for a biorelevant dissolution test design, there is a strong need to characterize the paediatric oral cavity and gastrointestinal tract in more detail taking into account both typical fasted and the fed state dosing conditions for each of the different age groups. Based on this information, and based on the experiences made in the biorelevant characterization of adult formulations, it should then be possible to compose age related test setups with sufficient *in vivo* predictivity and which will help to improve the quality of oral paediatric drug therapy.

6. Metabolism

First pass metabolic inactivation of drugs can affect the bioavailability of orally administered medicines; the intestine and liver are the most significant sites involved in first pass drug metabolism. There are many drugs whose oral bioavailability is reduced to half the administered dose as a result of first pass metabolism in the intestine and liver [120].

It is generally stated that the activity of drug metabolising enzymes is low at birth and reaches adult values by early childhood. Children in general have a larger liver size and hepatic blood flow per body weight than adults [121], which tends to increase hepatic clearance of chemicals when enzymatic activity is similar to that observed in adults. This has consequences in terms of dosage adjustment where scaling based on mg/kg is not appropriate.

The ontogeny of specific metabolic pathways has significant consequences for individual therapeutic agents and this needs better understanding when extrapolating adult data into paediatric populations. The example of the grey baby syndrome resulting from dosing chloramphenicol to neonates at doses extrapolated from adult data is often used to highlight the importance of understanding ontogeny of metabolic pathways [16]. This review will summarise existing knowledge on metabolic ontogeny with a focus on the intestine and liver as sites of metabolism. For more extensive reviews the reader is directed to alternative sources including; ontogeny of drug metabolising enzymes [14, 122]; and age related changes in the metabolism of drugs [14, 123-127]. This review will limit its focus to neonates up to adulthood with foetal data included as a point of reference rather than the focus of the review.

6.1. Drug metabolism in the gut lumen

The microflora of the intestinal lumen changes with age; this alteration in bacterial colonization has implications in terms of drug metabolism within the gut. This topic has been reviewed for adults previously (eg [128, 129]).

The greatest changes in colonisation occur in the hours and days immediately following birth. The pattern of bacterial colonization within the gut is influenced by feeding with differences observed in breast fed compared to formula fed infants [130]. There are known differences in bacterial composition based on age and diet [131], these factors may be important in predicting the free concentration of active drug within the gut lumen in infants and children. Andrieux *et al.* (2002) compared the faecal microflora from children (3-15 years) to adults and elderly adults to investigate levels of bacterial enzymes (β-galactoside, α-galactoside, β-glucoside, β-glucuronidase, neuraminidase, N-acetylgalactosaminidase, α-fucosidase, nitrate reductase and azoreductase). There were no significant differences in the enzyme activities between the three populations; although the data was more variable for the children [132]. This result is interesting as it suggests that luminal metabolism will match adult values from 3 years, however, there is still very limited data on infants and neonates. Other sources also agree that intestinal colonisation reaches adult-like composition by aged 1-4 years (eg [133-135]).

The maximum number of operational taxonomic units (OTUs) for children between 0 and 1 year is approximately 1000, whereas adults commonly exhibited between 1000 and 2000 OTUs [136]. However, interindividual variations are significantly greater among children than among adults: the microbiota of children is dominated by a few bacterial genera and species, with *Bifidobacterium* being most prevalent in infants [137].

The age dependant excretion of digoxin reduction products by inactivation within the gut lumen has been shown to increase with age; from 1-3% of infants; 7% of children; 10% of adolescents compared to 40% of adults [138]. However, Linday *et al.* (1987) also reported that the digoxin-inactivating bacteria (*Eubacterium Lentum*) were present as early as the second week of life. This suggests that it is the metabolic activity of the gut flora that needs to be understood rather than simply quantifying the species present.

Bile acids and neutral sterols are metabolised extensively within the lumen of the human intestine. Huang *et al.* (1976) compared faecal samples from infants <1.5 years children at 4 years and adults to look at metabolic products of bile acids and neutral sterols [135]. Their results suggested that metabolism by 7 α-dehyroxylation of bile acids increased significantly with age. Metabolism of cholesterol (by bacterial biohydrogenation and subsequent reduction) also increased with age with values at 4 years being similar to those found in adults [135]. This data agreed with previous reports that 7 α -dehydroxylation bacteria are not established in the gut before 12-18 months [139].

Previous studies reported that excretion of methane in breath, as a consequence of specific gut microflora, did not occur in children younger than 2 years of age and reached adult values at 10-14 years [140]. This finding is supported by research conducted by Rutili *et al.* (1996) showing that methanobacteria was not detected in faecal samples from children under 27 months of age; with an incidence of 40% at 3 years and 60% at 5 years [141].

These data indicate that metabolism within the gut is similar to adults at some point ranging from 12 months to 14 years. This finding demonstrates the need for additional research in this area to better understand bacterial colonisation as well as the impact of diet on luminal metabolism of drugs in paediatric populations.

6.2. Drug metabolism in the gut wall

Metabolism in the gut lumen and wall can decrease the bioavailability and the pharmacological effects of a wide variety of drugs including cyclosporine, nifedipine, midazolam, verapamil [142-145]. The mechanisms that underpin these processes have previously been reviewed in adults (eg [128, 146]).

It is primarily enzymes that are responsible for metabolism of drugs as they traverse the intestinal wall therefore the ontogeny of enzymatic activity can affect the fraction of drug absorbed escaping first pass metabolism within the gut wall (Fg).

Stahlberg *et al.* (1988) measured the activity of aryl hydrocarbon hydroxylase; epoxide hydrolase and glutathione peroxidase using small intestinal biopsies taken from children. The data showed that neither epoxide hydrolase nor glutathione peroxidase showed any change in activity with age [147]. However, aryl hydrocarbon hydroxylate showed a significant increase in activity with age with a correlation of 0.38 and values for <1year olds being 64 pmol/min/mg protein compared to 185 pmol/min/mg protein in 11-12 year olds [147]. However, other research has demonstrated that epoxide hydrolase activity does change with age as foetal gut activity of epoxide hydrolase was twofold higher compared to adults [148].

6.2.1. CYP 3 gene family

The cytochromes P450 (CYPs) constitute the major enzyme family capable of catalyzing the oxidative biotransformation of most drugs and are therefore of particular relevance for clinical pharmacology.

The CYP3A subfamily is predominant, accounting for approximately 70% of the cytochromes in the adult small intestine, and is involved in the metabolism of more than 70% of currently administered drugs [149].

