

**UCC Library and UCC researchers have made this item openly available.  
Please [let us know](#) how this has helped you. Thanks!**

<b>Title</b>	The pig as a pre-clinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms - a PEARRL review
<b>Author(s)</b>	Henze, Laura J.; Koehl, Niklas J.; O'Shea, Joseph P.; Kostewicz Edmund; Holm, René; Griffin, Brendan T.
<b>Publication date</b>	2018-04-10
<b>Original citation</b>	Henze, L. J., Koehl, N. J., O'Shea, J. P., Kostewicz, E. S., Holm, R. and Griffin, B. T. (2018) 'The pig as a preclinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms: a PEARRL review', Journal of Pharmacy and Pharmacology, 71 (4), pp. 581-602 doi:10.1111/jphp.12912
<b>Type of publication</b>	Article (peer-reviewed)
<b>Link to publisher's version</b>	<a href="https://onlinelibrary.wiley.com/doi/epdf/10.1111/jphp.12912">https://onlinelibrary.wiley.com/doi/epdf/10.1111/jphp.12912</a> <a href="http://dx.doi.org/10.1111/jphp.12912">http://dx.doi.org/10.1111/jphp.12912</a> Access to the full text of the published version may require a subscription.
<b>Rights</b>	<b>© 2018 Royal Pharmaceutical Society. This is the peer reviewed version of the following article: (2018), The pig as a preclinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms: a PEARRL review. J Pharm Pharmacol., which has been published in final form at <a href="https://doi.org/10.1111/jphp.12912">https://doi.org/10.1111/jphp.12912</a>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.</b>
<b>Item downloaded from</b>	<a href="http://hdl.handle.net/10468/5790">http://hdl.handle.net/10468/5790</a>

Downloaded on 2019-06-19T19:37:32Z

# **The pig as a pre-clinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms - a PEARRL review**

*Laura J. Henze<sup>1</sup>, Niklas J. Koehl<sup>1</sup>, Joseph P. O'Shea<sup>1</sup>,  
Edmund S. Kostewicz<sup>2</sup>, René Holm<sup>3</sup>, Brendan T. Griffin<sup>1,\*</sup>*

*<sup>1</sup> School of Pharmacy, University College Cork; Ireland*

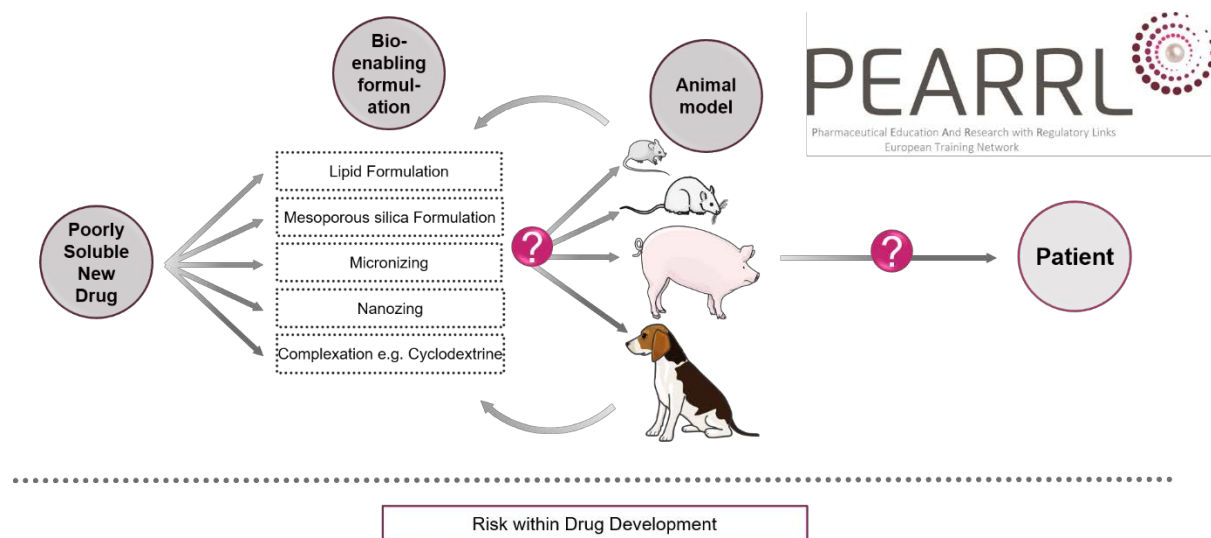
*<sup>2</sup> Institute of Pharmaceutical Technology, Goethe University, Max-von-Laue-Str.  
9, Frankfurt am Main, Germany*

*<sup>3</sup> Drug Product Development, Janssen Research and Development, Johnson &  
Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium*

\*Corresponding author. Brendan T. Griffin, Cavanagh Building, School of Pharmacy, University College Cork, College Road, Cork, Ireland. E-mail: [Brendan.griffin@ucc.ie](mailto:Brendan.griffin@ucc.ie)

Pig as a preclinical model of oral drug absorption

## Graphical Abstract



## **Abstract**

**Objectives** In pharmaceutical drug development, preclinical tests in animal models are essential to demonstrate whether the new drug is orally bioavailable and to gain a first insight into *in vivo* pharmacokinetic parameters that can subsequently be used to predict human values. Despite significant advances in the development of bio-predictive *in vitro* models and increasing ethical expectations for reducing the number of animals used for research purposes, there is still a need for appropriately selected pre-clinical *in vivo* testing to provide guidance on the decision to progress to testing in humans. The selection of the appropriate animal models is essential both to maximise the learning that can be obtained from such experiments and to avoid unnecessary testing in a range of species.

**Key findings** The present review, provides an insight into the suitability of the pig model for predicting oral bioavailability in humans, by comparing the conditions in the GIT. It also contains a comparison between the bioavailability of compounds dosed to both humans and pigs, to provide an insight into the relative correlation and examples on why a lack of correlation may be observed.

**Summary** While there is a general trend towards predicting human bioavailability from pig data, there is considerable variability in the data set, most likely reflecting species specific differences in individual drug metabolism. Nonetheless, the correlation between pigs vs humans was comparable to that reported for dogs vs. humans. The presented data demonstrate the suitability of the pig as a preclinical model to predict bioavailability in human.

**Keywords;** mini-pigs, oral drug absorption, physiologically based pharmacokinetic (PBPK), pigs, preclinical animal model

## 1 Introduction

In pharmaceutical drug product development, selection of the best lead drug candidate, defining how to formulate and to develop it into a newly licensed medicine is a complicated, costly, and risky process <sup>[1]</sup>. Over the last few decades, despite huge advances in therapeutic target discovery and the number of drug molecules entering development programs, the number of drug products (i.e. medicines) licensed for clinical use has decreased consistently <sup>[2]</sup>. Thus, there is a clear need to develop new technologies to improve the developability of emerging drug candidates and to unlock key bottlenecks stifling innovation in pharmaceutical development. New methods of reliably assessing the quality, safety and efficacy of emerging medicines are required to drive change in the drug product development process from the traditional ‘trial and error’ testing approach to selecting the most appropriate tests and avoiding costly development delays. Introducing more clinically relevant testing methods will make drug product development more cost-effective, reduce unnecessary testing, and ultimately facilitate earlier access to market for new medicines. This strategy towards more reliable pre-clinical testing has parallels with the recently proposed biopharmaceutics risk assessment roadmap (BioRAM) for optimising drug product development and clinical performance <sup>[3]</sup>. A key tenet of the BioRAM approach is to move from a “test and confirm” to a “learn and confirm” development paradigm, where predictions will be made in the ‘learn phase’ on how to maximise medical value for a new drug in advance of *in vivo* studies, and *in vivo* studies become ‘confirmatory’ rather than ‘exploratory’ <sup>[3]</sup>.

Operating within the conventional drug product development paradigm involves initially *in vitro* screening, pre-clinical testing followed by clinical evaluation in humans. There are two key stages where improvements in applying a ‘learn and confirm’ paradigm could be sought. Firstly, the link between the *in vitro* testing and the pre-clinical evaluation *in vivo*, there is a

## Pig as a preclinical model of oral drug absorption

need for an improved bio-relevance of screening conditions that truly reflect the pre-clinical animal model conditions, so the pre-clinical *in vivo* testing becomes confirmatory of *in vitro* tests. Secondly, there is a need to validate the reliability of pre-clinical models to predict performance in humans, so that clinical studies become truly confirmatory of pre-clinical investigations.

