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EXPERT REVIEW

A Physiologically Based Pharmacokinetic Model of the Minipig: Data Compilation and Model Implementation

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ABSTRACT In today's pharmaceutical research and development, physiologically-based pharmacokinetic (PBPK) modeling plays an important role in the design, evaluation and interpretation of pharmacokinetic, toxicokinetic and formulation studies. PBPK models incorporate *in vitro* physicochemical and biochemical data in a physiologically based model framework to simulate *in vivo* exposure. The comparison of simulated concentrations to those measured in *in vivo* studies can be used to gain insights into compound behavior and to inform PBPK based human pharmacokinetic predictions. The Göttingen minipig is gaining importance as a large animal model in pharmaceutical research due to its physiological and anatomical similarities to human and is increasingly replacing dog and non-human primate in preclinical studies. However, no PBPK model for minipig has yet been published. This review discusses the information available to establish the physiological database for this species and highlights the gaps in current knowledge. A preliminary PBPK model is created from this database and simulations for two drugs dosed both intravenously and orally are compared to measured plasma concentrations. Results support the validity of the model with simulated plasma concentrations within the range of the observations. In conclusion, the model will need to be refined as additional physiological data become available, but it can already provide useful simulations to assist pharmaceutical research and development in the minipig.

KEY WORDS absorption modeling · distribution · elimination · intestinal absorption · metabolism · minipig · physiologically based pharmacokinetic modeling · pig

ABBREVIATIONS

ACAT	Advanced Compartmental Absorption and Transit
BCS	Biopharmaceutics Classification System
CO	cardiac output
CYP	cytochrome P450
Fabs	fraction of dose absorbed
GFR	glomerular filtration rate
GI	gastrointestinal
PBPK	physiologically based pharmacokinetic
PK	pharmacokinetics
SEF	surface area enhancement factor

INTRODUCTION

Prediction of human pharmacokinetics (PK) of novel drug candidates is a major task of pharmaceutical research and relies on extrapolation from *in vitro* data and *in vivo* animal data (1,2). It has been shown that physiologically based pharmacokinetic (PBPK) modeling and simulation in animals leads to better understanding of the PK processes and *in vitro* to *in vivo* translation for a drug candidate and thus enables more reliable prediction of human PK (3). Furthermore, PBPK applied preclinically allows most efficient integration of experimental data to give mechanistic insight into underling processes and assist design of pharmacological and toxicological animal studies (4–6). For the most commonly used laboratory species, such as mouse, rat, and dog, physiological databases have been compiled and PBPK models established and applied (7–9). However, in the last decade, the pig has increasingly been used

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in preclinical studies as it shares numerous anatomical, physiological, genetic, and biochemical similarities to human (10). Although a commercial PBPK model for minipigs has been implemented in the generic software tool PK Sim (11), the details of this model are, to the best of our knowledge, not publicly available. Recently, a PBPK model for landrace pig was proposed to predict outcomes from multi-site sampling experiments (12). This sophisticated experimental setting allows sampling of portal and systemic blood as well as urine and bile from deeply anesthetized pigs (13–15). Although such a model is highly informative from a mechanistic point of view, the animals are unconscious and so orally administered drugs have to be directly dosed into the small intestine which is not appropriate for most preclinical and pharmaceutical development. In the standard preclinical setting, candidate drugs are administered orally as solutions, suspensions or capsules and therefore PBPK modeling of stomach passage and intestinal absorption is essential.

The Göttingen minipig is the predominant laboratory pig breed, due to favorable features including small size, well-defined health status, and strictly managed but not inbred genetics (16) (http://minipigs.dk/healthmonitoring). To integrate the minipig fully into preclinical research, including supportive modeling and simulation, a PBPK model needs to be developed based on reliable physiological data. It is the aim of this review to compile published literature on the Göttingen minipig with a special emphasis on data needed to build a PBPK model.

STRUCTURAL REQUIREMENTS FOR A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

Physiologically based pharmacokinetic models are distinguished from empirical approaches by their mechanistic mapping of model compartments to relevant anatomical structures (17). The generic minipig PBPK model we have created contains core components, such as absorption from the gastrointestinal (GI) tract, blood circulation and organs of metabolism and elimination. A schematic illustration of our preliminary model is given in Fig. 1.

Oral dosing is the preferred route for drug administration as it is safe, convenient and usually required for successful marketing. Therefore, much research has been devoted to prediction of intestinal absorption (18–20). The fraction of dose absorbed (fabs) is mainly determined by solubility, dissolution and membrane permeability as presented in the Biopharmaceutics Classification System (BCS) (21). However, the absorption process is complex and involves factors such as drug partitioning in micelles/lipid vesicles, chemical and enzymatic stability in the lumen, drug precipitation, gut metabolism and active transport. Several PBPK absorption models incorporating these factors have been defined for human (22,23) and animals (9,24) and we have followed these examples by collecting data for minipig intestinal surface area, residence times, fluid volumes, and regional pH.

Our PBPK absorption model follows the Advanced Compartmental Absorption and Transit (ACAT) model (22) by dividing the GI tract into a series of compartments with each compartment parameterized to capture the distinct properties influencing absorption, such as fluid volume, absorptive surface area, pH, and bile salt concentration (18,22,25,26). Transit through the GI tract is captured by transfer between adjacent compartments with the rate of transfer appropriately reflecting drug residence time in different regions.

To model distribution in the body, tissues, and organs are represented as individual compartments linked *via* the blood flow and appropriately parameterized with information on organ volumes, tissue to plasma partitioning, and blood perfusion. We have collected and summarized data on organ and tissue sizes as well as perfusion data obtained by Doppler sonography or with radiolabeled microspheres (27,28). For drug partitioning, models based upon the composition of tissues in terms of lipids, proteins and water and using readily measureable or predictable properties such as lipid partitioning, ionization and plasma protein binding (29–31) are used.

BACKGROUND ON THE GÖTTINGEN MINIPIG

Much of the available information on the Göttingen minipig was published during the 1960s and 1970s when the breed was established at the University of Göttingen by Haring and coworkers (32). However, this early data has to be reviewed cautiously as the breed underwent several changes before arriving at the laboratory species we use today.

Quite early after establishing the breed by crossing Minnesota minipigs (Hormel swine) with Vietnamese potbelly pigs, dominant white Landrace pigs were crossed in to obtain a white laboratory animal (32,33). However, a colored strain was bred in parallel until 1992 and it was stated that the two populations differed in growth rates and fertility (34). Therefore, if data are reported separately, information on the white strain should be used for establishing a representative model.

The change in breeding facility from free range to barrier condition in 1969 was accompanied by a significant weight gain. It was assumed that at that time the small size of Göttingen pig was rather due to environmental pressure than to genetic microsomia (34). By restrictive breeding the size could be reduced again in the following years. In 1992, the colored population was abolished in favor of the white one, which was moved to Denmark and used to repopulate breeding facilities in Germany and the US (16).

Ad libitum fed Göttingen minipigs (particularly female animals) grow fat and are an excellent model for obesity, showing all the characteristic comorbidity seen in humans (35,36).

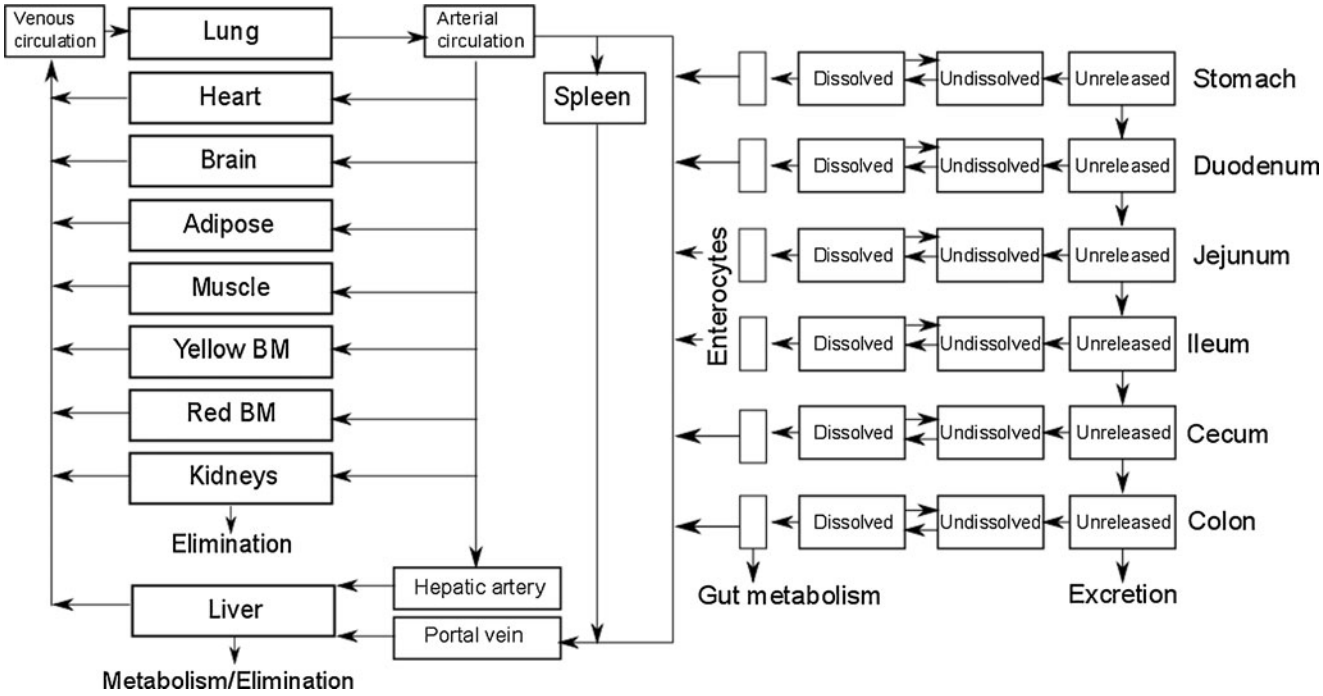

Fig. 1 Schematic view of a generic absorption and PBPK model. Yellow BM and red BM refer to yellow and red bone marrow, respectively.