CYP3A4 and CYP3A5 are abundantly present in the small intestine in adults; yet there is limited data regarding their expression in paediatric populations. A study to investigate localisation and expression of CYP3A in 59 normal duodenal biopsies from Caucasian children aged 1 month to 18 years; showed that CYP3A was expressed in all children 6 months and older and in half those up to 6 months [71]. However, there was a subsequent decline in CYP3A4 and CYP3A5 levels with age from 1-17 years [71]. A study by Johnson *et al.* (2001) investigated intestinal expression of CYP 3A with age; an increase in CYP3A expression was observed that was mirrored by a corresponding change in CYP3A4 enzyme activity [150].

6.2.2. GSTA1-1 Expression

Glutathione S-transferase alpha 1 (GSTA1–1) is the enzyme principally responsible for the conjugation of busulfan. Results from intestinal biopsies from children showed that busulfan-GSH conjugase activity was 77% greater in intestinal biopsies obtained from children under 5 years compared to those from 9-17 years old [151].

6.2.3. Sulfotransferases

Sulfotransferase (SULT) activity has been reported in both adult and foetal intestine; with mean activity values more than three times higher in foetal tissues compared to adult [152]. Cappiello *et al.* (1991) measured the activity of SULT isoforms from adult of foetal intestinal tissue samples using either p-nitrophenol or dopamine as substrates; their results found higher adult activity (ratio = 2.6) for p-nitrophenol substrate mediated activity and lower adult activity (ratio 0.8) for dopamine mediated activity [153].

6.2.4. Alcohol Dehydrogenases (ADH)

Using intestinal tissues samples from subjects ranging from 9 weeks gestation to 20 years, the expression of ADH was not observed to change appreciably with age [154].

6.2.5. Uridine 5'-diphosphate-glucuronosyltransferases (UGTs)

UGTs are highly expressed in the gastrointestinal tract, where they have the potential to influence the pharmacokinetics and biological effects of ingested drugs. However, there is little known about the ontogeny of UGT expression in the intestine.

These findings in 6.2.1-6.2.5 demonstrate that gut wall metabolism can vary with age depending on expression of specific enzymes at this site and their ontogeny. Further research is required to better understand the expression of these compounds as well as their activity with age and how this can affect bioavailability of many susceptible drug compounds.

6.3. Drug metabolism in the liver

There is a wealth of literature data on ontogeny of hepatic enzymes; Table 6 presents a summary of this data with the reader directed to other sources for more complete reviews (eg [14, 155, 156]). This table includes information on expression and/or activity of major enzymes although it is recognised that these factors are not correlative. Indeed, differences have been observed when reporting enzyme expression based on mRNA versus protein quantification versus enzymic activity which can lead to different conclusions regarding enzyme ontogeny [157, 158].

Table 6. Summary of expression/activity of hepatic enzymes in paediatric populations as a percentage of adult values

Enzyme	Foetus	Neonate	Infant	Child (2-5y)	Child (6-11y)	Adolescent (12-18y)	Comments	Reference
Human hepatic esterases	10		25					[159]
P450 family								
CYP 1A2		4-5	10-25	50-55	50-55	-	CYP1A2 activity may be delayed in breast fed infants [160]	[161]
		10	50					[162, 163]
		50		150		100		[164]
			30-81					[127]
						100		[165]
CYP 2A6						100		[165]
CYP 2B6	0		10					[163]
	present						Adolescents show reduced values compared to adults	[166]
CYP 2C9		30	100					[124]
CYP 2C19	12-15				100			[124]
CYP 2D6		5	30	70				[167]
			100					[168]
CYP 2E1	10-30		100					[169-171]
CYP 3A4	10		30-40	100				[172]

			50					[173]
		15	15	30		50		[168]
CYP 3A7	1200	700	200	100	100	100		[168]
Alcohol Dehydrogenase (ADH)			100				A switch occurs from foetal expression of ADH1A to adult expression of ADH1C	[15, 154]
Aldo-ketoreductases					75			[174]
Aldehyde Oxidase		10	100					[175]
Flavin mono-oxygenases (FMOs)			Expression measured		50	100	FMO expression switches from FMO1 expressed within the foetus to FMO3 within the adult	[176]
Epoxide Hydrolases	25-50							[177, 178]
Uridine 5'-diphospho-glucuronosyltransferase								
UGT1A1			100	100			Adult levels observed at 4 months	[179, 180]
			50			50		[181]
UGT1A4	0	present	100					[179, 182]
UGT16A	0	present			100		Adult levels observed at 14 months	[180, 183, 184]
						50		[181]
UGT1A9			45-60	100				[179]

						50		[162]
UGT2B4			40	40				[179]
UGT2B7	10-20		100	100				[179, 185]
		10	15-20	15-20	50-60	70		[186]
UGT2B17	3	13						[187]
Sulfotransferases (SULT)	100							[188]
SULT1A1	100							[188]
SULT1A3	>100	100						[189, 190]
SULT1E1	>100							[188, 190]
	<25							[152, 191]
SULT2A1		100						[188, 190]
N-acetyltransferases (NAT)							Present in neonates, increasing in infants with a reduction in children	[192]
NAT1	30			100				[193, 194]
Thiopurine S-methyltransferase (TPMT)	30							[195]
Glutathione S-transferases (GSTs)								
GSTA1	70	100						[196, 197]
GSTA2	25	60						[196, 197]

GSTM	20	100		[196]
GSTP1	Present at high levels	Present	Undetectable in adult samples	[197]

Note that all values are approximations and may refer to % expression or % activity relative to the adult value.

Table 6 highlights that the most clinical relevant phase 1 enzymes (CYPs) are generally present at low levels in neonates reaching adult values at 1-5 years depending on the isoform. Phase 2 enzymes also show a wide age range in reaching adult values; UGT reaching adult values are dependent on the isoform; SULT adult values are reached early for all isoforms; NAT is variable in infants and reduced in children reaching adult values between 2-5 years; there is limited data on TMPT in children.

Differences in enzyme expression and activity can result in altered oral bioavailability of drugs (eg midazolam and zidovudine [198, 199]) or production of metabolites in paediatric populations that are not observed in adults (eg caffeine production in neonates receiving theophylline, differences in metabolite production in children with valproic acid, paracetamol, chloramphenicol, cimetidine and salicylamide [13]).