When attempting to make *a priori* prediction whether a new drug product will be bioavailable *in vivo*, a thorough understanding of the factors influencing drug absorption is essential. However, the process of oral drug absorption is complex, which is influenced by numerous factors including (a) the physiochemical properties of the drug, (b) formulation related factors such as formulation type and excipients used, and (c) physiological, genetic, biochemical and pathophysiological factors in the gastrointestinal (GI) tract. While the utility of bio-relevant *in vitro* and *in silico* models for predicting absorption potential, based on physiochemical properties of the drug substance, are well established, there are gaps in our knowledge on the interplay of drug, gastrointestinal physiology and formulation excipients on *in vivo* drug absorption. These gaps limit the ability to reliably predict *in vivo* behaviour for formulated drug products. Therefore, pre-clinical *in vivo* assessment of prototype drug formulations is routinely required, involving potentially a range of pre-clinical animal species, such as rats, dogs, pigs and non-human primates.

When considering the most suitable animal model to predict bioavailability or bio-performance in humans, a detailed understanding of physiological, anatomical and biochemical differences between different animal models is required<sup>[4]</sup>. In pre-clinical ADMET studies, commonly two animal species are used – one rodent and one non-rodent. There are limiting factors in the use of rodent species as an animal model to investigate the impact of drug formulation on bioavailability, such as the small liquid volume in the gastrointestinal tract<sup>[5]</sup>, limited dose size

Pig as a preclinical model of oral drug absorption

and the generally higher metabolic rate <sup>[6]</sup>, where the latter may also provide an obstacle for reliably predictions of human pharmacokinetics. The most common rodent model is the rat. Rats are considered to be good predictors of intestinal permeability in humans and have been shown to display similar intestinal absorption profiles (i.e. correlation > 0.8 between permeability estimates in rat and humans for 16 compounds encompassing both passive and carrier mediated substrates) and similar transporter expression patterns in the small intestine <sup>[7]</sup>. However, there are major differences between the two species that limit the utility of rats for predicting bioavailability in humans, including distinct expression levels and patterns for metabolizing enzymes in the intestine, and anatomical differences such as size, gastrointestinal length, pH profile and transit time <sup>[7]</sup>.

There have been extensive discussions in the literature on the use of dog and non-human primate models to predict oral bioavailability in humans and the merits of pre-clinical models have been well described recently <sup>[8; 9]</sup>. In principle, non-human primates are considered the closest model to humans, notwithstanding discrepancies with respect to intestinal metabolism which have been recently reviewed <sup>[8]</sup>. However, apart from ethical considerations for using non-human primates for routine pre-clinical testing, studies involving non-human primates are prohibitively expensive. Dogs have traditionally been the most commonly used large animal model in pharmaceutical drug product development. However, there are notable difference in the gastrointestinal tract relative to humans that must be considered <sup>[10]</sup>. The pH in the fasted state canine stomach is considered to be on average higher and more variable than in humans, ranging between 1.5 and 6.8. As a result, it is common to pre-dose dogs with pentagastrin, whereby gastric pH is lowered.

In contrast, there is much less published information on the suitability of pigs for assessing bioavailability of oral drug products. The use of pigs in pre-clinical assessments has increased

## Pig as a preclinical model of oral drug absorption

in recent years <sup>[11; 12]</sup>. A principle advantage of the pig model is the similarity between gastrointestinal tract anatomy and physiology of humans and pigs <sup>[13; 14]</sup>. Pigs, like humans, are omnivorous <sup>[15]</sup>, they have similar digestive system <sup>[4; 16; 15]</sup> and they are a suitable model for evaluation of most ADMET endpoints <sup>[16]</sup>. Moreover, the intestinal microbiome of the colon and the digestion characteristics of the small intestines are considered to be similar to man <sup>[17]</sup>. Analogies among pigs and human include skin structure, cardiovascular system, urinary system as well as the immune system <sup>[13]</sup>. There are several breeds of pigs used in pharmaceutical research, ranging from the domestic Landrace (LR) to more selectively bred miniature-sized pigs, such as Göttingen, Yucatan, Hanford, Sinclair minipigs. Göttingen minipigs are the most commonly used, and were originally developed by crossbreeding the Minnesota minipig, Vietnamese potbelly pig and German Landrace pig <sup>[18]</sup>. While there are gaps in the literature on the possible breed specific difference in gastrointestinal physiology, the mostly widely characterised are the LR and Göttingen minipig <sup>[19]</sup>.

In summary, with the increasing emphasis on expediting drug product development (or a 'quick win, fast fail' paradigm), drug development scientists must operate in reduced time-lines to provide a drug formulation that provides efficient and reliable bioavailability *in vivo*. Selecting the most suitable pre-clinical animal model is essential to demonstrate the efficacy of the drug formulation *in vivo* and to provide early predictors of the likely performance in humans. While the merits and limitations of rat, dog and non-human primate models have been extensively reviewed in the literature, the purpose of this review is to focus on the pig and its suitability as a model in drug product development. Furthermore, an extensive review of the literature was completed to harness knowledge from previous studies involving bioavailability assessment of drugs in pigs with the objective of assessing the degree of correlation between human's and pig's bioavailability, which to the best of our knowledge have not been published elsewhere. A secondary goal of this work was to compare the pig model to the more commonly used dog



Pig as a preclinical model of oral drug absorption

model, which will facilitate the more critically informed decisions on which pre-clinical species to select in drug product development.

## **2 Comparing the gastrointestinal tract of pigs and humans**

To determine the suitability of the pig for pharmacokinetic studies, an understanding of the conditions in the GI tract is essential. Differences in the anatomical and physiological characteristics of the GI tract can impact formulation performance and drug absorption. The next section will directly compare the pig to human GI tract conditions with the focus on identifying key differences.

### **2.1 Gastrointestinal pH**

The impact of the GI pH on oral drug absorption in humans is well established by affecting parameters such as solubility, ionization and stability <sup>[20]</sup>. In particular, the acidic pH of the stomach can have a significant influence on drug product performance, particularly for drugs displaying pH dependent solubility. The gastric pH during the fasted state in pigs and humans is broadly similar, for example the stomach pH in the fasted pig is 1.2 - 4.0 <sup>[21]</sup>, compared to reported ranges for humans, of 1.0 - 3.5 <sup>[22]</sup>. A more detailed overview of the pH along the length of the GI tract is presented in Table 1 which demonstrates a similar increase in the pH pattern in the small intestine and a pH reduction in the colon compared with humans. Interestingly, the pH profiles between pigs and humans in both the fasted and fed states were comparable. This is one distinct advantage of the porcine model versus the canine model and particularly important in terms of suitability of the pig model for predicting the performance of drug dosage forms that are likely to be influenced by the pH. This is pertinent for modified release dosage forms, where a pH change can act as a trigger for drug release in the intestinal lumen, e.g. gastric resistant tablets or colonic delivery based upon a pH sensitive polymer.

## 2.2 Intestinal physiology

While there are external morphological differences between humans and pigs, their internal physiology is considerably similar [4; 15; 26]. At maturity, the total length of the pig intestine is reported to be longer in pigs (24 cm/kg [26]) compared to the human intestine which is estimated to be 14 cm/kg. However, the reported values of total GI tract length vary widely in pigs, with values as much as 23 m in length, depending on the size and weight of the pig, as well as the type of measurement technique employed in various studies [4; 15; 26]. Furthermore, the anatomical arrangement of the large intestine is different between pigs and humans. In humans the large intestine can be described in ascending, transverse and descending colon forming a square like configuration, which ends in the s-shape of the sigmoid colon [15]. In pigs, the large intestine is described as a spiral colon, where the distinct sections of the colon are arranged in a series of coils [13; 15]. Despite these differences, the relative length of each of the three major intestinal segments (as a % of overall length) are similar between pigs and humans, as illustrated in Figure 1. Merchant *et al.*, demonstrated that the length of each section in pigs with an average weight of 95-110kg is comparable to humans, comprising 82 %, 17%, 1% vs. 65%, 33% & 2.5% for the small intestine, colon and caecum, respectively [4; 26]. Normally, smaller pigs are used in preclinical studies, therefore, it is interesting to note, that similar studies in smaller landrace pigs (average weight 18 kg) showed that the intestinal sections are also relatively similar. Differences in relative % lengths of the GI tract could potentially influence the type of formulation to be studied in a pig model. Merchant *et. al* reported that the amount of water in the pig intestine (18g/kg body weight) was higher than in human intestine (8.2g/kg body weight) [26]. A greater amount of water within the porcine intestinal tract may also merit consideration in terms of the dosage form disintegration and potential impact on drug dissolution.