However for other purposes, such as pharmacokinetic or toxicological studies, the animals should be generally lean (normal weight) and so, diet has to be restricted. When compiling data, it is advisable to compare reported age and weight with growth curves provided by the animal supplier. Even if appropriately fed, Göttingen minipigs are fast growing, gaining 2 kg per month in the first year of life. Final adult weight is achieved around 2 years of age and ranges between 35 to 45 kg (37,38). Bores and sows are sexually mature around 3–4 and 4–5 months, respectively. Skeletal growth is completed after one and a half years of age (39). To economize compound usage, minipigs should enter PK or toxicological studies at the smallest size possible (after sexual maturation), which will be usually around the age of 6 months (14.2 kg body weight) and our PBPK model assumes this body weight.

GASTROINTESTINAL SYSTEM AND ABSORPTION

Stomach

The ratio of GI system size to body size in Göttingen minipig is not the same as in landrace pigs. Empty stomach weight in different landrace breeds was reported as 0.3% of body weight (415–500 g), while in sexually mature Göttingen minipigs the relative stomach weight accounts for 0.9-1% (180–252 g) of body weight (35,40). However, apart from its dimensions, the GI systems of landrace and minipig are functionally and anatomically similar. As in humans, the porcine stomach is monogastric but exhibits several species-specific anatomical features. The so-called *diverticulum ventriculi* is located at the top of the cardiac stomach and although its physiological function is still debated, this protrusion has implications for orally applied drugs. If given by gavage tube, formulations can be accidentally miss-dosed into this pocket and dissolution and gastric emptying might be delayed (41,42). The pylorus in pig is quite unlike that of human. In pig it comprises a semilunar sphincter and the pyloric torus, a protuberance consisting of fat and muscle fibers lying in the muscle-free gap of the sphincter (43). These components are thought to act together as reinforcement of closure. Whether this reinforced closing mechanism has implications on passage of solid and/or non-dissolving dosage forms has not been clarified although there is evidence that large, non-dissolving particles empty less rapidly from stomach in pigs and minipigs than in other species (44). Telemetric pH studies in minipigs and pigs showed that transmitters remained for a very long time in the stomach (45). Long residence times were reconfirmed by unpublished preliminary in house studies where we observed pH drops (from pH 8 to 1) up to 48 h after capsule application, indicating that the device remained in the stomach at termination of measurement. An explanation for this delayed transit in pigs could be the relatively large size of the transponder (6×5.5×25 mm (46)) in combination with the tight closing pylorus. It was shown that size and density of non-dissolving dosage forms had an impact on gastric transit times in pigs and minipigs (44). For plastic tablets (20 mm × 8 mm) gastric residence time was measured from 1 to up to 28 days.

A complicating factor in estimation of gastric transit in pigs is the bimodal and incomplete emptying pattern of the porcine stomach (47). Interestingly, in minipigs, gastric motility and emptying seem to depend on feeding regimen. The strong motoric activity of antrum and intestine after one big meal resembled that of a carnivore, while half as much motoric activity was observed upon two smaller daily meals and *ad libitum* fed pigs exhibited a ruminant stomach emptying pattern (48). Additionally, occasional bile reflux seemed to have a strong inhibitory effect on motoric activity of stomach, interfering with the regular gastric emptying pattern. Based on these observations, a twice-daily feeding regimen could be used to produce more human-like and predictable gastric emptying.

Gastric acid secretion takes place in the fundus, and as in humans, is regulated by humoral (histamine, acetylcholine, gastrin), neuronal (n. vagus), and psychological stimulation (49–51). During fasted conditions, the cardiac glands are reported to produce a slightly alkaline secretion with high buffering capacity, reaching maximum activity during the night (52–54). This means that the cardiac stomach and diverticulum retain a basic pH, leading to a relatively high baseline pH in the whole stomach under fasted conditions. This stands in contrast to the human situation, where average stomach pH under fasted conditions ranges between 1.5 at night and 3 at day time (55). In general, it was observed that gastric pH in pigs and minipigs shows high intra-individual variability regardless of the method selected for measuring (44,45,56).

A summary of data from current literature on regional pH in pigs is given in Table I.

Small Intestine

The porcine small intestine is histologically, anatomically, and functionally similar to human (57). The minipig duodenum shows typical circular folds (*plicae circulares*) and finger like villi which decrease in height towards the ileum. The villi exhibit the same cell types as found in human small intestine (enterocytes, goblet-, and crypt cells) as well as Peyer's patches. In most mammals, including the pig, ileum and duodenum are considered to be much shorter than the jejunum. However the exact discrimination of these anatomical compartments is technically challenging and we will therefore consider only total small intestinal length (58).

Table I Summary of pH Values Measured in Minipig Gastrointestinal Tract

Region of GI		pH
Stomach	Anterior (food status not specified)	2.95 (143)[a]
		4.3 (144)
	Posterior (food status not specified)	6 (143)
		2.2 (144)
	Anatomical region not specified	3.6 (45)[a] (24 h fasted)
		1.15-4.0 (44) (12 h fasted)
		4.4 (56)[b]
Duodenum		6.1 (56);6.55 (143)[a]; 6.0 (144)
Jejunum		6.45 (56)[a];7.5 (143); 6.2-6.9 (144)
Ileum		6.55 (56)[a];6.3 (143); 7.5 (144)
Caecum		6.1 (56);6.8 (143); 6.3 (144)
Colon		6.35 (56)[a]; 7.1 (143); 6.8 (144)

[a] Average value was reported. [b] *Ad libitum* fed landrace pig.

Small intestine *in vivo* length in 3 week old Göttingen minipigs was reported to be 420 cm with a corresponding average diameter of 1 cm (10). In adult animals *in vivo* diameter of 2 cm and post mortem total small intestine length of 832 – 900 cm was measured (10,11). These values are in line with our in house post mortem measurements where we found a mean value of 840 cm +/- 6.0 CV% in adult (6 month old) Göttingen minipigs (n=6). It has to be kept in mind that the intestine becomes remarkably elongated after death and effective *in vivo* length might be shorter (12). Comparing small intestine length to domestic breeds, landrace pigs exhibit two times longer small intestine (length 1500–1800 cm) for a 4 times higher body weight (13).

To appropriately describe absorptive intestinal surface area, one has to take into account the enormous expansion due to villi and microvilli. In the PBPK model this is accounted for by multiplying area (length x diameter) of the intestine with a surface area enhancement factor (SEF). For landrace pig, SEF have been determined for small intestine (59). These values are very close to those of human (3.66 for small intestine and 2.48 for colon while human cecum showed a slightly lower value of 1.79) (59). Currently, it has to be assumed that the SEF values for landrace pigs apply for miniature breeds as no specific data are available.

No pig-specific data on intestinal permeability could be found and the current model assumes that human and minipig are the same in this respect.

Transit times for porcine intestine are much more homogeneous than for the stomach. In landrace pig, the mean transit times for liquids and solids in small intestine ranged between 3 and 4 h, regardless of the dosage form (60,61). This is in good accordance to human small intestinal transit times (2–4 h) while dogs, for example, exhibit about half the transit time of humans, probably because of the relatively shorter small intestine (200 cm) (62). In the large intestine, pigs, showed shorter residence times for fluid, high fiber meals (24.9 h) while other fluids and solids had transit times ranging between 35 and 49 h (61,63). A comparison of GI transit times in the laboratory species pig and as well as in human given in Table II.

Table II Gastrointestinal Transit Times for Liquids and Solids in Three Different Species

Transit time (h)	Stomach		Small intestine		Large intestine
	Liquid	Solid	Liquid	Solid	Liquid and solid
Pig/minipig	0.8-0.9 (61) (LR); 0.4* (145) (LR)	1.0-1.3 (61)[a] (LR); >24 (133)[b] (MP)	3.9-4.4 (61) (LR)	3.7-4.3 (61) (LR)	24.9-44.4 (61) (LR)
Dog	1.5 (144)	0.8-1.5 (133)	1.3-2.6 (62, 146)		18.5 (147)
Human	0.16-0.25 (148)	1.2-2 (133, 148, 149)	2-4 (149)	3.0-4 (149)	33.5-61.5 (149)

All values reflect overnight fasted experiments except the one marked with *, where pigs had free access to food.[a] Transit times were assessed with normal meal and dissolving granules.[b] Transit times for enteric coated tablets. Values on physiologic transit times are mainly available from landrace pig as minipigs were mostly used to test non-disintegrating dosage forms. (*LR* landrace pig; *MP* minipig)

Large Intestine

Absorption from the large intestine is relevant when high doses are given or when slow-release formulations are being developed. In pigs, the cecum is relatively large compared to other species. Landrace pig cecum is about 21–23 cm long (40). In house, we measured a cecum length of 8.6 cm +/- 10 CV% and diameter of 3.13 cm +/- 15 CV% in 6 adult Göttingen minipigs which is comparable to a reported length of 13 cm (64). Besides water and electrolyte reabsorption, the porcine large intestine efficiently ferments carbohydrates, analogously to fore-stomach digestion in ruminants (65,66). The colon in adult landrace pigs measures about 450 cm in length (40) while in Göttingen minipigs total colon length was measured at 303 cm (15–30 months of age, weighing about 29 kg) (34). We measured colonic diameter of 2.7 +/-10 CV% in 6 adult minipigs. The remarkable arrangement of the porcine colon in a series of loops has no functional implication (67). Table III lists dimensions of GI system of laboratory species, such as pig, minipig, and dog, as well as human.