Liver blood flow is increased in paediatric populations due to the larger ratio of liver to total body mass in infants and young children [121]. This increased blood flow to the liver will increase hepatic clearance of drugs. The first pass effect where a drug is cleared on first passage through the liver may therefore be greater in paediatric patients although the level of enzymes present will also influence this rate. Recently the microsomal protein content within the liver has been reported to increase with age from an estimated of 26mg/g in neonates rising to a maximum of 40mg/g in a 30 year old adult [200].

6.4. Future work required to understand paediatric metabolism mechanisms

The information on ontogeny of metabolic pathways is very limited with patchy coverage of even the most important enzyme families (eg CYPs). More data has been reported from foetal samples and neonates with very limited information from infants and children. There is more data on liver

ontogeny compared to intestine; this may be expected due to the relative contribution of metabolism from these two organs. Some enzyme systems develop within the foetus demonstrating near adult values at birth (eg sulfotransferases); these cause less concerns, in terms of drug performance, compared to those with significant postnatal development and associated variability.

There are at least two examples where the bioavailability of drugs was increased in children compared to adults as a result of differences in metabolism; midazolam and zidovudine. De Wildt *et al.* (2002) reported that midazolam elimination in preterm infants was approximately 10-fold lower than that reported in older children and adults; this reduction followed the pattern of CYP3A4 ontogeny in both the intestine and liver. A comparison of orally and intravenously administered midazolam enabled the authors to conclude that both liver and intestinal metabolic pathways contributed to the difference in elimination observed [199]. The oral bioavailability of zidovudine was observe to decrease from 89% in neonates under 14 days to 61% in those over 14 days old [198].

There are several recognised knowledge gaps in this field where additional research is required to define the true ontogeny of many enzyme systems. Efforts are required to ensure that future studies embrace a wide range of ages rather than relying on small sample sizes with a snapshot of expression; also appropriate methodology to ensure that the enzyme activity measurements are robust.

7. **Paediatric Clinical Testing**

In paediatric drug development several factors need to be considered to justify the decision to proceed with a pediatric clinical program for a medicinal product. These include: the presence of a serious or life-threatening disease for which the medicinal product represents a potentially important advance in therapy; the novelty of the medicinal product; the existence of unique paediatric indication and the need for paediatric formulation.

In drug development, bioavailability clinical studies are needed for a novel compound for determination of: the best administration route (absolute bioavailability) and/or the best formulation (relative bioavailability), whereas bioequivalence studies determine the equivalence of formulations for novel/old compounds.

ICH E11 guidance on clinical investigation of medicinal products in the paediatric population [11] provides information on if and/or when pharmacokinetic, bioavailability and bioequivalence studies have to be performed to determine pharmacokinetic parameters in the paediatric population, to enable dosing recommendations and to support formulation development.

The FDA Pediatric Study Decision Tree [201], reproduced in Figure 1, provides a simple assumptions-based framework that can be a helpful starting point in determining the pediatric studies (excluding oncology studies) necessary for labeling based on the ability to extrapolate efficacy from adult or other data.

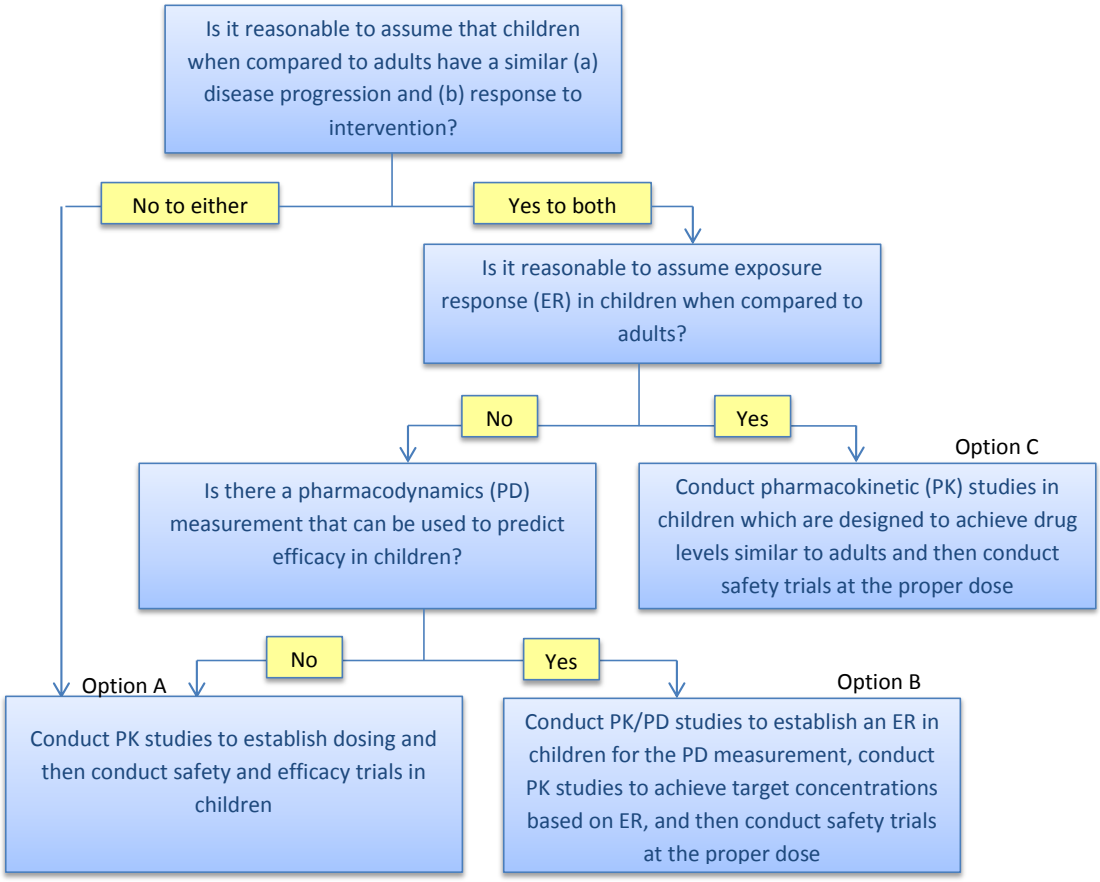

Figure 1: FDA paediatric study decision tree (reproduced based on [201])