## Pig as a preclinical model of oral drug absorption

Given that the surface area available for drug absorption is a critical factor for passive diffusion across the intestinal membrane, a comparison of the surface area of the pig versus human intestine is pertinent (Table 2). DeSesso and co-workers investigated the absorptive surface area in the lumen of the small intestine of pigs and compared this to human surface area. Using a basic cylindrical estimate of the luminal surface area, of the small intestine of a 70kg human versus a 47kg pig, the pig's intestinal surface area appears to be higher than the human's area, at 1.4m<sup>2</sup> versus 0.42 m<sup>2</sup> respectively. However, given that the apical 'brush border' on enterocytes of both humans and pigs is composed of plicae, villi and micro-villi, a more accurate estimate of the total surface area that take these factors in account, suggested that the intestinal absorptive surface area of humans and pigs are comparable, at 252 m<sup>2</sup> and 168-210 m<sup>2</sup> respectively [27].

### 2.3 Bile composition

Bile salts are amphiphilic molecules and intrahepatic bile acids undergo further conjugation with glycine and taurine [28]. Once synthesized and conjugated in the hepatocyte, bile acids are transported into the biliary tract, secreted through the hepatic duct into the gallbladder. During food intake the bile fluid is secreted into the duodenum, mediated by cholecystokinin and secretin [29]. In some animal species bile is secreted continuously (e.g. rats), whereas pigs have a secretion pattern similar to humans [4; 30]. Once secreted into the intestine, the central function of bile acids is the solubilisation of dietary lipids and promotion of their digestion and absorption. Thus, bile salts can have a significant influence on drug absorption, and in particular promote the solubilisation of poorly water soluble drugs [31].

Bile acids can be classified into primary and secondary bile acids; chenodeoxycholic acid and cholic acid are primary bile acids in many species, including pigs and humans [32]. Secondary bile acids are formed by bacterial hydrolysis of conjugated bile acids in the large intestine. The

## Pig as a preclinical model of oral drug absorption

major bile acids present in pigs and humans are summarized in Table 3 and a comparison of the bio-synthesis pathway for bile salts between the two species is presented in Figure 2. A key difference between bile composition between pigs and humans is hyocholic acid, which is the major primary bile acid in pigs. Hyocholic acid is synthesized in pigs by 6 $\alpha$ -hydroxylation of chenodeoxycholate <sup>[32]</sup>, whereas in humans only small amounts of hyocholic acid and hyodeoxycholic acid exists <sup>[33; 34]</sup>. In humans, CYP3A4 has been identified as the relevant enzyme for the 6 $\alpha$ -hydroxylase reaction of hyocholic acid biosynthesis <sup>[35]</sup>. In pigs, the primary structure of the porcine 6 $\alpha$ -hydroxylase enzyme showed 75% identity with members of the CYP4A subfamily in human <sup>[32]</sup>.

In both humans and pigs, the major bile components are conjugated with taurine or glycine <sup>[36]</sup>, with the majority (~80%) being conjugated with glycine<sup>[37]</sup>. In pigs, the rank order of prevalence of bile salts in the intestine is glyco-hyoicholate (44.4%), glyco-chenodeoxycholate (31.2%), glycol-hyodeoxycholate (8.2%), tauro-hyoicholate (6.8%) and tauro-chenodeoxycholate (6.6%), as illustrated in Figure 3 <sup>[38; 39]</sup>. By comparison, the most prevalent intestinal bile salts in humans are glyco-cholate (36.5%), glycol-chendeoxycholate (33%), glyco-deoxycholate (10%), tauro-cholate (6.8%) and tauro-chendeoxycholate (6.7%).

Given the key role bile acids play in the drug absorption process it is still unclear what impact, if any, these compositional differences in bile have on drug absorption. Also given that bile composition is highly influenced by fasted and fed states in humans, there is a need to improve knowledge on bile secretion profiles in the fasted and fed porcine intestine.

## 2.4 Gastrointestinal transit time

Formulations that enter the GI tract must pass through several sections, of which some are specialised for absorption and others for holding and digesting food. The stomach is used for

## Pig as a preclinical model of oral drug absorption

holding food and therefore can delay transit through the small intestine. The small intestine is the main absorptive site of drugs and nutrients in both pigs and humans. A comparison of the different transit times through the sections is crucial to increase the understanding of the pig as a pre-clinical animal model. The small intestine transit time is the most important time for drug absorption during the GI tract, it is reported to be similar in pigs and humans, 3-4 h in pig compared to 2-4 h in humans <sup>[10; 6; 41]</sup>. As described in previous sections the physiology of the small intestine is also comparable, consequently, the drug absorption in the small intestine in pigs is thought to be similar to the absorption in humans.

The gastric emptying time is an issue which has received much discussion when considering the suitability of pigs for assessing oral pharmacokinetics. The gastric emptying rate in pigs is considered to be longer and more variable than in humans. Human gastric transit is reported to be 10 – 15 minutes for liquids and can be up to 2 hours for indigestible solids <sup>[24]</sup>. By comparison, reported values of gastric emptying in pigs display wide variability. This may reflect a physiological difference of the stomach of pigs, which has a muscular outpouching called the torus pyloricus that can cause food to be retained in the stomach for prolonged period of up to 24 hours <sup>[15]</sup>. Alternatively, methodological differences in how the gastric emptying rate is determined can lead to variable estimates. Hossain *et al.* <sup>[21]</sup> reported an extraordinary long and highly variable gastric emptying time of 1 – 28 days measured by the emptying of a solid dosage form from the stomach. A trend towards an increased gastric emptying time was observed with increasing density of the dosage form. However, this study was limited in that it involved only two pigs that were repeatedly dosed for a prolonged period. In another study Oberle and Das <sup>[25]</sup> reported a shorter gastric emptying time in pigs of 6-24 hours, albeit still highly variable and longer than comparable values in humans <sup>[21; 25]</sup>. In contrast. Davis *et al.* <sup>[6]</sup> reported that the gastric emptying time after a light meal ranged between 1.5 - 6 hours in landrace pigs, and therefore was closer to human estimates, based upon investigations of the

## Pig as a preclinical model of oral drug absorption

gastric emptying of liquids and solid dosage forms in pigs using gamma scintigraphy <sup>[6]</sup>. Davis et al. <sup>[6]</sup> also reported the mean time to empty 50% of a dosed liquid to be 1.4 hours in pigs, compared to reported estimates of 11 -14 minutes for a solution in man <sup>[42]</sup>. The gastric emptying rate of pellets in pigs (pellet size of 0.85 -1.4 mm), was 2.2. h for 50% of the pellets to enter the small intestine and 4 hours after dosing a non-disintegrating tablet <sup>[6]</sup>. The small intestinal transit time in the pigs was reported to be constant on 3-4 hours for the three dosage forms (solution, pellets and tablet), the total transit time was between 24 and 48 hours for three of the 4 pigs, one pig showed an unusually long total transit time of 72- 96hours <sup>[6]</sup>.

More recently, Christiansen et al. <sup>[43]</sup> investigated the possibility of using Göttingen mini-pigs to investigate fasted versus fed state differences in oral bioavailability, and as part of the study, co-administered paracetamol was used as a marker for gastric emptying. Christensen and co-workers reported gastric emptying rates in the fasted animals to be 4-6 hours. After administration of FDA standard breakfast to mini-pigs (5 or 10 g/kg) or a nutritional drink (10 or 20 g/kg) the gastric emptying rate did not increase, but rather tended to decrease slightly. A possible explanation for this apparent lack of impact of food on gastric emptying may indicate that fasted pigs retain food in the stomach for prolonged periods, and hence are not dosed in a 'truly' fasted state. This hypothesis was further explored by Suenderhauf *et al.* <sup>[44]</sup> where pigs were pre-dosed with the pro-kinetic drug metoclopramide, followed by a post mortem determination of the stomach contents weight. In metoclopramide pre-treated pigs, the weight of the stomach contents was lower compared to the non-treated groups, suggesting that this is a potential approach to mimic a fasted state condition. In contrast, however, a recent study by Henze *et al.* <sup>[45]</sup> did not observe an effect of pre-treating with metoclopramide on the gastric emptying rate in minipigs, based upon analysis of paracetamol absorption both in the fasted and different fed state conditions (FDA breakfast vs. nutritional drink vs. normal pig food). The study found that the shortest and most consistent gastric emptying rate was observed in the case

## Pig as a preclinical model of oral drug absorption

where the animals had access to normal pig food at the normal times of the day. It would appear therefore that further studies are required to elucidate whether the pig model is suitable for exploring fasted versus fed effects on oral bioavailability of drug products.