PHYSIOLOGY

After a compound has been absorbed and passed *via* the portal vein through the liver it is distributed to the various in PBPK modeling, tissues and organs of the body are mapped to interconnected compartments, which are parameterized with partition coefficients and volumes. Relevant volumes can be calculated from weight and density data. While specific organ densities have not been established for pigs, it is reasonable to assume that these values do not significantly vary among mammals (7,68) so values of 0.98 kg/L for adipose and 1.05 kg/L for non-fat tissues have been taken (69). A compilation of organ weights in adult Göttingen minipigs (n= 20) is available from Ellegaard (http://minipigs.dk/backgrounddata) while blood flow and cardiac output (CO) determined with radiolabeled microspheres is given in (28,70). The sources report CO in juvenile (3 kg) and adult (21.5 kg) animals to be 717 ml/min and 3147 ml/min, respectively. To scale these values appropriately to a 14.2 kg physiology the allometric power law (Y=aM^b) was applied, where a is a constant, Y is CO, M is the body mass and the exponent b is 0.75 (71). Accordingly, a CO of about 2304 ml/min (16.2 ml/min/100 mg BW) is calculated for a 14.2 kg minipig. Interestingly, it could be shown that the scaling exponent of 0.75 in landrace pigs does not hold above a body weight of 75 kg, due to disproportional growth of adipose and muscle tissue (72,73). However, in the body mass range in which Göttingen minipigs are used disproportionalities are not expected.

The total blood volume of an adult 14.2 kg minipig is about 923 ml, which corresponds to 6.5% of its body weight

Table III Comparison of Gastrointestinal System Dimensions of Pigs and Minipigs, Dog, and Human

	Small intestine		Cecum		Colon	
	Length (cm)	Diameter (cm)	Length (cm)	Diameter (cm)	Length (cm)	Diameter (cm)
Landrace pig	1500-2000 (40,62,144)	2.5-3.5 (62)	21-30 (40,143,144)	8-10 (62)	450-500 (40,144);	8-10 (62)
Minipig	832-900 (57,150) 840 +/-6.0 CV%[a]	2 (150)	13-20 (34,64) 8.6 +/-10 CV%[a]	3.13 +/-15 CV%[a]	303 (34)	2.7 +/-10 CV%[a]
Human	300-325 (62); 680-700 (62,151) (post mortem)	3-4 (62)	7 (62,151)	6 (62)	93 (151)	6 (62)
Dog	150 (62); 414 (144)	2-2.5 (62)	12-15 (62); 8 (144)	-	25-60 (144)	2-2.5 (62)

[a] post mortem in house measurements in 6 adult Göttingen minipigs (6 months of age, 13.3 kg +/- 9 CV% body weight).

(74). Typical hemoglobin values for adult male minipigs are 8.3 mmol/l, hematocrit is 40.7% and serum albumin ranges between 30.1 and 40.3 g/L (http://minipigs.dk/backgrounddata). Table IV summarizes relative organ weights of landrace and minipigs as well as their blood perfusion. Table V gives an overview of CO across different laboratory species and human.

For some tissues, data were not available and had to be extrapolated. This applies especially for tissues that are not easily accessible or diffusely distributed such as fat and muscle, skin, bones, and bone marrow. As these are important compartments in PBPK models, the approaches used to deduce relevant parameter values are presented below in more detail.

Fat and Muscle Tissue

Johansen *et al.* performed dual energy X-ray scans of 9–10 month old lean and obese Göttingen minipigs (36). Lean pigs consisted of 10% body fat, while this value increased to 15% in obese animals. These findings were in line with earlier determined values (75). Interestingly, landrace pigs have a higher fat content then minipig breeds 45% of body weight was skeletal muscle which was reconfirmed by a more recent study, where total muscle was determined by subtraction of other body components (75,76). Johansen *et al.* reported that 87.8% of the body weight in Göttingen minipigs corresponded to lean body mass (body weight without bones and adipose tissue) (36) which is in good agreement with our collection of literature data for these tissues. Perfusion of adipose and skeletal muscle was measured to be 11.1 ml/min/100 g, and 13.5 ml/min/100 g, respectively (70,77).

Skin

Porcine skin architecture, composition, vascularization, lymphatic drainage as well as tight attachment to subcutaneous tissue closely resemble those of human (78–81). Consequently, the pig model is frequently used for dermal toxicology and pharmacokinetic studies (82,83). In addition, minipig was recently shown to be a valuable model for subcutaneous drug testing (84) and so, physiologically based absorption are of increasing interest.

Reported skin volume or weight for common laboratory species vary considerably between authors (*e.g.*, from 9 to 16% of body weight in dog) (85,86). This probably stems from ambiguity in definition for example, whether fur, subcutaneous fat tissue, and other appendages should be assigned to the skin compartment or whether the more stringent anatomical definition of dermis and epidermis is used. For the purpose of building a PBPK model it seems reasonable to adhere to the latter, as skin appendages are often pharmacologically inert and do not share perfusion and composition characteristics of dermis and epidermis. In humans, it is generally agreed that this compartment makes up about 3% of total body weight. To our knowledge, there is only one reported value on skin weight for minipigs, dating back to 1981 (34). Here, a weight of 4.79 kg was measured for boars, which corresponds to 16% of total body weight. Compared to human and other laboratory species this seems a very high value and most probably included skin appendages like hooves.

Price *et al.* proposed to estimate human skin volume from body surface area and average skin thickness (69). To use this method an estimate of body surface area of minipigs is required. A value of 0.74 m^2 is given for micropigs in FDA Guidance (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf). However, the Göttingen minipig is the smallest of all minipig breeds and the micropig body weight of 21.5 kg is high compared to the typical weight of Göttingen minipigs entering PK studies. As an alternative, surface area of pigs might be more appropriately calculated according to the following formula (BW in kg) (87):

Table IV Organ Size and Blood Perfusion for Göttingen Minipig (6 month old male, 14.2 kg, N=20) and Juvenile Landrace Pig (~2.5 Month Old, 25 kg)

	Organ weight (% of body weight)			Perfusion (ml/min/100 g)		
	Minipig (CV%) (14.2 kg)	Pig (25 kg)	Human (70 kg)	Minipig(14.2 kg)	Pig (25 kg)	Human (70 kg)
Lung	0.566 [I](6.89)	1 (152)	1.42 (68)	CO[a]	CO[a]	CO[a]
Heart	0.52 [I](9.62)	0.37 (153)	0.47 (68)	118 (70,77)	120 (154)	72.7 (68)
Liver	1.67[I](9.58)	2.94 (152)	2.57 (68)	167 (70,77)	107 (152)	80.5 (68)
Spleen	0.16[I](16.88)	0.20 (155)	0.25 (68)	297 (70,77)	220 (156)	42.7 (68)
Kidneys	0.47[I](12.77)	0.40 (152)	0.44 (68)	361 (70,77)	429 (157)	400 (68)
Brain	0.44[I](13.86)	0.40 (152)	1.9 (68)	75.8 (70,77)	76.0 (152)	50.0 (68)

[a] It is assumed that the whole cardiac output (CO) perfuses the lung (7). [I] Data from http://minipigs.dk/backgrounddata

Table V Cardiac Output as Reported for Different Laboratory Species and Human

	Minipig (3 kg)	Minipig (20 kg)	Pig (25 kg)	Dog (10 kg)	Human (70 kg)
Cardiac output (ml/min/100 g BW)	23.3 (70)	14.6 (77)	20 (152,157)	12 (68)	8 (68)

For Göttingen minipig values for juvenile (3kg BW, about 1 month of age) and adult animals (20kg BW,10 months of age) were available

$$\text{Surface area } \left(\text{m}^2\right) = \frac{70 * \text{BW}^{0.75}}{1000}$$

For a 14.2 kg minipig this results in a body surface area of, which is the same as in dog (68). Qvist and coworkers reported an average skin thickness for 6 month old Göttingen minipigs of 2.34 mm (82). These values correspond well with measurements in sexually mature Yucatan minipigs where flank and dorsal skin thickness was measured between 1.5 and 2 mm, respectively (88). The resulting skin volume for Göttingen minipigs would then be 1198 ml or 8.4% of total body weight which is in line with skin volumes found in other species (86).

Skin perfusion in Göttingen minipigs was reported to be 7.9 ml/min/100 g tissue (corresponding to 5% of CO) (70,89). In landrace pigs, measures were in the same range (between 3 ml/min/100 g tissue in dorsal and 10–11 ml/min/100 g tissue on ventral areas) (90). For predicting kinetics of transdermal, intra- and subcutaneous administrations, knowledge on lymph flow can be of importance. Intra dermally injected tracer was transported in young landrace pigs with a speed of 3.3–4.6 mm/s in lymph vessels (79). As a comparison, human lymphatic transport occurred at a speed 16 mm/s (91,92).