Bridging procedures can be used if disease progression, exposure-response relationships, and clinical endpoints are similar in adults and children and in these circumstances, confirmatory efficacy trials in children are not necessary [11, 202] and only safety trials have to be conducted. Otherwise efficacy trials need to be conducted in the paediatric population due to a lack of any appropriate pharmacodynamic measurement (Figure 1) [12, 23, 202]. Safety profiles cannot be transferred directly to children even when the disease processes are the same [203]. The regulatory criteria for accepting extrapolation and/or modeling as pivotal evidence may take into account factors other than the ones mentioned in the decision tree (for example feasibility of trials, ethical constraints, and unmet medical need) [204]. The extrapolation of efficacy from adult and other data to the pediatric population minimizes the exposure of children to clinical trials; increases the speed and efficiency of pediatric drug development and allows pediatric patients timely access to safe and effective medicines [201]. The experience of the US FDA in extrapolating efficacy in paediatric drug-development programs has shown that for 370 paediatric studies submitted to the FDA between 1998 and 2008, extrapolation of efficacy from adult data occurred for 82.5% and defined as complete for 14.5% and partial for 68% of them. When extrapolation was used, 61% of the drug products obtained a new paediatric indication or extension into a new age group whereas this number decreased to 34% when there was no extrapolation [201].

Disease and disease progression models need to be considered when comparing drug response and kinetics in adults and children as well as formulation bridging. Disease models can be applied to simulate treatment response and in combination with drug models, it is possible to explore the implications of different algorithms for dose adjustment [205].

The most favorable bridging scenario is when safety and efficacy has already been established in adults for the same indication; however an alternative extreme scenario exists when a drug is intended only for paediatric indication and prior adult data is very limited. A two-step approach starting with an exploratory dose-finding/PK study for primary efficacy, safety objectives and PK assessment (ideally to be conducted with the final paediatric formulation) followed by a confirmatory efficacy/safety trial (ideally to be conducted with the final paediatric formulation) has been suggested for such extreme cases [206, 207].

7.1. Methodology for pharmacokinetic studies

Following determination that a paediatric pharmacokinetic (PK) study is necessary, the methodological approach has to be decided. Some aspects to consider within the PK study design for a paediatric population that are described in the relevant regulatory guidelines are presented in Figure 2 [11, 208].

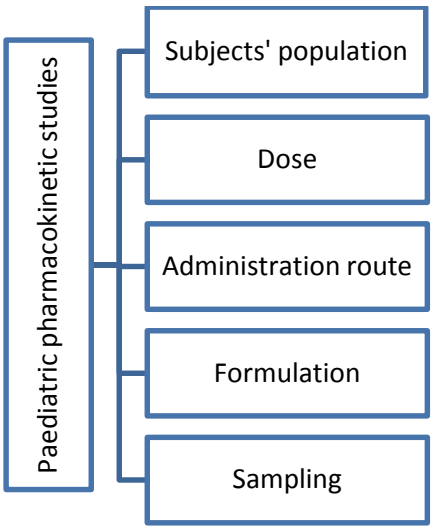

Figure 2. Aspects of PK study design to consider within a paediatric population

The traditional pharmacokinetic approach ('data-rich') in clinical study design involves administration of single dose (in case of linear PK) or multiple doses for determination of steady-state concentration (in case of non-linear PK) of the drug to a relatively small group of subjects with relatively frequent blood and sometimes urine sample collection [209].

Methodological issues and ethical concerns represent the major obstacles that have limited traditional PK approaches in paediatric clinical research. Alternative and innovative approaches to clinical trial design in small populations have been developed in the last few decades which are referenced in relevant regulatory guidelines [11, 23]. These novel methods go some way to overcome the limitations of small sample numbers and of the ethical-acceptability of the trial. A variety of alternative designs such as sequential design, adaptive design, bayesian approach, randomised withdrawal design, randomised placebo-phase design, three-stage clinical trial design have been described for determination of efficacy and safety in the paediatric population [210].

The population PK approach ('data sparse') method for clinical study design involves infrequent sampling (as few as 2-4 samples per subject) of blood from a larger patient population (eg n = 50) with analysis via 'population methodology' to ensure that the maximum amount of information is extracted with minimal disruption to the patients [209]. Recently modeling approaches based on physiological based pharmacokinetic (PBPK) modeling, PK/PD modeling have been applied to the design of paediatric clinical studies [211]. For example, PK/PD modelling techniques have been applied for the improvement of antimicrobial prescribing [212].

7.1.1. Subjects' population

The subjects' population includes paediatric patients in age ranges in whom the medicinal product is likely to be used. A high inter subject variability is expected due to the involvement of patients, yet this reflects clinical observation. In some cases variability can be reduced by use of phenotyping of patients [11, 208].

7.1.2. Dose

Estimation of a safe and effective dose in paediatric patients is a challenge. Determination or prediction of the paediatric dose–response relationship presents difficulties and additional time is required to complete studies in children as compared in adults [213]. Paediatric doses of medicinal products have traditionally been scaled from adult doses, using functions related to body weight, height, or age. The development of allometric approaches was an advance when compared with the use of Clark's Rule and Young's Rule 40 years ago and may have potential clinical utility in children older than 8 years of age and in adolescents [214]. But these approaches may be questionable when complex absorption and disposition processes are encountered and can fail to predict exposure accurately, particularly in paediatric patients younger than 1 year of age in which dramatic age-related differences in drug disposition are observed [215-217]. A more mechanistic approach combining the ADME in adult population with the physiological development in the paediatric population should be used for the assessment of the 'first-in-children' dose [211].

7.1.3. Administration route

Intravenous administration of a true solution or a very fine emulsion or dispersion of the active ingredients in adequate and non-toxic solvent is suggested for new compounds. In case that the intravenous administration is not possible, then the formulation should be administered either by the oral route or by an adequately justified other chosen route [11].

7.1.4. Formulation

A relative bioavailability study is required for new formulations introduced as highlighted previously in Section 7 (Paediatric clinical development) [11].

7.1.5. Blood Sampling

Minimal blood volume sampling coupled with sensitive assays are required for clinical studies in the paediatric population. Technological advances that allow accurate assays with smaller sample sizes and alternative sampling strategies (eg, dried blood spots or saliva samples) are of great importance

for paediatric clinical studies [209]. Population-based pharmacokinetic methodologies offer advantages in sampling, as less frequent sampling than in traditional pharmacokinetic studies is endorsed [209, 218].