Another factor for consideration in relation to transit time of dosage forms is the age of the pigs. Snoeck *et al.* <sup>[46]</sup> compared the investigated transit time of pellets in young suckling piglets to piglets in different post-weaning states. The gastric emptying rate in sucking pigs (3 week old 4-6kg pigs) was faster compared to post-weaning pigs (4-7 week old 10-13kg pigs), with 75% of the pellets having emptied from the stomach within 1.5-3.5 hours. Three days postweaning, the gastric emptying time is markedly prolonged (75% of the pellets having emptied from the stomach within 25-34 hours). This delayed intestinal transit was most likely related to the change in diet (i.e. from sow's milk to a dry diet) and social stress as a result of weaning. Three weeks post weaning, the gastric emptying rate appeared to return to norm with transit times similar to values reported in growing and adult pigs (75% pellets emptied within 7 hours). While the majority of pellets were emptied from the stomach within 24 hours in 3-week-weaned pigs, ~5% of pellets retained in the stomach 31 hours post dose. Furthermore, while transit of pellets in the small intestine and caecum were relatively rapid and complete, prolonged retention of pellets in the colon was also evident (85% pellets excretion within 50.5 hour). The authors therefore suggested that for certain types of pelletised dosage forms, extended retention in the porcine stomach and colon may need to be considered <sup>[46]</sup>.

The colonic transit time is highly variable in both pigs and humans. In humans, estimates can range from 20 hours to > 2 days <sup>[47: 48]</sup>. Overall, the total gastrointestinal transit time in pigs, measured by roentgenography, was reported to be between 2 and 33 days <sup>[6]</sup>. Even though this would appear longer than in humans, this may also reflect the slow and variable gastric emptying rate as discussed previously.

## Pig as a preclinical model of oral drug absorption

Given the significant variability observed with transit time as described previously, this may be due to different study protocols used as gastric emptying could depend on their normal feeding cycle, the fasting regime, the age and size of the pigs and amount and type of food. Therefore, a better standardisation of the study protocols would be favourable to obtain more comparable results of the different studies in order to improve the understanding of the gastric emptying in pigs. Further, the above results from various studies show that further studies with more subjects are needed to get a full understanding of the gastric emptying in pigs <sup>[21; 25; 6; 46]</sup>.

In conclusion, the gastric emptying rate in pigs appears to be slower than in humans, whereas the small intestine transit time in pigs is similar to the ones reported in humans, for solids and liquids <sup>[41]</sup>. Given that a drug is absorbed in the small intestine and that the area for absorption in pigs is similar to the area in humans, pigs seem to be a suited species for investigation of drug absorption, with the precaution that the plasma sampling should be planned to take the potential slower gastric emptying rate into account.

### 2.5 Intestinal metabolism and transporters.

Another key consideration that can affect oral bioavailability is the prevalence of intestinally mediated first pass metabolism and/or efflux which can collectively limit drug access to the systemic circulation <sup>[49]</sup>. In humans it is known that CYP3A enzymes are responsible for metabolising approximately 48 % of the drugs currently on the market and account for more than 70 % of CYP enzymes present in the GI tract <sup>[50; 51]</sup>. Therefore, any differences in expression levels of intestinal enzymes and efflux transporters between humans and pigs may have a profound effect on the predictability of drug absorption in humans.



## Pig as a preclinical model of oral drug absorption

While there is some data published in the literature regarding the expression profiles of important drug transporters (e.g. P-glycoprotein) and metabolising enzymes (e.g. CYP3A) in the GI tract of pre-clinical animals, the available data is limited. In the cases where these systems are involved in the metabolism or transport of certain drugs, a difference between humans and the animal models can be used to explain a lack of correlation between the species. For example, Cao *et al.* compared the transporter expression and metabolic enzyme expression in the duodenum between rat and human. The expression profile of 16 intestinal transporters displayed a moderate correlation ( $r^2 = 0.56$ ), whereas no correlation was found for the expression of metabolising enzymes between rat and human intestine [7]. This suggests a difference in intestinally metabolic capacity between the two different species and the challenges in predicting oral bioavailability in human. Relative to the more commonly utilised rat and dog models, studies reporting expression levels of intestinal transport/metabolising enzymes in pigs are sparse. With respect to the nutritional transporters Nøhr *et al.* [52] have demonstrated the presence of the proton coupled amino acid transporter (PAT1) in the small intestine and rectum in mini-pigs, a transporter also present in the human small intestine [53]. The level of PAT1 expression have, however, to the best of our knowledge not been quantified in mini-pigs. In general, the extend of homology between pigs and humans with respect to intestinal drug metabolising enzymes, efflux and influx transporters on both a qualitative and a quantitative level is unclear. Therefore, further mechanistic studies are required to identify inter-species commonalities and differences in transporter and/or metabolic profiles.

### 2.6 Hepatic metabolising enzymes

There are several families of CYP enzymes, each of which have subfamilies and unique isoforms. CYP isoforms are only found in one species, so a specific isoform of humans may have an orthologue in pigs, but an identical isoform is not found to date [54]. CYP3A in humans is the most important enzyme family metabolizing around 27% of drug compounds and

Pig as a preclinical model of oral drug absorption

comprising 30% of the total CYP System <sup>[55]</sup>. The enzyme seems relatively well conserved in pigs and shows a very high similarity with the CYP3A4 in humans <sup>[56]</sup>. In particular, it seems that there are no major differences among CYP1A1, 1A2, 2B, 2E1 and 3A in pigs when compared to humans <sup>[57]</sup>. Hence, the pig may be a suitable pre-clinical model for drugs that are mainly metabolized by these CYP enzymes. In contrast the pig model is less suited for studies involving drugs that are metabolized by CYP2C and CYP2D. In humans the CYP2C and CYP2D metabolize about 22% and 12% of the drugs, respectively <sup>[55]</sup>. However porcine CYP2C and CYP2D display a distinctly different substrate specificity. A human CYP2C substrate, S-mephenytoin, was not metabolized by the pig, using liver microsomes. Furthermore, diclofenac and tolbutamid, two CYP2C substrates in humans <sup>[58]</sup>, were metabolized to a lower extent in pigs compared to humans <sup>[57; 59]</sup>. The CYP2D activity generally seems higher in pigs than in humans <sup>[25; 29; 60; 61]</sup>. It is also worth considering that the various pig breeds may display different activity of hepatic enzymes. As recently reviewed, the Landrace pig demonstrated a presence and activity of CYP2D6 and CYP2C9 enzymes whereas Göttingen minipigs showed no activity of CYP2D6 and CYP2C9 enzymes <sup>[54]</sup>.

### 3 Oral bioavailability of drugs: comparing pigs versus humans

To assess how well pig and human bioavailability correlate, a systematic review of the published literature was performed to identify previous studies where absolute bioavailability of drugs in pigs have been reported. Studies and compounds were only included where both oral and intravenous data was available, hence the absolute bioavailability could be calculated. Data from various pig breeds were included. The determination of absolute bioavailability was based on the traditional comparison between the area under the curve (AUC) after intravenous and oral administration:

$$F_{abs} = \left( \frac{AUC_{oral}}{AUC_{i.v.}} \right) * \left( \frac{Dose_{i.v.}}{Dose_{oral}} \right)$$

## Pig as a preclinical model of oral drug absorption

In total, 20 compounds were identified to fulfil these criteria and the pharmacokinetic data for each compound in pigs and humans are summarized in Table 4. The degree of correlation of  $F_{abs}$  between the pig and human data are presented in Figure 4. A general positive trend towards predicting human bioavailability based on values in pigs was obtained, with a coefficient of determination  $r^2$  of 0.36.