Mineral Bone and Bone Marrow

Most PBPK models assume the skeletal system to be composed of three compartments. These are mineral bone, yellow and red bone marrow. Mineral bone is generally not included in PBPK models as it is assumed to be pharmacologically inert. Nonetheless, knowledge of this compartment can be useful to establish mass balance for the whole physiology. The minipig skeleton makes up about 2% of total body weight, which for a 14.2 kg minipig corresponds to 283 g (36). Mineral bone density was reported to be 530 +/- 82.6 mg/cm^3 (39).

To our knowledge no data has been published so far on total bone marrow volume, although minipigs are considered a good source for hematopoietic stem cells (93). In humans, yellow and red bone marrow constitute about 27% and 12% of total wet bone weight, respectively (69). If the same percentages are used for minipigs, this results in 0.5% (76.4 g) and 0.2% (34 g) of body weight for yellow and red bone marrow, respectively.

In 3–4 month old landrace pigs, femoral head perfusion was measured at 11 ml/min/100 mg (94). As the trabecular bone of the femoral head consists mainly of red marrow, this value for perfusion should be generally applicable for the red bone marrow compartment. Yellow marrow is mainly constituted from fat and as an approximation, perfusion of adipose tissue can be used (11.1 ml/min/100 mg).

Liver

Anatomically, the porcine liver is constituted of six lobes in contrast to the four lobes in human (67). Additionally, the portal triads are linked *via* connective tissue, which results in a lobulation, typically seen in. However, these peculiarities are not expected to have an impact on functionality. Relative liver weight in minipigs is 1.67% of body weight, which is lower than in human (2.6%) or dog (3.3%) (7). Portal liver blood flow is reported to be 23% of CO, while the hepatic artery makes up 3.3% of CO (70).

Pigs and minipigs have a gall bladder for bile storage and although the composition of porcine bile is very close to that of human, the concentration capacity of the gallbladder seems to be less efficient, leading to a three times less concentrated bile (95). A quantitative analysis of bile conjugates in landrace pigs revealed that glycohyocholate (GHC; 22.5 mM), glycochenodeoxycholate (GCDC, 17.4 mM), and glycohyodeoxycholate (GHDC, 13.7 mM) were the main constituents (96). Total bile salt concentration in the gallbladder was 156 mM (97). Bile flow was reported to be 9 µl/min/kg, which is in the range of human values (1.5–15 µl/min/kg) (98). Biorelevant dissolution media such as have been developed for human (99) and successfully used to generate solubility values for input into PBPK absorption simulations (100) have not yet been designed for pigs or minipigs.

Kidney

The porcine kidney physiology strongly resembles that of human (101). The relative kidney volume of pigs and minipigs (0.4% – 0.47% of BW) lies between human (0.34%) and dog values (0.53%). Glomerular filtration rate (GFR) and perfusion of porcine kidney are comparable to human (see Table VI) (34,68,102,103) as well as urine pH (7–9) which can influence elimination of ionizable compounds (104).

Table VI lists physiological values of minipig and landrace pig kidney.

Generally, not much is known concerning drug transporter expression in pigs and minipigs. Porcine orthologues for human OAT1 and OAT3 were identified in pig kidney and have been successfully cloned (105,106). Of all common laboratory species, it seems that the pig variant provides the highest amino acid identity (89%) to human OAT1. In human kidney, the efflux transporters P-glycoprotein and MRP2 play a key role in elimination of bulky anionic compounds (107) and the uptake transporters of the solute carrier family (*e.g.*, OATP) have importance for reabsorption of bile salts and other, organic charged molecules. There are indications from mRNA expression studies that porcine kidney expresses P-glycoprotein as well as MRP2 (108).

METABOLISM

Metabolic clearance in PBPK models is generally scaled up from *in vitro* measurements. For example, drug kinetics measured in incubations with liver microsomes or hepatocytes are input into a liver model to predict hepatic clearance. Scaling of such data requires a way to relate *in vitro* metabolic activities to the *in vivo* situation and the quantification of enzyme abundance is one way to do this (109). Protein rather than mRNA quantification is required, as transcription and translation are controlled separately and protein and mRNA levels do not necessarily correlate.

For landrace pig, protein abundance of liver Cytochrome P450 (CYP) was quantified by means of liquid chromatography mass spectrometry (110). CYP2A and CYP2D were the most prominently expressed isoforms accounting for 34% and 26% of total P450 protein content. Compared to human, CYP3A and CYP2C were lower expressed in pigs, reaching only 14% and 16% of total CYP content, respectively. It could be shown that human and landrace pig isoforms of CYP1A and CYP2E share almost identical protein sequences and corresponding isoforms of CYP3A, CYP2A, and CYP2C are highly homologous (110). To our knowledge, CYP expression data has not been established for the Göttingen minipig. Broadly, minipig metabolic activity seems to compare relatively well to landrace pig and human (111) although there are some marked differences: Göttingen minipig liver microsomes exhibit a total CYP content of 0.81 nmol/mg protein (112) which is considerably higher than the one of landrace pig (0.22–0.57 nmol/mg protein, (113,114)) or human liver microsomes (0.26–0.43 nmol/mg protein, (115)) Additionally, the Göttingen minipig shows more sex-related expression and activity modulations than other pig breeds (116). For example, activity of CYP1A2 and CYP2E1 was measured as 4 times higher in female than in male Göttingen minipigs although males exhibited similar activity levels to landrace pigs (116). Additionally, male minipigs showed 70-fold lower activity for CYP2A compared to females, which exhibited the same activity as landrace pigs (116,117). Such gender differences are not observed in humans. However, CYP2A shows similar substrate specificity in human and pig breeds, metabolizing test nicotine in both species (118).

Table VI Relative Kidney Weight, Perfusion and Glomerular Filtration Rate (GFR) for Several Species

Species	Organ weight (% of body weight) (2 Kidneys)	Perfusion (ml/min/100 g organ weight)	GFR (L/h × kg)
Minipig	0.47[a]	360.70 (70,77)	0.1-0.15 (34)
Landrace pig	0.40 (152)	429.4 (157)	0.06-0.1 (102,103)
Dog	0.55 (7,68)	432 (68)	0.36 (68)
Human	0.44 (68,69)	400 (68)	0.1 (68)

[a] Value from http://minipigs.dk/backgrounddata

For porcine CYP2C and CYP2D, substrate specificity seems to be different to the human isoenzymes. Porcine CYP2C metabolized diclofenac and tolbutamide but to a markedly lower extent than the human forms (119,120) and other typical human CYP2C substrates, such as S-mephenytoin were not metabolized at all in pig (117). Common inhibitors and inducers of human CYP2C seem to have low to no effect on the porcine isoform (120,121). In contrast to the other sex-related CYP modulations observed in Göttingen minipig, CYP2C shows higher activity in male animals (116,118). Whether the same degree of polymorphism is present in minipig isoenzymes as found in human, remains to be elucidated.

Although cloned porcine CYP2D exhibited a strong amino acid identity with other mammals, pig and minipig human prototypic reactions (122). Apparently, many of these reactions were catalyzed in preference by porcine CYP2B (123). Thus, common human inhibitors such as quinidine had only weak effects on the porcine isoenzymes (124).

The most abundantly expressed human cytochrome, CYP3A4, seems to be relatively well conserved in pigs and minipigs. Besides monkey CYP3A8, porcine CYP3A29 shows the highest protein similarity of all laboratory species to human CYP3A4 (125). Furthermore, purified minipig CYP3A29 showed comparable activity to its human counterpart in hydroxylation of prototypic substrates nifedipine and testosterone (125). Besides CYP3A29, there are four other less studied porcine CYP3A sequences available, CYP3A22, CYP3A39, CYP3A46, and CYP3A88. Tissue distribution pattern of CYP3A enzymes in minipig is comparable to human: Porcine CYP3A mRNA was found readily expressed in liver and brain capillaries as well as in the intestine (120). In

the latter, a similar expression gradient as in human was observed, decreasing from small intestine to colon (126).

Information on conjugation capacity in minipigs is scarce. Comparative studies indicated that minipig glucuronosyltransferases were much more efficient than in man (111,127). Diclofenac PK in minipigs was reported to resemble more dog than human metabolism, as diclofenac was glucuronidated and extensively entero-hepatically recycled, leading to a prolonged exposure (128). Moreover, a recent assessment of raloxifen kinetics in landrace pig indicated that intestinal glucuronidation capacity was high (129).

MODEL CONSTRUCTION, VERIFICATION, AND REFINEMENTS

As no PBPK model of Göttingen minipig has yet been published it has been the aim of this review to collate the needed physiological data to allow construction of such a model. As it has been outlined above, much data is available but important gaps still exist. For example some absorption related parameters, particularly for effective permeability was not available to us. The current model uses therefore the assumption that minipig and human are the same in this respect. Furthermore, much of the reported data covered wide ranges and based on the source, it was not always possible to determine whether these ranges reflect inter-individual variation, measurement imprecision or experimental differences. Further work is needed to complete and better define the relevant physiological data and such efforts are currently ongoing in our own and other groups. In parallel to this work, we are also pursuing a strategy to validate predictions of minipig PK for a set of reference drugs. Simulated PK profiles are compared to *in vivo* data and mismatches are using sensitivity analysis to determine the parameters most likely to be responsible. Better definition of uncertain model parameters is then possible and adjustments can be made to bring about a better match to the *in vivo* data. Careful selection of the reference compounds is needed to facilitate this process. For example, acetaminophen is readily absorbed and the rate limiting step for appearance of drug in the systemic circulation is gastric emptying. Thus, it can be used to characterize gastric emptying time (130,131). Furthermore, dosing of different dosage forms such as solutions, tablets, or capsules should allow assessment of the impact of formulation. This information can then be added to the generic model in a physiologically relevant manner and used to inform future simulations.