7.2. Bioequivalence studies

A change from the formulation used in the confirmatory trials requires a bioequivalence study. Bioequivalence studies for paediatric formulations can be performed in adults given that there are no differences in metabolism in the paediatric population, and the administration of two or more formulations to adults is suggested in order to increase sensitivity.

However, official FDA and EMA guidelines [19] [22] state that *in vivo* bioequivalence studies may be exempted if an assumption of equivalence in *in vivo* performance can be justified by satisfactory *in vitro* data. Currently, a BCS-based biowaiver is restricted to highly soluble drug substances with known human absorption formulated in solid oral IR formulations.

7.3. Paediatric Labelling

In response to paediatric legislative initiatives a new paediatric labeling information database has been developed from FDA, in which key paediatric information from the studies submitted are highlighted and examples of negative paediatric trials are included [6]. In a recent presentation Dr Reigner shared that according to the FDA database 33% of paediatric trials were negative until 2011 revealing a need to increase the success rates of paediatric trials [219]. In the same presentation it was noted that, according to the FDA, the biggest obstacle to efficient trials relates to poor design and planning (poor selection of dose range, poor selection of endpoints, high placebo response rates [220], responders vs non/poor responders) and absence of learning from prior trials [219].

Recently the FDA initiated a review for aspects of paediatric studies and changes in product labelling resulting from Best Pharmaceuticals for Children Act (BPCA) and Pediatric Research Equity Act

(PREA) and their predecessor policies, as well as for assessment of the incentives for paediatric studies of biologics [221]. Better information about the efficacy, safety, and appropriate prescribing of drugs provided to clinicians, recommendations against a product's use in children due to unexpected harm and development of new formulations were identified after the application of the Acts.

8. *In silico* modelling of clinical data

Regulatory requirements make the application of model-based approaches an essential step in paediatric drug development and can be used as decision tools, as study optimisation tools and as data analysis tools [205]. Modelling and simulation techniques can be used to optimize trial designs, to characterize and predict pharmacokinetic-pharmacodynamic (PK-PD), to select dose level and dosing regimens, to develop sampling schemes, and to select outcome measures. These applications are usually based on adult data and the use of extrapolation approaches that incorporate PK and PD data from preclinical species, *in vitro* experiments, and paediatric data from pilot studies or studies in older paediatric groups can be included [164, 211]. Modelling approaches have led to a reduction and replacement of animal studies during drug discovery and development [205]; the same principles can be applied to refine and replace paediatric clinical testing.

8.1. PK-PD models

Two modelling approaches can be used. The "bottom–up" approach brings together all the information at a subsystem level and the structure of the whole system is identified, whereas the "top–down" approach starts from an observable and clinically relevant behaviour and the biological components that cause this behaviour are identified [205]. For the "bottom-up" approach, integration of age-dependent mechanistic models of drug ADME is possible using a whole-body physiologically based pharmacokinetic (PBPK) model, but further research is needed for their development [222].

PK-PD relationships are believed to be similar in adult and most paediatric populations. However, age-related PD differences are rarely reported in the literature, and one of the few examples is the increased sensitivity to d-tubocurarine (an antagonist of nicotinic neuromuscular acetylcholine receptors) in neonates and infants compared with children and adults [223]. PD measurements are non-invasive, and specific devices exist for measuring various pharmacodynamic measures in children, for example: body temperature, heart rate, blood pressure, visual analogue scale for pain, EEG, scales for depression, seizure counts, respiratory peak flow, bone density [224]. The use of each PD endpoint has to be validated for use in children. An example is the development of an observational scale for the measurement of pain in young children, since they are not able to report their pain using a visual analogue scale. Following validation such a scale can be used as a PD endpoint for the development of PD models for pain in children of different ages [223]. Placebo and PD models that link efficacy in children with drug concentrations from adults have been developed to minimize the need for paediatric PK or PD studies [225]. PD models can also be linked to indirect models where the drug kinetics is described by a single compartment involving a single rate constant (referred as 'kinetic' models) [224, 226]. Applications of population approaches in paediatrics could be extended by further development of PK-PD models. [224]

PK-PD modelling can be used to generate virtual clinical study outcomes via clinical trial simulation (CTS) [227]. A virtual clinical trial is generated based on a PK-PD model; a disease progress (for chronic diseases) or placebo-effect model and trial subject demographic covariates [227]. Several examples of paediatric CTSs have been published (eg [228-230]). For example, Jadhav *et al.* (2009) [230] showed that use of clinical trial simulations with prior knowledge from adult patients and paediatric data from other trial of drug with similar indication could assist in the development of anti-hypertensive agents [230]. The lack of knowledge about the mechanisms underlying treatment response in many therapeutic indications has prevented the development of mechanistic PK-PD models, and most examples often refer to standard statistical models that do not allow for inferences about age-related differences in PK [205].

As noted previously, modelling and simulations can be used for determination of an appropriate dose in children. Examples based on a PK criterion only (to estimate the paediatric dose that will achieve the same exposure as in adults) have been undertaken for asparaginase and montelukast [231, 232] and on a PK-PD criterion (achieve a given value of a surrogate marker of efficacy) for acyclovir [233]).

Based on a recent review of the Paediatric Investigation Plans (PIP) from EMA [204], it was shown that models are mainly used to compare concentration response between age groups to inform dose finding but not to define an extrapolation strategy. This could be attributed to the fact that when designing the PIP there is insufficient PK-PD data from the product in the paediatric population to enable model building for robust and sensitive PK-PD comparisons.

8.2. Population models

Population approaches are now established as the method of choice for analysing data collected in paediatric PK and PK-PD studies in order to get maximum information from the limited data collected in paediatric studies and to allow the exploration of model-based dosing recommendations [209, 211]. Advantages include the ability to analyse studies with sparse and unbalanced PK data collection, and the ethically acceptable wide range of clinically relevant covariates [209, 234, 235]. The population design (i.e. total number of subjects, number of groups, number of individuals per group, dosing and sampling schedules) should be chosen carefully before performing a prospective analysis by a parametric method. Optimal design theory that leads to true optimization of the design, or stochastic simulations that allow evaluation of a given design planning are used in the design of population studies [224]. For the paediatric population, the population model must include covariates such as bodyweight and age [224]. Size adjustments are based on allometric or empiric approaches, where the allometric approach is more mechanistically and physiologically based [235].