Looking at the individual drug level, the bioavailability in pigs is comparable to that in humans for the majority of drugs. However, there are specific drugs where the oral bioavailability data in pigs differed substantially from human data, which lowered the correlation observed overall between humans and pigs. On closer examination of this latter set, metoprolol and diclofenac appeared to display substantial differences in pigs relative to humans. This, most likely reflecting the different metabolic pathways for these drugs between the two species. Absolute oral bioavailability of metoprolol in pigs was reported to be as low as 3 %, whereas the buccal administration lead to a bioavailability of 58-107% <sup>[29]</sup>. These findings were in accordance with data published by Mogi et al. <sup>[61]</sup>, who could not detect the plasma concentration of metoprolol after oral administration to pigs, as the plasma concentration were under the quantitation limit of the used bioanalysis. In humans the bioavailability of metoprolol is approximately 50%, a difference suggested to be driven by a difference in the metabolic pathway between the species. Metoprolol undergoes  $\alpha$ -hydroxylation and O-demethylation through interactions with CYP2D6. As previously described, CYP2D has a higher activity in pigs than in humans, hence this difference most likely reflects extensive first pass metabolism of metoprolol in pigs. Interestingly, metoprolol bioavailability improved substantially to 58-107% following buccal administration to pigs, which supported the observations that first pass metabolism is the major limiting factor of metoprolol bioavailability in pigs <sup>[29]</sup>. Mogi and co-workers reported in vitro data of the oxidation process of metoprolol by liver enzymes from pigs and humans. The O-

Pig as a preclinical model of oral drug absorption

demethylation process of metoprolol was 10-fold faster by the pig microsomal system when compared to human liver microsomal system. This phenomenon is also reported for other compounds that are metabolised by CYP2D6, like dextromethorphan which shows a rapid and extensive metabolism in the pig and a O-demethylation process that is a 10-fold greater in pig liver microsomes compared to human liver microsomes <sup>[60]</sup>. It is important to mention that CYP2D6 metabolizes about 12% of all compounds, including  $\beta$ -blocker, antipsychotic as well antidepressant drugs <sup>[54]</sup>. Differences in the metabolic rate may also go in the other direction, i.e. humans metabolising faster than pigs. Diclofenac is a CYP2C substrate. Oberle and co-workers <sup>[25]</sup> observed a higher bioavailability in pigs compared to human data., which was concluded to be a reflection of a different metabolism profile between the two species. Collectively, these examples underline the importance of looking at the hepatic metabolism when selecting the non-clinical species, but also provide an explanation why quantitative predictions and degree of correlations between pigs and humans is low ( $r^2= 0.36$ ). In general a head to head comparison of bioavailability across species may be difficult.

Similar correlations comparing human bioavailability to other pre-clinical animal species have been described in the literature. Musther and co-workers <sup>[63]</sup> investigated the correlation of bioavailability data from dog, monkey, mouse, and rat with human bioavailability data. The correlation was conducted based upon a published data-set from Sietma et al. <sup>[64]</sup>, where data from these animal models were reported. The data visualized the breadth of difference that exist between animal species and humans in terms of oral bioavailability. Musther et al. <sup>[63]</sup> highlighted the gaps in the correlation between the bioavailability in animals and humans and concluded that a quantitative prediction was not possible when comparing the bioavailability head to head. The extent of correlation was for monkey ( $r^2= 0.69$ , 40 drugs), rat ( $r^2= 0.29$ , 121 drugs;  $r^2= 0.21$ , 48 drugs), mouse ( $r^2$  of 0.25, 30 drugs) and dog ( $r^2 = 0.38$ , 128 drugs;  $r^2 = 0.15$ , 49 drugs) <sup>[7; 9; 63; 65]</sup>. Based on the findings in the current study, the porcine model displayed a

## Pig as a preclinical model of oral drug absorption

higher overall correlation than both rodent models. The porcine model also compares favourably to the canine model, in terms of a similar coefficient previously reported for dogs. It should be noted that the number of drugs available with absolute bioavailability in pigs was lower (20) relative to the other species, reflecting key knowledge gaps in the literature and a need for further investigations to demonstrate more conclusive relationship. Moreover, sorting the data to exclude justifiable outliers, such as the species specific CYP2D metabolic difference could facilitate more informed conclusion. For example, excluding the values of Diclofenac and Metoprolol, which are two substances not suitable in the pig model, from the correlation illustrated in Figure 4, improved the correlation from  $r^2 = 0.36$  to  $r^2 = 0.52$ .

Additionally, to provide an insight on how the rate of drug absorption compares between pigs and humans,  $t_{max}$  values were compared. Previously,  $t_{max}$  has been used to evaluate gastric emptying, as a delayed emptying leads to a longer  $t_{max}$  <sup>[43]</sup>.

Within the group of drugs presented in Table 4,  $t_{max}$  values were reported for only twelve of these drugs.  $T_{max}$  values in pigs ranged from 0.6-5.0 (median 2.0) whereas in humans the range of  $t_{max}$  values was 0.25-2.5 (median 1.9). The larger range of  $t_{max}$  values in pigs compared to humans most likely reflects a more variable gastric emptying in pigs, as discussed previously. Figure 5 illustrates the  $t_{max}$  ratio between pigs and humans for each drug. As a general guide,  $t_{max}$  in pigs was on average 2-fold higher than the corresponding value in humans. While there are individual drugs which do not follow this general guide, such as Ketoprofen where  $t_{max}$  was 5-fold higher in pigs, and omeprazole was 4 fold higher in humans <sup>[73]</sup>, these individual cases most likely reflects species specific differences in drug metabolism leading to extremes  $t_{max}$  estimates. It should also be noted that while  $t_{max}$  is useful in the evaluation of gastric emptying, there are limitations to its reliability as an estimate of rate of drug absorption. Differences in fasting/fed state conditions in the study design could affect gastric emptying and therefore need to be tightly controlled to ensure that appropriate

## Pig as a preclinical model of oral drug absorption

comparisons can be made. In addition, the value of  $t_{max}$  is highly influenced by the frequency of sampling within the study, and ideally most frequent sampling should be conducted near the expected peak plasma concentrations. The findings presented here would indicate that when designing plasma sampling studies involving pigs, frequent plasma sampling may be required for time periods up to 2 times the expected  $t_{max}$  in humans.

### **4 Pig versus dog in predicting human bioavailability: How do they compare?**

While the extents of correlation obtained for pigs vs. humans are comparable to values reported for dogs vs. humans, it should be acknowledged that there is a substantially more diverse drug data set available in the literature for dogs. In order to more reliably compare between species, the relationships of human bioavailability to both dogs and pigs were evaluated using only drugs which were common to both. In total a group of 15 drugs (from the 20 drugs of Table 4) were identified from the literature where absolute bioavailability was available in dogs, pigs and humans. Figure 6 presents the relationships obtained for these common set of drugs between pigs versus humans and dogs versus humans. For the set of 15 drugs identified, the coefficients of determinations obtained for pigs was  $r^2 = 0.49$ , whereas for dogs  $r^2 = 0.35$ . Within both relationships, specific drugs could be identified where the oral bioavailability data in the animal correlated poorly to human data.

In the case of absorption rate, it has previously been discussed that on average  $t_{max}$  was longer in pigs than humans, most likely reflecting a slower gastric emptying in pigs. In the case of dogs, gastric emptying is considered to be more comparable to humans, however, few studies directly compare rates of absorption between the species. Within the group of 15 drugs where

## Pig as a preclinical model of oral drug absorption

both canine, porcine and human data is available, there were only three drugs where a direct comparison of  $t_{max}$  was possible.  $T_{max}$  of moxifloxacin was  $2.0 \pm 1.9$  hours,  $4.0 \pm 1.0$  hours and  $1.6 \pm 2.9$  hours in humans, pigs and dogs, respectively, confirming that while dogs and humans are similar, absorption in pigs appear to be marginally slower [66]. A similar trend is evident for ketoprofen with  $t_{max}$  values of 0.8 hours,  $3.9 \pm 1.1$  hours and  $1.1 \pm 0.3$  hours in humans, pigs and dogs, respectively [72,73]. In the case of diclofenac differences are also prominent, a  $t_{max}$  of 0.3 hours, 0.8 hours and  $1.2 \pm 0.6$  hours was reported for humans, pigs and dogs, respectively [25,62].

In summary, while the number of drugs in the data-sets is limited, the findings demonstrate that pigs compare more favourably than dogs at predicting the extent of absolute bioavailability (Fabs) in humans, based on this limited set of comparable studies. The presented data therefore provide further evidence of the suitability of the pig in pre-clinical assessment of oral dosage forms. However, the rate of drug absorption in pigs is tending to be slower in pigs than humans, whereas in dogs,  $t_{max}$  is more comparable to human estimates. In the case of drugs and/or formulations where a delayed rate of absorption might have a significant impact on in vivo performance (e.g. sustained release products or fast acting dosage forms), the canine model may offer advantages, notwithstanding the clear limitation where canine gastric pH is considerable higher than humans and pigs.

## **5 The use of the pig model for assessing in vivo performance of oral dosage forms**

In drug formulation development it is crucial to assess the bioavailability of orally delivered drugs and in particular how this can be influenced by the use of different formulations in order

## Pig as a preclinical model of oral drug absorption

to support the formulation development work. Conductance of pre-clinical *in vivo* studies as well as *in vitro* and *in silico* approaches are often used in combination in order to understand the mechanisms that drive the absorption from the formulations tested before they are brought into human trials. Optimizing the steps from the *in vitro* work in the lab, linked to *in vivo* data from the animal studies can therefore help to expedite the process of development.