With in view, we have model which represents our current view of the most relevant values. It will be refined, as new data arrive and based on its performance in simulating reference drugs. The model implementation is given in the Supplementary Material

In order to provide a first validation of our preliminary model we simulated plasma pharmacokinetics for two marketed antibiotics, moxifloxacin and griseofulvin, and compared to published data obtained in Göttingen minipigs (132,133). Table VII summarizes the input parameters for these simulations. Moxifloxacin, a synthetic fluoroquinolone, is a readily absorbed compound exhibiting moderate to high lipophilicity (logP = 2.9) and moderate solubility.

The source literature (132) provided the mean of plasma concentration profiles obtained in three Göttingen minipigs after intravenous dose of 2.8 mg/kg and oral dose of 9.2 mg/kg, both administered as solution. No estimates of variability were given. The data were extracted from the graphs using a custom program developed in Matlab (http://www.mathworks.com). Total weight adjusted clearance was calculated from the extracted profiles to be 9 L/h for a 14.2 kg physiology. For compound distribution, predicted tissue partitioning using an adaptation of the approach proposed by Rodgers (134) underestimated the volume of distribution and underpredicted measured partition coefficients in the rat (135). Therefore we used the measured rat partition coefficients for muscle, lung, bone, and fat to replace the calculated values in the minipig model. This optimized volume of distribution of 3.7 L/kg compared well to the value of 4.1 L/kg obtained by non-compartmental analysis of the observed data. Simulated plasma concentrations were in good agreement with observed data for both intravenous (Fig. 2a) and oral doses (Fig. 2b). Linear representation of simulated and measured concentration profiles is provided in Supplementary Material.

Table VII Overview of Input Parameters for Model Validation

Compound	Moxifloxacin	Griseofulvin
Molecular weight (g/mol)	401.4	352.8
Dose (mg/kg)	i.v.: 2.8	i.v.: 6.0
	p.o.: 9.2 as a solution	p.o.: 6.0 as a tablet
LogP	2.9[b]	2.9 (137)
pKa	6.4 (acid); 9.5 (base) (158)	Neutral
Water solubility (mg/ml)	0.168[b]	0.063[a]
Particle size radius (μm)	Not applicable	3.1 +/-1.8 (140)
B/P ratio	1.25 (in rats) (132).	0.722[a]
Fup (%)	63 (132)	15.3[a]
Human intestinal permeability (cm/s × 10E-4)	0.203[a]	0.321[a]
Clearance (L/h × kg)	0.645 (132)	0.724 (133)

[a] predicted using ADMET Predictor (159). We assume that minipig and human have the same permeability

[b] values from http://www.drugbank.ca/drugs

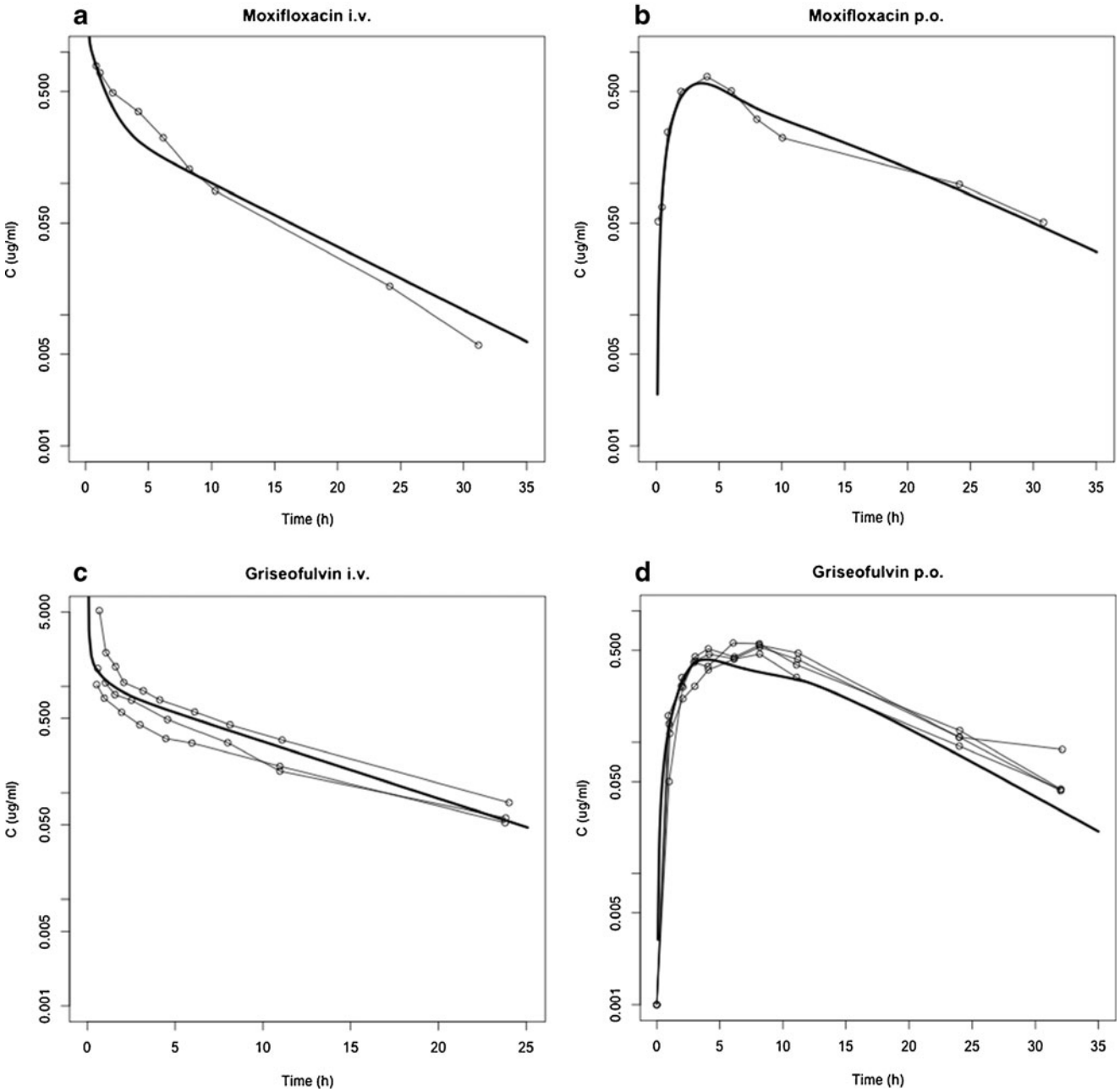

Fig. 2 Simulations (bold lines) of intravenous (**a**) and oral (**b**) pharmacokinetics of moxifloxacin and griseofulvin (**c**, **d**) are shown on a semilogarithmic scale.

Griseofulvin is an antibiotic with antifungal activity (136). The compound is poorly soluble, but highly permeating (BCS class II), exhibiting a solubility in biorelevant media of 0.019 mg/ml (137,138), and a moderate to high lipophilicity (LogP of 2.9) (137). Griseofulvin is in all laboratory species almost completely eliminated *via* metabolism and excreted in the urine (139). The data source provided plasma concentration profiles after intravenous dosing to three Göttingen minipigs. We extracted the data from the graphs and determined, using non-compartmental analysis, a weight adjusted clearance of 8.7 L/h for a 14.2 kg minipig. The data source provided averaged oral PK profiles of commercially available, micronized griseofulvin tablets. As no further formulation details were given, we obtained particle size data from a different reference (140). As expected, modeling oral absorption of the compound was challenging. Griseofulvin is inefficiently and variably absorbed (only 30–70% in rats and human) despite micronization or/and fat co-administration (141). In our simulation, Tmax was predicted within the observed range. However, Cmax was under predicted. A parameter sensitivity analysis showed that the simulated Cmax was very sensitive to the solubility value used. Bile salt concentrations play a considerable role in

absorption of Griseofulvin and other BCS class II compounds (142). However, information on intestinal bile salt concentrations in pigs or minipigs could not be found in the public domain and so we did not include them in the preliminary model factor was that in particle form which impedes a clear separation of dissolution and solubility processes.

Our preliminary PBPK model of the minipig exhibited encouraging performance in this first validation procedure. Obviously some major gaps remain, notably the establishment of scaling factors from *in vitro* metabolism data to predict hepatic clearance and better understanding of the physiological factors determining oral absorption of poorly soluble molecules in pigs. However, the current model provides a solid basis for further work.

OUTLOOK AND CONCLUSION

Minipig is increasingly used in preclinical research and modeling tools are needed to guide study design and interpretation. This review has collated available data to allow construction of a preliminary PBPK model for Göttingen minipig. An advantage of the physiologically based approach is that the model can be continuously updated and refined as new information becomes available, in particular on absorption and metabolism. Furthermore, by adjusting the model to better describe *in vivo* data for well-chosen reference molecules and different formulations a gradual improvement in predictive performance of the model is expected.