8.3. PBPK models

Mechanistic whole body PBPK modelling can be used in clinical development to (i) predict plasma/tissue PK; (ii) to provide a mechanistic understanding of the ADME properties of the drug; (iii) to develop PK/PD relationships based on target concentrations and to extrapolate between species, dose, route of administration and formulations [236] [224]. Their advantages over empirical includes the incorporation of compound-related information and compound-independent anatomical and physiological information [211, 237]. The events that take place in each organ, tissue or group of tissues are described mathematically using the tissue size, vascular perfusion, permeability of tissue membranes to the drug, binding or partition of the drug between components in blood or tissue, as well as elimination processes [238].

PBPK modelling can be advantageous for the prediction of drug behaviour in paediatric age groups, as known maturation processes can be integrated. Physiological parameter values for all ages and corresponding regression equations can also be incorporated [224]. PBPK paediatric models developed until now are mainly based on prediction after intravenous drug administration [164, 239-241]. There is a need for further research for development of appropriate PBPK paediatric models following oral administration. The need for better absorption models in the adult population has previously been highlighted [242], and the developmental changes of the gastrointestinal tract in the paediatric population for the PBPK models of oral absorption in paediatrics are an added complicating factor [70].

8.3.1. Building paediatric PBPK models

In order to build a successful PBPK model all developmental changes affecting drug absorption, distribution, metabolism, and elimination (ADME) need to be incorporated. A common approach in developing a paediatric PBPK model is to modify a PBPK model that has been validated with adult PK data and then to incorporate the differences in growth and maturation [243].

An age related absorption model should account for the factors contributing to developmental changes within the GI tract [82]. Physiological absorption models have been developed for some animals and adults [244] [245] [246]. Features of the absorption models in integrated PBPK modeling software (eg GastroPlus®, PK-Sim®, Simcyp®) include: pH-solubility and pH-logD profiles; drug solubility in biorelevant media; drug dissolution as a function of pH; particle size; diffusivity; particle density; ability to handle controlled release formulation using the Weibull equation or an empirical *in vitro* profile; chemical degradation; transit along the gastrointestinal tract; absorption as a function of surface area and effective permeability estimated either by *in silico* predictions, mechanistic equations or by conversion from *in vitro* permeability measurements; enterohepatic cycling; saturable gut and liver metabolism; saturable carrier-mediated transport in the gut by influx and efflux proteins [247]. Absorption models developed for the adult population are usually combined with *in vitro* biorelevant dissolution data for prediction of the performance of oral formulations [88, 248]. It should be noted that to date there are no reported validated models and *in vitro* biorelevant dissolution methods appropriate for use in children to predict drug absorption as a function of age.

For the distribution model, prediction of distribution volume as a function of age is estimated using allometry. The drawback of this method is that the interaction of the drug and the paediatric physiology is not taken into account. The use of organ: plasma partition coefficients could provide a mechanistic assessment of the dependence of distribution volume on drug and organism-specific parameters.

For the elimination model several approaches have been proposed: (i) scaling total clearance by allometry [249] when only size differences exist and should only be used alone when the age range of the participants is greater than the age at which the primary pathway of clearance reaches similar activity to adults; (ii) adult–child clearance scaling [250] based on the knowledge of the pathways of clearance and the ontogeny of those pathways; and (iii) *in vitro–in vivo* extrapolation in children for

prediction of clearance due to intestinal and hepatic metabolism [239]. Knowledge of the pathways of clearance and the ontogeny of those pathways are needed for (ii) and (iii). Empirical methods linked with allometry are used for the prediction of renal clearance, but in this case active transport processes are not included [250]. To date, mechanistic approaches for the prediction of paediatric clearance via the kidneys are not available.

8.3.2. Use of paediatric PBPK models

PBPK modelling is used in paediatric drug development for: selection and optimization of dosing schedule and sampling times; simulation-based trial design for virtual populations; risk assessment related to drug–drug interactions and target organ toxicities and PK–safety assessment [204, 211, 243, 251, 252].

Optimization of the paediatric age-specific dose and paediatric clinical trials can be performed through a learn-and-confirm approach using a minimal number of PK studies in any age group and reassessment with a PBPK model [253]. The use of the PBPK model for the estimation of dose is mainly “exploratory,” and validation with prospectively collected data is needed in order to become “confirmatory”. Furthermore, sampling times can be suggested based on simulations by a PBPK model [254]. PBPK models can also be used to address complex clinical drug-drug interactions and to explore “what if” scenarios [251]. PBPK models can be linked with PD models for the prediction of drug effects in case that child-specific disease outcomes are available and used along with the developmental changes [211].

Modelling and simulation has the potential to enable paediatric clinical bridging and increase success rates. The FDA vision is that design of paediatric studies will be performed 100% by simulation in the future [219, 230].

9. Paediatric Formulations

Lack of age-appropriate medicines for children is a global problem, which significantly affects developing countries. Furthermore, off-label use of drugs (prescribing outside the terms indicated in the product license) in the paediatric population ranges from 60 to 90% with the highest percentage being in infants (<1 year of age), indicating that drug treatment in children is still driven by empiricism [205, 217].

In response to these challenges, the WHO launched its 'Make Medicines Child Size' program in December 2007, and guidelines for the development of medicines for paediatric use have been issued from the regulators [255]. An ideal dosage form for paediatric patients of all ages should allow both safe and accurate dose administration [256, 257]. Development of medicines for the paediatric population is mandatory for sponsors, with a difference on the time required in US and EU: the Pediatric Research Equity Act in US is required at the time of filing the New Drug Application whereas in EU the paediatric investigation plan is required prior to filing the Marketing Authorization Application [258] [259, 260]. Bioequivalence of the paediatric formulation to the adult formulation, is desirable but not required.

A lot of interest has arisen for new approaches to paediatric formulations development and new methods of drug delivery in paediatrics. There may be no single formulation that is ideal for paediatric patients of all ages and a range of dosage forms may be required to cover all age groups. The development and selection of an age appropriate formulation is related to several factors: (i) sufficient bioavailability; (ii) minimal dosage frequency; (iii) minimal impact on the life style of the child; (iv) non-toxic excipients in the formulation; (v) convenient and reliable administration; (vi) stability; (vii) ease of the production process and (viii) cost of the formulation [82]

The magnitude of doses required through childhood can vary 100-fold, thus, if a medicinal product is to be used in all age groups, theoretically, a range of different dosage forms should be available providing different strengths or concentrations to allow simple, accurate and safe dosing [257].