The most common pre-clinical models to assess oral dosage forms, include, rats, dogs, pigs and less frequently non-human primates. Knowing which pre-clinical model to use in order to being able to compare different formulations as well as to predict oral bioavailability in humans is an important decision to generate data where conclusions can be drawn and the right formulation selected and taken into clinical trials in humans. However, there remains a lack of clarity in species selection for a specific formulation technology. This lack of clarity is particularly problematic for drug substances displaying low solubility and/or permeability (i.e. BCS Class II and IV), which generally requires bio-enabling formulations to improve oral bioavailability. While there are numerous oral drug delivery platform technologies to enhance oral bioavailability, it becomes increasingly clear that each formulation technology cannot be universally applied to all poorly water-soluble drugs and the selection of the correct formulation technology is often complicated (Figure 7.). As a result, a key question that needs to be addressed in selecting the most suitable pre-clinical model is: Which animal model will reliably predict the *in vivo* performance of a selected formulation technology, in particular bio-enabling formulation technology?

Table 5, summarizes data from different formulation technologies with *in vivo* data from pigs, which has been reported in the literature. The objective is to summarise where the pig model has been utilised to demonstrate *in vivo* performance of specific formulation technology, and to act as a guide on which formulation technologies can be assessed using the pig model. Most



## Pig as a preclinical model of oral drug absorption

of the cases identified in Table 5 involve studies using formulation technologies that enhance oral bioavailability of poorly soluble drugs belonging to the BCS class II. Given the increasing number of new drugs in development with solubility limitations it is only a natural reflection of current focus in pharmaceutical research. The formulation technologies that have been assessed using pigs include micronized and nanosized formulations, as well as hot melt extrusion, lipid based and mesoporous silica formulation.

The use of lipid based formulations as an approach to enhance oral bioavailability has increased in recent times. The drug absorption of lipid and surfactant based formulations can be affected by a variety of factors including characteristics on dilution in the intestinal fluids, solubilisation capacity of the drug during transit through the intestine, as well as the presence of food [91; 92]. It has been demonstrated that the lipid based formulations can not only enhance the solubility of the drug in the intestinal fluids, but also enhance the lymphatic uptake of the drug, which circumvents the first pass metabolism of the drug. This is predominantly present when the drug is given with long chain triglycerides and the drug has a  $\log P > 5$  and a solubility in triglycerides  $> 50 \text{ mg/mL}$  [93; 94], but exceptions also exist from this rule of thumb [95]. There are a variety of factors that must be considered in designing optimal lipid based formulations, for example the composition (commonly described by the LFCS classification system), the proportion and type of lipid surfactants and/or co-solvents. Upon digestion and the introduction of bile salts, the mixture of drug, lipids, surfactants and cosolvents is undergoing continual changes from an emulsion to lamellar and hexagonal phases and finally generating a pre-absorptive mixed micellar state. Hence, in order to predict the behaviour of the drug in the GI tract it is crucial to have an advanced understanding of how the drug is solubilized and in which phases of the colloidal structure in the GI tract it can be found. It is a complex relation between lipid formulation and their pharmacokinetic properties. Several studies using lipid-based formulations have utilised a pig model to assess *in vivo* performance, see Table 5. Griffin and

## Pig as a preclinical model of oral drug absorption

co-workers showed for the lipophilic drug fenofibrate, that the pig model was suitable to assess the *in-vivo* performance of lipid-based formulations, showing predictably high oral absolute bioavailability in pigs (65-72%)<sup>[96]</sup>. T max values in pigs were 2.4-4.6 hours, which were similar to estimates in humans<sup>[97]</sup>. The *in vivo* results from the pig provided a better insight into the performance of different lipid based formulation classes, and enhanced the understanding on *in vitro* behaviour as well as the predictability of *in vitro* results. In addition, an *in silico* biopharmaceutical model was developed from this data to predict drug and lipid absorption during digestion of lipid based formulations, therein supporting the utility of pigs in mechanistic studies involving lipid based formulations<sup>[98]</sup>. In the case of fenofibrate, the pig model was also employed to compare novel mesoporous formulation to the commercially available nanosized Lipantil ® Supra. Interestingly the absolute bioavailability for nanosized Lipantil ® Supra observed in pigs was 71%, which was similar to the reported human data of 69%<sup>[69; 70]</sup>. Also the range of tmax in pigs matched the human data<sup>[70,71]</sup>.

It should also be noted that a recent study by Thomas *et al.* exploring oral bioavailability of fenofibrate from a series of lipid based formulations in minipigs indicated that fenofibrate absorption in minipigs was considerably delayed<sup>[100]</sup>. T max of fenofibrate was 8 hours (0.5-30 hours) from lipid based formulations<sup>[100]</sup>. Furthermore, t max of the micronized commercially available Lipanthyl 200 M capsules was 24 hours (8-72 hours)<sup>[100]</sup>. This may reflect differences in the study design (e.g. fed/fasting conditions, which affect gastric emptying), but also differences related to the breed of pigs used in the two studies, i.e. landrace and mini pigs, where the latter may display different pharmacokinetic properties for fenofibrate relative to humans and landrace pigs<sup>[101]</sup>.

The pig model has also been widely used to assess *in vivo* performance of a range of modified release formulations, see Table 5. For controlled release formulations, the mechanism of drug

## Pig as a preclinical model of oral drug absorption

release is dependent on the rate of degradation of the polymer, which controls the extent of drug diffusion through the polymer matrix. They are developed with the aim to reduce the frequency of dosing with a short terminal elimination half-life and to minimize the degree of fluctuation in the drug's plasma concentration over the dosing intervals. It is likely that some of the difference in the GI physiology can influence the release and bioavailability of controlled-release dosage forms. From a physiological perspective, pigs seem to be a suited surrogate for humans when evaluating controlled release formulations as each intestinal section, i.e. duodenum, jejunum, ileum and colon, is comparable. Furthermore, the bacterial flora of the colon is considered similar to man<sup>[17; 23]</sup>. For sustained release formulations a few studies have been reported in the literature where the pig was used as an animal model. For formulations with glipizide, the pig was reported to be a suitable model to predict modified release formulation performance. Kulkarni and co-workers<sup>[102]</sup> reported that the porcine absorption kinetics were consistent with the published clinical data, conversely the beagle dog was less consistent compared to humans. Kostewicz *et al.* investigated nifedipine sustained formulations in pigs, as described in Table 5, the obtained data was similar to data from a previously performed human study. Kostewicz and co-workers came to the conclusion that the obtained results suggested that the pig can be a useful model in differentiating the release profiles of nifedipine for the fed state in humans<sup>[104]</sup>. Another type of sustained release that has been tested in pigs are entero coated formulations. An important factor for these kind of systems is the pH in the stomach and the pH change upon entry into the small intestine. Pigs have a slower gastric emptying rate than humans, as discussed above, however a very similar pH in the stomach and the small intestine. The study conducted with entero coated formulations in pigs therefore resembled the release profile observed in humans well and pigs should in general be a suited model for evaluation of these systems.

Pig as a preclinical model of oral drug absorption

Though very limited data are available comparing different formulation systems in pigs, they all indicate that pigs can be useful when evaluating both sustained release formulations as well as bio enabling formulations. This clearly demonstrates the relevance of the pig as a model to predict pharmacokinetics in human and the opportunity to use the pig for various formulation optimisation purposes.

## **6 Role of *in vitro* and *in silico* models for predicting the suitability of pig models and the gaps in the current knowledge**

A key driver of the drug and formulation development processes is the ability to predict human pharmacokinetics (PK) as early as possible, in order to select candidates with the best developability, while rejecting those with a high probability of failure <sup>[108]</sup>. The intention of conducting *in vivo* pre-clinical studies in drug development is therefore to mitigate risk of developing medicines that are unsafe and/or ineffective in subsequent tests in humans, but equally important is to reduce the number of animals used for research purposes by appropriate application of *in vivo* studies. This is both an ethical and legislative obligation and the choice and number of animals has to be appropriately justified and must be in accordance with the 3 R's principle of replacement, reduction and refinement. In addition to the 'reduction' principle, 'replacement' options should always be considered, and this is where *in vitro* or *in silico* models can play a key role. Improvements in the correlation from *in vitro* data will help to reduce the number of animals needed. While the goal is to reduce the number of animals used, this reduction should not compromise the statistical outcome of the study, i.e. an underpowered study, as it makes the data unsuitable for decision making and therefore in principle unethical. Hence, the study should always include sufficient experimental units to obtain a precise and robust result that can lead to a correlation or prediction of the drug performance in humans.