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REFERENCES

1. Sinha VK, *et al.* From preclinical to human–prediction of oral absorption and drug-drug interaction potential using physiologically based pharmacokinetic (PBPK) modeling approach in an industrial setting: a workflow by using case example. Biopharm Drug Dispos. 2012;33(2):111–21.
2. Rowland M and Benet LZ. Lead PK commentary: Predicting human pharmacokinetics. J Pharm Sci. 2011.
3. Jones HM, *et al.* Simulation of human intravenous and oral pharmacokinetics of 21 diverse compounds using physiologically based pharmacokinetic modelling. Clin Pharmacokinet. 2011;50(5):331–47.
4. Jones HM, *et al.* A novel strategy for physiologically based predictions of human pharmacokinetics. Clin Pharmacokinet. 2006;45(5):511–42.
5. Parrott N, Lave T. Applications of physiologically based absorption models in drug discovery and development. Mol Pharm. 2008;5(5):760–75.
6. Lave T, *et al.* Challenges and opportunities with modelling and simulation in drug discovery and drug development. Xenobiotica Fate Foreign Compd Biol Syst. 2007;37(10–11):1295–310.
7. Brown RP, *et al.* Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Heal. 1997;13(4):407–84.
8. Poulin P, Theil FP. Prediction of pharmacokinetics prior to *in vivo* studies. II. Generic physiologically based pharmacokinetic models of drug disposition. J Pharm Sci. 2002;91(5):1358–70.
9. Parrott N, *et al.* Predicting pharmacokinetics of drugs using physiologically based modeling–application to food effects. AAPS J. 2009;11(1):45–53.
10. Forster R, *et al.* The RETHINK project on minipigs in the toxicity testing of new medicines and chemicals: conclusions and recommendations. J Pharmacol Toxicol Methods. 2010;62(3):236–42.
11. Dressman JB, Thelen K, Willmann S. An update on computational oral absorption simulation. Expert Opin Drug Metab Toxicology. 2011;7(11):1345–64.
12. Sjogren E, Bredberg U, Lennernas H. The pharmacokinetics and hepatic disposition of repaglinide in pigs: mechanistic modeling of metabolism and transport. Mol Pharm. 2012;9(4):823–41.
13. Bergman E, *et al.* Enterohepatic disposition of rosuvastatin in pigs and the impact of concomitant dosing with cyclosporine and gemfibrozil. Drug Metabolism Dispos Biol Fate Chem. 2009;37(12):2349–58.
14. Thorn HA, *et al.* Different effects of ketoconazole on the stereoselective first-pass metabolism of R/S-verapamil in the intestine and the liver: important for the mechanistic understanding of first-pass drug-drug interactions. Drug Metabolism Dispos Biol Fate Chem. 2009;37(11):2186–96.
15. Sjodin E, *et al.* Intestinal and hepatobiliary transport of ximelagatran and its metabolites in pigs. Drug Metabolism Dispos Biol Fate Chem. 2008;36(8):1519–28.
16. Simianer H, Kohn F. Genetic management of the Gottingen Minipig population. J Pharmacol Toxicol Methods. 2010;62(3):221–6.
17. Nestorov I. Whole-body physiologically based pharmacokinetic models. Expert Opin Drug Metab Toxicology. 2007;3(2):235–49.
18. Yu LX, *et al.* Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. Adv Drug Deliv Rev. 1996;19(3):359–76.
19. Parrott N and Lave T. Computer Models for Predicting Drug Absorption, in Oral Drug Absorption, J.B. Dressman and C. Reppas, Editors. 2010, Informa.
20. Suenderhauf C, *et al.* Combinatorial QSAR modeling of human intestinal absorption. Mol Pharm. 2011;8(1):213–24.
21. Amidon GL, *et al.* A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res. 1995;12(3):413–20.
22. Agoram B, Woltosz WS, Bolger MB. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv Drug Deliv Rev. 2001;50 Suppl 1:S41–67.
23. Thelen K, *et al.* Evolution of a detailed physiological model to simulate the gastrointestinal transit and absorption process in humans, part 1: oral solutions. J Pharm Sci. 2011;100(12):5324–45.
24. Willmann S, Edginton AN, Dressman JB. Development and validation of a physiology-based model for the prediction of oral absorption in monkeys. Pharm Res. 2007;24(7):1275–82.

25. Thelen K, *et al.* Evolution of a detailed physiological model to simulate the gastrointestinal transit and absorption process in humans, part II: extension to describe performance of solid dosage forms. J Pharm Sci. 2012;101(3):1267–80.
26. Jamei M, *et al.* Population-based mechanistic prediction of oral drug absorption. AAPS J. 2009;11(2):225–37.
27. Cosgrove D, *et al.* Quantification of blood flow. Eur Radiol. 2001;11(8):1338–44.
28. Levine BA, Sirinek KR, Gaskill 3rd HV. The radiolabeled microsphere technique in gut blood flow measurement–current practice. J Surg Res. 1984;37(3):241–55.
29. Rodgers T, Rowland M. Mechanistic approaches to volume of distribution predictions: understanding the processes. Pharm Res. 2007;24(5):918–33.
30. Rodgers T, Rowland M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci. 2006;95(6):1238–57.
31. Rodgers T, Leahy D, Rowland M. Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. J Pharm Sci. 2005;94(6):1259–76.
32. Haring F, *et al.* Miniature swine development for laboratory purpose in Proceedings of a symposium on swine in biomedical research. Richland: Battelle Memorial Institute, Pacific Northwest Laboratories Division; 1965.
33. Glodek P, *et al.* Das Göttinger Minischwein - ein Laboratoriumstier mit weltweiter Bedeutung. Zuchtungskunde. 1977;49:21–32.
34. Glodek P and B Oldigs eds. Das Göttingen Minischwein. ed. P. Glodek and B. Oldigs1981, Paul Parey: Berlin. 32–43 and 75–85.
35. Bollen PJ, *et al.* Growth differences of male and female Gottingen minipigs during *ad libitum* feeding: a pilot study. Lab Anim. 2005;39(1):80–93.
36. Johansen T, *et al.* The obese Gottingen minipig as a model of the metabolic syndrome: dietary effects on obesity, insulin sensitivity, and growth hormone profile. Comp Med. 2001;51(2):150–5.
37. Kohn F, Sharifi AR, Simianer H. Modeling the growth of the Goettingen minipig. J Anim Sci. 2007;85(1):84–92.
38. Bollen P, Andersen A, Ellegaard L. The behaviour and housing requirements of minipigs. Scand J Lab Anim Sci. 1998;25(1):23–6.
39. Tsutsumi H. In: McAnulty PA *et al.*, editors. Skeletal System, in The Minipig in Biomedical Research. Boca Raton: CRC Press, Taylor & Francis Group; 2012. p. 33487–2742.
40. Kühn U. Vergleichende anatomische Untersuchungen des Darmtraktes und des darmassoziierten lymphatischen Gewebes (GALT) bei alten Hausschweinerassen und einer modernen Fleischrasse in *School Vet. Med.* Hannover: Tierärztlichen Hochschule Hannover; 2001.
41. S, K, Auswirkungen der Vermahlungsintensität (grob, fein) und Konfektionierung (schrotförmig, pelletiert) des Mischfutters auf die Milieubedingungen im Mageninhalt von Schweinen, in Tierärztliche Hochschule Hannover 2009, University of Hannover: Hannover. p. 225.
42. Hänichen T. Stomach ulcers in swine. Tierärztliche Praxis. 1975;2:107–91.
43. Bal HS and Ghoshal NG. Histomorphology of the torus pyloricus of the domestic pig (Sus scrofa domestica). Zentralblatt fur Veterinarmedizin. Reihe C: Anatomie, Histologie, Embryologie. 1972;1(4):289–98.
44. Hossain M, *et al.* Gastrointestinal transit of nondisintegrating, nonerodible oral dosage forms in pigs. Pharm Res. 1990;7(11):1163–6.
45. Oberle RL, Das H. Variability in gastric pH and delayed gastric emptying in Yucatan miniature pigs. Pharm Res. 1994;11(4):592–4.
46. Schneider JH, *et al.* Ambulatory pH: monitoring with a wireless system. Surg Endosc. 2007;21(11):2076–80.
47. Rerat A. Quantitative measurement of carbohydrate absorption in pigs after ingestion of corn starch. Med Chir Digest. 1975;4 suppl 2:49–51.
48. Ruckenbusch Y, Bueno L. The effect of feeding on the motility of the stomach and small intestine in the pig. Br J Nutr. 1976;35 (3):397–405.
49. Schubert ML. Gastric exocrine and endocrine secretion. Curr Opin Gastroenterol. 2009;25(6):529–36.
50. von Rosenvinge EC, Raufman JP. Gastrointestinal peptides and regulation of gastric acid secretion. Curr Opin Endocrinol Diabetes Obes. 2010;17(1):40–3.
51. C VG. Safety Assessment in the Minipig - Principal Body Systems, in The Minipig in Biomedical Research, P.A. McAnulty, *et al.*, Editors. 2012, CRC Press: Boca Raton, FL 33487-2742. p. 211–236.
52. Köttendorfer S. Auswirkungen der Vermahlungsintensität (grob, fein) und Konfektionierung (schrotförmig, pelletiert) des Mischfutters auf die Milieubedingungen im Mageninhalt von Schweinen, Tierärztliche Hochschule Hannover. 2009, Deutsche Veterinärmedizinische Gesellschaft Service GmbH: Gießen. p. 1–225.
53. Höller H. Untersuchungen über Sekret und Sekretion der Cardiadrüsenzone im Magen des Schweines. Zentralblatt für Veterinärmedizin Reihe A. 1970;17:685–711.
54. Köttendorf S. Auswirkungen der Vermahlungsintensität (grob, fein) und Konfektionierung (schrotförmig, pelletiert) des Mischfutters auf die Milieubedingungen im Mageninhalt von Schweinen. Gießen: Deutsche Veterinärmedizinische Gesellschaft Service GmbH; 2009.
55. McLauchlan G, *et al.* Comparison of gastric body and antral pH: a 24 hour ambulatory study in healthy volunteers. Gut. 1989;30 (5):573–8.
56. Merchant HA, *et al.* Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea pig, rabbit and pig, and implications for their use in drug development. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2011;42(1–2):3–10.
57. Kurihara-Bergstrom T, *et al.* Characterization of the Yucatan miniature pig skin and small intestine for pharmaceutical applications. Lab Anim Sci. 1986;36(4):396–9.
58. Adeola O, King DE. Developmental changes in morphometry of the small intestine and jejunal sucrase activity during the first nine weeks of postnatal growth in pigs. J Anim Sci. 2006;84(1):112–8.
59. Snipes RL. Intestinal absorptive surface in mammals of different sizes. Adv Anat Embryol Cell Biol. 1997;138:III–VIII. 1–90.
60. Davis SS, Illum L, Hinchcliffe M. Gastrointestinal transit of dosage forms in the pig. J Pharm Pharmacol. 2001;53(1):33–9.
61. Wilfart A, *et al.* Digesta transit in different segments of the gastrointestinal tract of pigs as affected by insoluble fibre supplied by wheat bran. Br J Nutr. 2007;98(1):54–62.
62. Dressman JB, Yamada K. Animal models for oral drug absorption. Drugs Pharm Sci. 1991;48:235–66.
63. van Leeuwen P, *et al.* An animal model to study digesta passage in different compartments the gastro-intestinal tract (GIT) as affected by dietary composition. Curr Nutr Food Sci. 2006;2(1):97–105.
64. McRorie J, Greenwood-Van Meerveld B, Rudolph C. Characterization of propagating contractions in proximal colon of ambulatory mini pigs. Dig Dis Sci. 1998;43(5):957–63.
65. Argenzio RA, Lebo D. Ion transport by the pig colon: effects of theophylline and dietary sodium restriction. Can J Physiol Pharmacol. 1982;60(7):929–35.
66. Yasuda K, *et al.* Cecum is the major degradation site of ingested inulin in young pigs. J Nutr. 2007;137(11):2399–404.
67. Bode G, *et al.* The utility of the minipig as an animal model in regulatory toxicology. J Pharmacol Toxicol Methods. 2010;62 (3):196–220.
68. Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharm Res. 1993;10(7):1093–5.