Consequently, a variety of different oral dosage forms, such as solutions, syrups, suspensions, powders, granules, effervescent tablets, orodispersible tablets, chewable tablets and gums, mini tablets, innovative granules, conventional immediate release and modified release tablets and capsules could be available for children of all age categories. However, since safe and effective oral drug therapy requires patient compliance, the availability of a broad spectrum of formulations is sometimes not enough and palatability is another important parameter to consider in oral paediatric drug therapy. To improve palatability, before oral application, dosage forms are often diluted with beverages (eg fruit juice or milk) or dispersed in fluids or semisolid meals to mask their taste. Based on these considerations, it can be summarized that both the variety of dosage forms required in oral paediatric drug therapy and the dosing conditions are essentially different to oral drug therapy in adults.

EMA has proposed draft guidance that indicates preferred formulations as a function of the age, based on a matrix that combines different age groups and conventional dosage forms. It is important to note that this guide reflects some general aspects of acceptability of various dosage forms as it is not an in-depth, evidence-based guide, but is based on a questionnaire for hospital paediatricians, pharmaceutical scientists and parents [24]. Recent data from South Africa suggest that there is no significant difference in refusal rates of children receiving liquid and solid formulations [104].

During formulation development, solubility and stability data are useful initial parameters. Solubility can determine the potential for a solution formulation. The required solubility is such that the upper dose is soluble in a convenient volume; a volume of ≤10 mL was proposed by Strickley (2008) [102].

Table 7 summarises a range of oral paediatric formulations with considerations regarding their use and limitations.

Table 7. Oral paediatric formulations: considerations and limitations of use [24, 104, 221]

Formulation	Considerations/comments
Oral solutions/ Oral suspensions	Easy administration and wide range for dose adaptation Appropriate for all age groups Limited availability of safe excipients Physico-chemical characteristics of the suspension Dosing: use of measuring device required for dose accuracy; issues with re-suspension when sedimentation occurs Palatability issues
Powders/ Granules	Appropriate for all age groups when manipulated to form a liquid Appropriate from 6 months when administered as solid, or co-administered with semi-solid food Dosing: use of measuring device required if not in a sachet Issues with aspiration, choking
Tablets	Size and shape may affect acceptability Small sizes available (mini-tablets; 3mm) Different strengths for children of different ages will be required Acceptability can be improved with training in tablet swallowing
Capsules	Can be administered intact (size and shape should be considered) Can be opened when justified (co-administration with food, liquids) Size and shape may affect acceptability Different strengths for children of different ages will be required
Orodispersible tablets	Issues with direct swallowing, not dispersed in liquid prior to administration, choking Poor drug load resulting in large unit forms Palatability issues
Chewable tablets	Issues with direct swallowing, chocking Palatability issues
Effervescent formulations	Need to be dissolved or dispersed in liquid prior to administration Palatability issues Bicarbonate may lead to gastrointestinal malfunctions
Oral film strips	Film area and height limits drug load to 15–25 mg
Oromucosal formulations	Requires use of applicator Requires adhesive properties for retention within the oral cavity Issues with swallowing

Use of liquid dosage forms in some developing countries has limitations associated with long-term storage or transport under the conditions of extreme temperatures [261]; flexible solid dosage forms such as multi-particulates (for example, granules and pellets) can be advantageous in these circumstances. Swallowing issues associated with solid dosage forms can be solved by crushing tablets or opening capsules and adding the resulting powder to beverages or soft food [104]. Mixing with foods or drinks should be explained and justified. Different foods or drinks may have different properties and differ in their effect on the medicinal product and may affect product performance

and drug pharmacokinetics [24]. For example 6-mercaptopurine is completely degraded within minutes by milk or milk products, administration of crushed isoniazid tablets with apple sauce may be associated with impaired gastrointestinal absorption and treatment failure [104, 262].

9.1. "Enabling" formulations

Preliminary "enabling" formulations might be used in early paediatric clinical trials, as the development of paediatric formulations with optimized properties is often a challenging, time and resource intensive process [206]. A simple formulation, such as the dispersion of available granulates/powder mixes (or the marketed adult formulation) with a liquid vehicle can be used as "enabling" formulation.

Formulation bridging is independent to clinical bridging. In cases where an "enabling" or other formulation is used that is not the final paediatric formulation, formulation bridging needs to be managed either by a clinical study or by reference to BCS-based biowaivers [263]. If an "enabling" formulation is used for the exploratory dose finding study, then two relative BA studies are needed: one study with the reference adult formulation (before the first study in children) and one study with the final marketed paediatric formulation. Formulation bridging studies are performed in adults unless: the drug is unsafe in healthy volunteers; the PK of the compound is different in patients (but if the impact of the disease on PK is expected to be similar in both test and reference formulation then a relative BA in healthy volunteers is conducted); the PK of the compound is different in children (but if the effect on PK is expected to be of the same extent with both formulations a relative BA in adult population is conducted) [206]. For paediatric formulations of drugs with linear elimination PK that are similar to the adult formulation and differ only in the dosage strength similarity can be shown with *in vitro* dissolution studies [264] [206].

Biopharmaceutic risk associated with formulation changes also involves the BCS classification (permeability and solubility) of the drug, taste properties, stability, dissolution rate-limiting effects, dosage forms for various age groups with dosing instructions, and variability of PK parameters [263].

9.2. Excipients used in paediatric formulations

Excipients are substances added to confer a suitable consistency or form to a drug and are usually considered to be inert that is, not affecting the intended action of the therapeutically active ingredients [265]. They can be added as diluents, wetting agents, solvents, fillers, binders, emulsifiers, absorption enhancers, sustained release matrices, preservatives, sweeteners, stabilising, colouring or flavouring agents. In 1937 deaths of more than 30 children from "Elixir Sulfanilamide" were reported when the manufacturer tried to create a suitable formulation for young children and others who could not swallow pills and included diethylene glycol (a toxic substance found in antifreeze) in the formulation [221]. In 1985 the Committee on Drugs recommended that the FDA to mandate labelling of over-the-counter and prescription formulations to include a qualitative list of inactive ingredients due to adverse reactions reports associated with pharmaceutical excipients [265]. Issues related to excipients used in oral paediatric formulations are presented in Table 8.