## Pig as a preclinical model of oral drug absorption

Prediction of human pharmacokinetics generally relies on interpretation and extrapolation from *in vitro* and preclinical *in vivo* data <sup>[41]</sup>. There has been significant development in computational approaches to predict human pharmacokinetics, ranging from classical compartmental PK through to more complex physiologically based pharmacokinetic (PBPK) models, which can integrate data from both these sources to give an estimate of human PK <sup>[109]</sup>. A schematic presentation of the process is illustrated in Figure 8. The application of *in silico* modelling, with a specific focus on the use in pigs is discussed below.

### 6.1 Reliable *in vitro* methods - biorelevant media

The first step to characterize the dissolution and solubility behaviour of a drug, are reliable and appropriate *in vitro* models <sup>[110]</sup>. The selection of the media is a crucial step, biorelevant media simulating the human's gastrointestinal conditions is a useful tool to get a first impression of the dissolution properties of a drug <sup>[111]</sup>. More important, with regard to the aim to reduce the number of animals, is to mimic the pre-clinical animal conditions. For common pre-clinical animal models, like dogs and rats, biorelevant dissolution media has been developed to simulate the gastrointestinal tract fluids of these species <sup>[112]</sup>. Properties such as pH, buffer capacity, osmolality, surface tension and hydrodynamic conditions were included to improve forecast and interpretation of pre-clinical results. For the pig the data is limited, there is a clear lack about the composition of porcine gastric and intestinal fluids, hence no biorelevant media is available for pigs why further work is needed to characterize intestinal porcine conditions. All in all, media specially designed for pre-clinical animal models will be essential to guide the selection of animal species suited for *in vivo* investigations of a drug candidate.

Pig as a preclinical model of oral drug absorption

## 6.2 Computational models and *in vitro*- *in vivo* correlations (IVIVC)

The simplest methods of predicting *in vivo* results is by means of an *in vitro*- *in vivo* correlation (IVIVC). Kesisoglou *et al.* demonstrated the use of a simple level C IVIVC in a retrospective analysis of the *in vivo* performance of extended release matrix and multi-particulate preparations of a BCS class III development candidate (MK-0941) with different targeted release rates (8hr, 12hr and 16 hr) in Yucatan minipigs. A good correlation between the *in vitro* release and bioavailability was reported [105]. Similarly, Keohane *et al.* demonstrated an *in vitro*-*in vivo* relationship (IVIVR) for coated microspheres containing Ciclosporin A. The resultant IVIVR demonstrated a strong linear correlation between *in vitro* release and *in vivo* absorption [82]. McCarthy *et al.*, meanwhile, demonstrated the possibility of obtaining a level A IVIVC using such an approach, by optimising the biorelevance of the dissolution test for fenofibrate, as a model for poorly soluble compound. McCarthy and co-workers demonstrated that computational *in silico* methods could be used to deconvolute the oral absorption process from the pharmacokinetic profile, correlate this with *in vitro* release and model *in vivo* pharmacokinetics by re-convolution [70]. Govender *et al.* have recently proposed a similar approach to describe the absorption of amoxicillin from a delayed release, dual-biotic system [113].

Computational models based on the release and distribution kinetics of drug from formulations, modelled along with compartmental PK have also been implemented. Stillhart *et al.* developed a pharmacokinetic model in MatLab, based on simulating the intraluminal concentration and solubility of fenofibrate when delivered in three distinct lipid based formulations to fasted, juvenile Landrace pigs [98]. Using this biopharmaceutical model it was possible to accurately model plasma profiles, while also allowing a prediction of the intraluminal solubilisation, supersaturation and precipitation of the administered formulation. Using a model that considered the absorption of both the drug and lipid excipients after oral administration it was

Pig as a preclinical model of oral drug absorption

possible to mechanistically investigate the *in vivo* performance in a manner not possible using simple *in vitro* digestion tests, which do not consider the impact of continuous absorption [98].

### 6.3 Physiologically based pharmacokinetic models

The distinguishing feature of PBPK models, relative to empirical computational models, is the application of prior physiological knowledge in the mechanistic mapping of model compartments and in the processes that determine absorption [104; 41]. This physiological knowledge, incorporating parameters such as gastrointestinal transit, pH and luminal volume, is combined with physiochemical measurements, e.g. dissociation constants and partition coefficients, and *in vitro* measurements, such as solubility and dissolution rates and enzymatic degradation kinetics, into the PBPK model in order to provide a simulated PK profile [109; 41; 114]. PBPK models allow predictions of drug disposition based on a series of mass balance equations, which incorporate physiological, physiochemical and *in vitro* data within an *in silico* model [115]. Numerous commercial PBPK software systems are available, most notably Simcyp, GastroPlus™ and PK-Sim®, while there is also widespread use of user-built models, built using packages such as MATLAB®, Berkeley Madonna, MoBi®, STELLA® or acsIX™ [114].

PBPK has a long history of use in veterinary pharmacology and toxicology. These studies have a particular focus on analysing xenobiotic disposition for the purposes of estimation of meat withdrawal periods based on residue depletion in edible tissue after either accidental exposure to a toxin or after off-label use of a veterinary preparation. Numerous reports of PBPK models being used to extrapolate disposition studies between species [115-119], estimate withdrawal

## Pig as a preclinical model of oral drug absorption

periods based on probabilistic methods <sup>[120; 121]</sup> and assessment of the potential for drug-drug interactions due to altered protein binding <sup>[122; 123]</sup> have been published.

The growing use of the pig as a preclinical species of choice has led to significant developments in porcine PBPK models. Jones *et al.* developed a generic PBPK model for the prediction of clinical pharmacokinetics using preclinical data from various sources, including pigs, which proved superior to allometric scaling <sup>[108]</sup>. While significant differences remained in some cases, particularly when certain assumptions of the PBPK model were violated, the use of PBPK modelling provided insight into potential reasons for poor predictability, which allometric methods did not <sup>[110]</sup>.

The most significant development in PBPK modelling in pigs was the minipig PBPK model developed using the advanced compartmental absorption (ACAT) model. By using a series of mass-balance equations that describe the specific physiology of the minipig, a porcine PBPK model was generated to simulate oral PK. The proposed model was initially validated with griseofulvin and moxifloxacin, with encouraging results <sup>[41]</sup>. However, the authors also identified areas where this model can further be refined, particularly in the areas of absorption related parameters and bile salt profiles within the minipig intestine. Further physiological characterisation along with pharmacokinetic analysis of well-chosen reference compounds, and adjustment of the model to reflect *in vivo* PK, was suggested to contribute to model refinement <sup>[41]</sup>. Subsequent work using paracetamol as a marker of GI motility and gastric emptying, was used to update this model. The updated model was subsequently validated on a number of PK studies in minipigs using omeprazole, caffeine, midazolam and warfarin. The prolonged gastric emptying in the re-parameterised PBPK model accurately predicted pharmacokinetics of this validation dataset in minipigs <sup>[44]</sup>. Studies such as those by Lignet *et al.* can further add to this



Pig as a preclinical model of oral drug absorption

knowledge space, aiding the development and validation of this PBPK model by characterising the bioavailability of a set of reference compounds in the minipig model <sup>[86]</sup>.

O'Shea *et al.* used the GastroPlus™ minipig ACAT PBPK model to simulate bioavailability of fenofibrate from a commercial micronized formulation and novel lipid based formulation in fasted Landrace pigs <sup>[99]</sup>. Using this model, the authors successfully simulated fasted bioavailability for both formulations by incorporating the intravenous pharmacokinetic data, along with biorelevant *in vitro* solubility and dissolution measures into the mechanistic model. The model was subsequently used to extrapolate this data to the fed state, where the elimination of a food dependent increase in fenofibrate bioavailability utilising the novel formulation was predicted <sup>[99]</sup>. Kesisoglou *et al.* have also used the GastroPlus™ minipig model, in conjunction with modelling of dog and human data, in the formulation development of a modified release preparation of gaboxadol <sup>[124]</sup>. The authors successfully incorporated *in vitro* dissolution data and preclinical pharmacokinetic data within the PBPK models to guide formulation development. Subsequently, it was possible to use the minipig PBPK model to develop an IVIVC in order to project formulation performance <sup>[124]</sup>. Using regional permeability data measured in dogs and clinical pharmacokinetics from human studies, the minipig ACAT model was optimised using an immediate release dry filled capsule as a reference. Using this optimised model, the *in vivo* dissolution was deconvoluted from the simulated plasma profile for two modified release formulations. This *in vivo* dissolution profile was subsequently plotted against the *in vitro* release profile resulting in a linear IVIVC <sup>[124]</sup>.