69. Price PS, *et al.* Modeling interindividual variation in physiological factors used in PBPK models of humans. Crit Rev Toxicol. 2003;33(5):469–503.
70. Wyler F, *et al.* The Gottinger minipig as a laboratory animal. 5. Communication: cardiac output, its regional distribution and organ blood flow (author's transl). Research in experimental medicine. Zeitschrift fur die gesamte experimentelle Medizin einschliesslich experimenteller Chirurgie. 1979;175 (1):31–6.
71. West GB, Brown JH. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. J Exp Biol. 2005;208(Pt 9):1575–92.
72. van Essen GJ, *et al.* Cardiovascular performance of adult breeding sows fails to obey allometric scaling laws. J Anim Sci. 2011;89 (2):376–82.
73. van Essen GJ, *et al.* Does cardiovascular performance of modern fattening pigs obey allometric scaling laws? J Anim Sci. 2009;87 (6):1991–7.
74. Diehl KH, *et al.* A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol. 2001;21(1):15–23.
75. Holtz W, Kallweit E. In: Glodek P, Oldigs B, editors. Körperbau und Entwicklung (Anatomy and Development), in Das Göttinger Miniaturschwein (The Goettingen Miniature Pig). Berlin: Verlag Paul Parrey; 1981. p. 32–43.
76. Wittsiepe J, *et al.* Bioavailability of PCDD/F from contaminated soil in young Goettingen minipigs. Chemosphere. 2007;67 (9):355–64.
77. Beglinger R, *et al.* [The Goettingen miniature swine as an experimental animal. 1. Review of literature, breeding and handling, cardiovascular parameters]. Research in experimental medicine. Zeitschrift fur die gesamte experimentelle Medizin einschliesslich experimenteller Chirurgie. 1975;165(3):251–63.
78. Monteiro-Riviere NA, Stromberg MW. Ultrastructure of the integument of the domestic pig (Sus scrofa) from one through fourteen weeks of age. Anat Histol Embryol. 1985;14(2):97–115.
79. Sharma R, *et al.* Quantitative imaging of lymph function. Am J Physiol Heart Circ Physiol. 2007;292(6):H3109–18.
80. Hammond SA, *et al.* Transcutaneous immunization of domestic animals: opportunities and challenges. Adv Drug Deliv Rev. 2000;43(1):45–55.
81. Makin A, Mortensen JT, Brock WJ. In: McAnulty PA *et al.*, editors. Dermal Toxicity Studies, in The Minipig in Biomedical Research. Boca Raton: CRC Press, Taylor & Francis Group; 2012. p. 186.
82. Qvist MH, *et al.* Evaluation of Gottingen minipig skin for transdermal *in vitro* permeation studies. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2000;11(1):59–68.
83. Gschwind HP, *et al.* Pimecrolimus: skin disposition after topical administration in minipigs *in vivo* and in human skin *in vitro*. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2008;33(1):9–19.
84. Zheng Y, *et al.* Minipig as a potential translatable model for monoclonal antibody pharmacokinetics after intravenous and subcutaneous administration. mAbs. 2012;4(2).
85. Warner RL, McFarland LZ. Integument, in The Beagle as an Experimental Animal 1970, Iowa State University Press. p. 126–148.
86. Quillen EW, Reid IA. Effect of intravertebral angiotensin II on cardiac output and its distribution in conscious dogs. Circ Res. 1988;63(4):702–11.
87. Swindle MM. Swine in hte laboratory: surgery, anesthesia, imaging and experimental techniques. 2nd ed. Boca Raton: CRC Press; 2007.
88. Fujii M, *et al.* Evaluation of Yucatan micropig skin for use as an *in vitro* model for skin permeation study. Biol Pharm Bull. 1997;20 (3):249–54.
89. Bulow J, Madsen J, Hojgaard L. Reversibility of the effects on local circulation of high lipid concentrations in blood. Scand J Clin Lab Investig. 1990;50(3):291–6.
90. Monteiro-Riviere NA, *et al.* Interspecies and interregional analysis of the comparative histologic thickness and laser Doppler blood flow measurements at five cutaneous sites in nine species. J Investig Dermatol. 1990;95(5):582–6.
91. Fischer M, *et al.* Flow velocity of single lymphatic capillaries in human skin. Am J Physiol. 1996;270(1 Pt 2):H358–63.
92. Nathanson SD, Nelson L, Karvelis KC. Rates of flow of technetium 99 m–labeled human serum albumin from peripheral injection sites to sentinel lymph nodes. Ann Surg Oncol. 1996;3(4):329–35.
93. Peterbauer-Scherb A, *et al.* Isolation of pig bone marrow mesenchymal stem cells suitable for one-step procedures in chondrogenic regeneration. J Tissue Eng Regen Med. 2010;4 (6):485–90.
94. Schneider T, *et al.* Dynamic gadolinium-enhanced MRI evaluation of porcine femoral head ischemia and reperfusion. Skeletal Radiol. 2003;32(2):59–65.
95. Nakayama F. Composition of gallstone and bile: species difference. Journal Lab Clin Med. 1969;73(4):623–30.
96. Legrand-Defretin V, *et al.* Ion-pair high-performance liquid chromatography of bile salt conjugates: application to pig bile. Lipids. 1991;26(8):578–83.
97. Farthing MJ, Keusch GT, Carey MC. Effects of bile and bile salts on growth and membrane lipid uptake by Giardia lamblia. Possible implications for pathogenesis of intestinal disease. J Clin Investig. 1985;76(5):1727–32.
98. Martinez M, *et al.* Applying the biopharmaceutics classification system to veterinary pharmaceutical products. Part II. Physiological considerations. Adv Drug Deliv Rev. 2002;54 (6):825–50.
99. Jantratid E, *et al.* Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. Pharm Res. 2008;25(7):1663–76.
100. Jones HM, *et al.* Predicting pharmacokinetic food effects using biorelevant solubility media and physiologically based modelling. Clin Pharmacokinet. 2006;45(12):1213–26.
101. Dalmose AL, *et al.* Surgically induced urologic models in swine. J Investig Surg Off J Acad Surg Res. 2000;13(3):133–45.
102. Cibulskyte D, *et al.* The pharmacokinetics and acute renal effects of oral microemulsion ciclosporin A in normal pigs. Int Immunopharmacol. 2006;6(4):627–34.
103. Lodrup AB, *et al.* The association between renal function and structural parameters: a pig study. BMC Nephrol. 2008;9:18.
104. Preusse C, Skaanild MT. In: McAnulty PA *et al.*, editors. Minipigs in Absorption, Distribution, Metabolism, and Excretion (ADME) Studies, in The Minipig in Biomedical Research. Boca Raton: CRC Press, Tylor &Francis Group; 2012. p. 143–58.
105. Hagos Y, *et al.* Functional expression of pig renal organic anion transporter 3 (pOAT3). Biochimie. 2005;87(5):421–4.
106. Hagos Y, *et al.* Cloning of the pig renal organic anion transporter 1 (pOAT1). Biochimie. 2002;84(12):1221–4.
107. Giacomini KM, *et al.* Membrane transporters in drug development. Nat Rev Drug Discov. 2010;9(3):215–36.
108. Goh LB, *et al.* Endogenous drug transporters in *in vitro* and *in vivo* models for the prediction of drug disposition in man. Biochem Pharmacol. 2002;64(11):1569–78.
109. Rostami-Hodjegan A, Tucker GT. Simulation and prediction of *in vivo* drug metabolism in human populations from *in vitro* data. Nat Rev Drug Discov. 2007;6(2):140–8.
110. Achour B, Barber J, Rostami-Hodjegan A. Cytochrome p450 pig liver pie: determination of individual cytochrome p450 isoform contents in microsomes from two pig livers using liquid chromatography in conjunction with mass spectroscopy. Drug Metabolism Dispos Biol Fate Chem. 2011;39(11):2130–4.