Table 8. Issues related to excipients used for oral formulations relevant to paediatric patients. [257, 265] [266] [104, 267]

Excipient	Use	Characteristics	Toxicity	Recommendations regarding usage
Aspartame	Sweetener (artificial)	dipeptide of aspartic acid and a methyl ester of	rare hypersensitivity reactions	not in homozygous autosomal recessive

	chewable tablets, sugar-free formulations	phenylalanine	cross-reactivity with sulfonamides.	phenylketonuria patients in patients without dietary restrictions <5 mg/kg/d Labelling is required for prescription and non-prescription products for phenylalanine content.
Benzyl Alcohol	Preservatives injectable medicinal products and solutions		Gasping syndrome: accumulation of metabolites in blood (metabolic acidosis) and brain (neurotoxicity)	neonates: not be given due to their immature metabolismup to three years old: should be carefully evaluated and may best be avoided
Dyes	Colouring agents	azo dyes (sunset yellow), quinoline dyes (quinoline yellow), triphenylmethane dyes (FD&C blue), xanthene dyes (erytrhosine)	gastrointestinal intolerance, abdominal pain, vomiting, and indigestion hypersensitivity	avoid unless necessary
Ethanol	Solvent, preservative in oral liquid preparations	rapidly absorbed from the GI tract and metabolised to acetaldehyde, which is then	acute intoxication with accidental overdose and chronic toxicity associated with routine use for chronic medical	co-administration of ethanol may alter drug absorption or metabolism of drugs and may result in drug interaction

		oxidised to acetate PK in preterm and term infants not well understood	conditions CNS depressant, respiratory/ cardiovascular toxicities at high concentrations long-term effects of low ethanol doses under discussion	max amount in OTC medicines (USA): 0.5% v/v for <6 years of age 5% v/v for 6–12 years of age 10% v/v for >12 years of age.
Fructose	Sweetener		an elevation in blood glucose concentration laxative effects at high oral doses	Not in patients with diabetes, hypoglycaemia, hereditary fructose intolerance
Lactose (milk sugar)	Filler, diluent in tablets, capsules and to give bulk to powders	disaccharide of glucose and galactose	severe prolonged diarrhoea, dehydration, metabolic acidosis in lactose intolerance	intake of <3g may provoke the described symptoms sensitivity to lactose varies in severity
Methacrylic acid/ethylacrylate copolymer	Coating Materials high-strength coated pancreatic enzyme formulation		fibrosing colonopathy	

Propylene glycol (PG)	Solvent, solubilizer	metabolised to lactic and pyruvic acid, excreted unchanged in urine; accumulation of PG. in neonates $t_{1/2}$: 16.9 hr (adults $t_{1/2}$: 5hr).	neurotoxic effects in adolescents and school-children;in low-weight newborns and pre-term babies numerous deaths, severe brain damage and life-long handicaps; metabolic acidosis; hyperosmolality that may cause laxative effect	Up to 25 mg/kg/day in adults acceptable limit in neonates unknown Products with high levels of PG not to be administered <4 years (limited metabolic pathway- alcohol dehydrogenase). not in paediatric dialysis patients
Propyl-parabens	Preservative	agonistic activity at hormone receptors		Up to 5 mg/kg/day for children > 2 years with mature metabolic capacity. recently deleted from the list of permitted food additives in the EU
Saccharin	Sweetener (artificial) in solid and liquid oral dosage forms			not included in drug labeling
Sorbitol	Diluent in	metabolised to	absorbed from GI tract	no available

	tablets, plasticiser for gelatine in capsules, vehicle and stabiliser in oral liquid formulations	fructose and glucose	more slowly than sucrose	recommendation infants and children; <20 g/day in adults contraindicated in paediatric patients with hereditary fructose intolerance and hypoglycaemia
Sucrose disaccharide	Sweetener	Converted to fructose and glucose	decrease in dental plaque pH, dissolving tooth enamel and promoting dental caries	avoided for paediatric patients with hereditary fructose intolerance, diabetes; for long-term therapy large amounts of sucrose replaced by sugar-free formulations
Sulfites	antioxidants		primary exposure in children is through foods, serious reactions have also occurred after oral, administration of sulfite-containing drugs	

*The American Academy of Pediatrics recommended removal of ethanol from more than 700 liquid preparations for children in 1984. This recommendation seems to have been adopted by many manufacturers in the USA; the commonly used oral medicines including furosemide listed no ethanol as an excipient, unlike in the UK.

For adult products, excipient levels are based on the stated amount in an FDA database called "Inactive Ingredients Guide" (IIG) and in the case that the excipient doesn't have a history of prior use, safety tests are needed. For paediatric products, no such IIG database exists, and selection of an excipient for paediatric formulations is difficult [261]. A single comprehensive and readily accessible database of safety and toxicity of excipients for paediatrics (STEP) is under development [268]. Significant exposure of infants to potentially harmful excipients (eg ethanol) is common [269]. Safety of excipients needs to be determined in paediatric patients and especially in infants whose metabolic and elimination pathways may not be fully developed or who may be more susceptible to adverse effects during the first few weeks and months after birth [265] [266].

Recently the EMA has published a concept paper on the need for revision of the guideline on excipients in the label and package leaflet of medicinal products for human use (CPMP/463/00) in order to include safety concerns regarding excipients that have been identified which are not currently addressed in the guideline and the need to cover the paediatric population is noted [270].

10. **Conclusions**

Paediatric biopharmaceutics is crucial in optimisation of the design and development of age-appropriate oral medicines.

Successful biopharmaceutic tools for paediatric populations require reliable clinical experimental data coupled with mechanistic understanding of all ADME processes. With specific research required on: ontogeny of various biological components; maturation of biliary excretion of drugs; metabolic capacity of gastrointestinal tract; carrier mechanisms; drug transporters in the gastrointestinal tract; first-pass metabolism; protein binding capacity of a drug in children and age-related changes in PD [217] [251]. Until such data is available existing biopharmaceutical measurements rely on allometric scaling of adult values which is of limited and as yet unknown value. The barriers to clinical research

in paediatric patients (or healthy volunteers) need to be carefully considered to ensure that the ethical concerns surrounding safe and effective medicines for all paediatric patients is balanced with the ethics associated with involvement of children in clinical trials. The most vulnerable paediatric group are neonates and infants below 6 months as these individuals are most different to adults and where predictivity from adult parameters is least robust. Research needs to be prioritised for this youngest, most at risk, population. Greater access to existing paediatric clinical data would be useful in the validation of new age-appropriate tools that will be developed specifically to understand the biopharmaceutics processes in children.

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