111. Anzenbacherova, E., *et al.*, Minipig as a model for drug metabolism in man: comparison of in vitro and in vivo metabolism of propafenone. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia. 2003;147(2): 155–9.
112. Skaanild MT. Porcine cytochrome P450 and metabolism. Curr Pharm Des. 2006;12(11):1421–7.
113. Nebbia C, *et al.* Comparative expression of liver cytochrome P450-dependent monooxygenases in the horse and in other agricultural and laboratory species. Vet J. 2003;165(1):53–64.
114. Myers MJ, *et al.* Identification of multiple constitutive and inducible hepatic cytochrome P450 enzymes in market weight swine. Drug Metabolism Dispos Biol Fate Chem. 2001;29(6):908–15.
115. Shimada T, *et al.* Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther. 1994;270 (1):414–23.
116. Skaanild MT, Friis C. Cytochrome P450 sex differences in minipigs and conventional pigs. Pharmacol Toxicol. 1999;85(4):174–80.
117. Bogaards JJ, *et al.* Determining the best animal model for human cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey and man. Xenobiotica Fate Foreign Compd Biol Syst. 2000;30(12):1131–52.
118. Skaanild MT, Friis C. Analyses of CYP2C in porcine microsomes. Basic Clin Pharmacol Toxicol. 2008;103(5):487–92.
119. Anzenbacher P, *et al.* Presence and activity of cytochrome P450 isoforms in minipig liver microsomes. Comparison with human liver samples. Drug Metabolism Dispos Biol Fate Chem. 1998;26 (1):56–9.
120. Puccinelli E, *et al.* Expression and inducibility by phenobarbital of CYP2C33, CYP2C42, CYP2C49, CYP2B22, and CYP3As in porcine liver, kidney, small intestine, and nasal tissues. Xenobiotica Fate Foreign Compd Biol Syst. 2010;40(8):525–35.
121. Ioannides C. Cytochrome p450 expression in the liver of food-producing animals. Curr Drug Metab. 2006;7(4):335–48.
122. Puccinelli E, Gervasi PG, Longo V. Xenobiotic metabolizing cytochrome P450 in pig, a promising animal model. Curr Drug Metab. 2011;12(6):507–25.
123. Skaanild MT, Friis C. Is cytochrome P450 CYP2D activity present in pig liver? Pharmacol Toxicol. 2002;91(4):198–203.
124. Hosseinpour F, Wikvall K. Porcine microsomal vitamin D(3) 25-hydroxylase (CYP2D25). Catalytic properties, tissue distribution, and comparison with human CYP2D6. J Biol Chem. 2000;275 (44):34650–5.
125. Anzenbacherova E, *et al.* Model systems based on experimental animals for studies on drug metabolism in man: (mini)pig cytochromes P450 3A29 and 2E1. Basic Clin Pharmacol Toxicol. 2005;96(3):244–5.
126. Nishi K, *et al.* The expression of intestinal CYP3A4 in the piglet model. Transplant Proc. 2004;36(2):361–3.
127. Marini S, *et al.* Xenobiotic-metabolizing enzymes in pig nasal and hepatic tissues. Xenobiotica Fate Foreign Compd Biol Syst. 1998;28(10):923–35.
128. Oberle RL, *et al.* Pharmacokinetics and metabolism of diclofenac sodium in Yucatan miniature pigs. Pharm Res. 1994;11(5):698–703.
129. Thorn HA, *et al.* Extensive intestinal glucuronidation of raloxifene in vivo in pigs and impact for oral drug delivery. Xenobiotica Fate Foreign Compd Biol Syst, 2012.
130. Heading RC, *et al.* The dependence of paracetamol absorption on the rate of gastric emptying. Br J Pharmacol. 1973;47(2):415–21.
131. Clements JA, *et al.* Kinetics of acetaminophen absorption and gastric emptying in man. Clin Pharmacol Ther. 1978;24(4):420–31.
132. Siefert HM, *et al.* Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: a comparison in humans and other mammalian species. J Antimicrob Chemother. 1999;43(Suppl B):69–76.
133. Aoyagi N, *et al.* Bioavailability of griseofulvin from plain tablets in Gottingen minipigs and the correlation with bioavailability in humans. J Pharmacobio-dynamics. 1984;7(1):7–14.
134. Rodgers T, Leahy D, Rowland M. Tissue distribution of basic drugs: accounting for enantiomeric, compound and regional differences amongst beta-blocking drugs in rat. J Pharm Sci. 2005;94(6):1237–48.
135. Beckmann J, *et al.* Tissue concentrations of vancomycin and Moxifloxacin in periprosthetic infection in rats. Acta orthopaedica. 2007;78(6):766–73.
136. Sobue S, Sekiguchi K, Nabeshima T. Intracutaneous distributions of fluconazole, itraconazole, and griseofulvin in Guinea pigs and binding to human stratum corneum. Antimicrob Agents Chemother. 2004;48(1):216–23.
137. Takano R, *et al.* Oral absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test. Pharm Res. 2006;23 (6):1144–56.
138. Fujioka Y, *et al.* Prediction of oral absorption of griseofulvin, a BCS class II drug, based on GITA model: utilization of a more suitable medium for *in-vitro* dissolution study. J Control Release Off J Control Release Soc. 2007;119(2):222–8.
139. Lin C, Symchowicz S. Absorption, distribution, metabolism, and excretion of griseofulvin in man and animals. Drug Metab Rev. 1975;4(1):75–95.
140. Mosharraf M, Nystrom C. The effect of dry mixing on the apparent solubility of hydrophobic, sparingly soluble drugs. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 1999;9(2):145–56.
141. Rowland M, Riegelman S, Epstein WL. Absorption kinetics of griseofulvin in man. J Pharm Sci. 1968;57(6):984–9.
142. Bates TR, Gibaldi M, Kanig JL. Solubilizing properties of bile salt solutions. II. Effect of inorganic electrolyte, lipids, and a mixed bile salt system on solubilization of glutethimide, griseofulvin, and hexestrol. J Pharm Sci. 1966;55 (9):901–6.
143. DeSesso JM, Williams AL. In: Macor JE, editor. Contrasting the gastrointestinal tracts of mammals: factors that influence absorption, in annual reports in medicinal chemistry. The Netherlands: Academic; 2008. p. 353–71.
144. Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos. 1995;16(5):351–80.
145. Treacy PJ, Jamieson GG, Dent J. Pyloric motor function during emptying of a liquid meal from the stomach in the conscious pig. J Physiol. 1990;422:523–38.
146. Lui CY, *et al.* Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans. J Pharm Sci. 1986;75(3):271–4.
147. Hernot DC, *et al.* Evaluation of association between body size and large intestinal transit time in healthy dogs. Am J Vet Res. 2006;67(2):342–7.
148. Adkin DA, *et al.* The effects of pharmaceutical excipients on small intestinal transit. Br J Clin Pharmacol. 1995;39(4):381–7.
149. Graff J, Brinch K, Madsen JL. Gastrointestinal mean transit times in young and middle-aged healthy subjects. Clin Physiol. 2001;21 (2):253–9.
150. Bahr R, Flach A. Morphological and functional adaptation after massive resection of the small intestine: experiments using minipigs of the Gottingen strain. Prog Pediatr Surg. 1978;12:107–42.
151. Snyder WS, *et al.* eds. Report of the Task Group on Reference Man. ICRP publication1975, Pergamon Press: New York.

152. Buur JL, *et al.* Development of a physiologic-based pharmacokinetic model for estimating sulfamethazine concentrations in swine and application to prediction of violative residues in edible tissues. Am J Vet Res. 2005;66(10):1686–93.
153. Upton RN. Organ weights and blood flows of sheep and pig for physiological pharmacokinetic modelling. J Pharmacol Toxicol Methods. 2008;58(3):198–205.
154. Duddy J, *et al.* Physiological model for distribution of sulfathiazole in swine. J Pharm Sci. 1984;73(11):1525–8.
155. Phuc BHN, Hieu LT. "A" molasses in diets for growing pigs. Livest Res Rural Dev. 1993;2(3):75–8.
156. van Woerkens LJ, *et al.* Effect of epinine on systemic hemodynamics and regional blood flow in conscious pigs. J Cardiovasc Pharmacol. 1992;19(4):580–6.
157. Lundeen G, Manohar M, Parks C. Systemic distribution of blood flow in swine while awake and during 1.0 and 1.5 MAC isoflurane anesthesia with or without 50% nitrous oxide. Anesth Analg. 1983;62(5):499–512.
158. Carceles CM, *et al.* Pharmacokinetics and milk penetration of moxifloxacin after intramuscular administration to lactating goats. Vet J. 2007;173(2):452–5.
159. SimulationsPlus, ADMET PredictorTM manual, 2010a.