Lennernäs and co-workers have also developed porcine PBPK models, with a particular focus on simulating data from Landrace and Yorkshire pigs, which have multiple blood sampling sites. By measuring plasma concentrations in the hepatic, portal and femoral veins, along with biliary and urinary concentration, a detailed analysis of the ADME process was obtained <sup>[125]</sup>.

## Pig as a preclinical model of oral drug absorption

Sjögren *et al.* developed an 11 compartment PBPK pig model, to model drug concentrations in these sampling sites and estimate tissue distribution throughout a whole body PBPK model. This mechanistic PBPK model, in combination with appropriate *in vitro* assessment of enzyme kinetics and cellular disposition, allowed successful prediction of the influence of metabolism and carrier mediated processes on the hepatic disposition of repaglinide <sup>[125]</sup>. Similar models were used by Lennernäs and co-workers to investigate a potential drug-drug interaction between verapamil and fexofenadine <sup>[126]</sup> and to mechanistically investigate the effect of intracellular binding of doxorubicin on its PK in pigs <sup>[127]</sup>.

The use of PBPK modelling during drug and formulation development is an emerging field for the prediction of preclinical and clinical PK using physiochemical and *in vitro* measurements. However, thus far the use of PBPK modelling has been largely confined to the retrospective, mechanistic analysis of preclinical data. While some work has focused on the extrapolation of these models to alternative formulations or dosing scenarios (e.g. in fed versus fasted state), and extrapolating between different preclinical species, there is still a lack of prospective models used in formulation design. There remains a need for systematic studies utilising PBPK models as part of a ‘learn and confirm’ paradigm before the full benefit of this approach is realised.

## 7 Conclusion

This review has investigated the potential advantage and suitability of the pig as a pre-clinical model within pharmaceutical sciences. From an anatomical and physiological perspective, the pig model is widely recognized and displays high similarities to the GI conditions of humans. There remain striking gaps in our knowledge about the pig model and its utility to predict human bioavailability of oral dosage forms, as discussed above. Analysis of the available

## Pig as a preclinical model of oral drug absorption

literature for absolute bioavailability studies conducted in pigs, dogs and humans demonstrated that the pigs compared favourably to dogs in terms of predicting human bioavailability.

As a result of the limited data available in the literature, it is currently not possible to make quantitatively predictions of human bioavailability.

While this review has provided specific examples of limitation of pigs, such as drugs metabolised extensively by the CYP2D system, there is overall a need to harness knowledge from a wider range of drug molecules (both successes and failures) to develop better guides as to which drug formulation that are best suited for testing in pigs. There is also an opportunity to improve the link between *in vitro* screening and *in vivo* testing in preclinical animals. This could be the development of species specific biorelevant screening tool, which is currently not available for pigs.

Clearly, there is no “one size fits all” choice in the context of preclinical animal modelling, hence there is no “ideal” species that represent all aspects of human GI conditions <sup>[9]</sup>. With the data presented in the present work it is reasonable to state that the pig model is similar to most physiological conditions in man and hence a model suitable for considering when conducting *in vivo* studies.

## 8 Acknowledgments

The authors are part of the PEARRL European Training network, which has received funding from the Horizon 2020 Marie Skłodowska-Curie Innovative Training Networks programme under grant agreement No. 674909.

## References:

1. DICKSON M, GAGNON JP. Key factors in the rising cost of new drug discovery and development. *Nat Rev Drug Discov* 2004; 5: 417-29.
2. HUGHES JP et al. Principles of early drug discovery. *Br J Pharmacol* 2011; 6: 1239-49.
3. SELEN A et al. The biopharmaceutics risk assessment roadmap for optimizing clinical drug product performance. *J Pharm Sci* 2014; 11: 3377-97.
4. KARARLI TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory- animals. *Biopharmaceutics & Drug Disposition* 1995; 5: 351-380.
5. GROVE M et al. Bioavailability of seocalcitol I: Relating solubility in biorelevant media with oral bioavailability in rats - Effect of medium and long chain triglycerides. *J Pharm Sci* 2005; 8: 1830-1838.
6. DAVIS SS et al. Gastrointestinal transit of dosage forms in the pig. *J Pharm Pharmacol* 2001; 1: 33-9.
7. CAO X et al. Why is it Challenging to Predict Intestinal Drug Absorption and Oral Bioavailability in Human Using Rat Model. *Pharm Res* 2006; 8: 1675-1686.
8. SJOGREN E et al. In vivo methods for drug absorption - Comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. *European Journal of Pharmaceutical Sciences* 2014: 99-151.
9. HATTON GB et al. Animal Farm: Considerations in Animal Gastrointestinal Physiology and Relevance to Drug Delivery in Humans. *J Pharm Sci* 2015; 9: 2747-2776.

## Pig as a preclinical model of oral drug absorption

10. GARDNER N et al. Development and validation of a pig model for colon-specific drug delivery. *J Pharm Pharmacol* 1996; 7: 689-93.
11. BODE G et al. The utility of the minipig as an animal model in regulatory toxicology. *Journal of Pharmacological and Toxicological Methods* 2010; 3: 196-220.
12. COLLETON C et al. The Use of Minipigs for Preclinical Safety Assessment by the Pharmaceutical Industry: Results of an IQ DruSafe Minipig Survey. *Toxicol Pathol* 2016; 3: 458-466.
13. SWINDLE MM, SMITH AC. Comparative anatomy and physiology of the pig. *Scand J Lab Anim Sci* 1998: 11-21.
14. SWINDLE MM. *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques, 3rd Edition*, Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques, 3rd Edition, Boca Raton: Crc Press-Taylor & Francis Group, 2016.
15. PATTERSON JK et al. The pig as an experimental model for elucidating the mechanisms governing dietary influence on mineral absorption. *Exp Biol Med* 2008; 6: 651-664.
16. VAN DER LAAN JW et al. Regulatory acceptability of the minipig in the development of pharmaceuticals, chemicals and other products. *J Pharmacol Toxicol Methods* 2010; 3: 184-95.
17. ROWAN AM et al. Comparison of the ileal and fecal digestibility of dietary amino-acids in adult humans and evaluation of the pig as model animal for digestion studies in man. *Br J Nutr* 1994; 1: 29-42.
18. SIMIANER H, KOHN F. Genetic management of the Gottingen Minipig population. *Journal of Pharmacological and Toxicological Methods* 2010; 3: 221-226.

## Pig as a preclinical model of oral drug absorption

19. FORSTER R et al. The RETHINK project Minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment. *Journal of Pharmacological and Toxicological Methods* 2010; 3: 158-159.
20. KATAOKA M et al. Effects of gastric pH on oral drug absorption: In vitro assessment using a dissolution/permeation system reflecting the gastric dissolution process. *European Journal of Pharmaceutics and Biopharmaceutics* 2016: 103-111.
21. HOSSAIN M et al. Gastrointestinal transit of nondisintegrating, nonerodible oral dosage forms in. *Pharm Res* 1990; 11: 1163-1166.
22. DRESSMAN JB et al. Upper gastrointestinal (GI) pH in young, healthy- Men and women. *Pharm Res* 1990; 7: 756-761.
23. DRESSMAN JB, YAMADA, K.,1991. Animal model models for oral drug absorption. In *Welling, PG, Tse, FLS, Dighe, SV (eds) Pharmaceutical bioequivalence, vol 48 Dekker, New York, pp 729-739.*
24. BRENER W et al. Regulation of the gastric emptying of glucose. *Gastroenterology* 1983; 1: 76-82.
25. OBERLE RL, DAS H. Variability in gastric pH and delayed gastric emptying in Yucatan miniature pigs. *Pharm Res* 1994; 4: 592-4.
26. 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. *European Journal of Pharmaceutical Sciences* 2011; 1-2: 3-10.
27. DESESSO JM, WILLIAMS AL. Contrasting the Gastrointestinal Tracts of Mammals: Factors that Influence Absorption. In: Macor, J. E. ed. *Annual Reports in Medicinal Chemistry, Vol 43.* 2008: 353-371.
28. BURRIN D et al. DIGESTIVE PHYSIOLOGY OF THE PIG SYMPOSIUM: Intestinal bile acid sensing is linked to key endocrine and metabolic signaling pathways. *J Anim Sci* 2013; 5: 1991-2000.

Pig as a preclinical model of oral drug absorption

29. HOLM R et al. In vitro, ex vivo and in vivo examination of buccal absorption of metoprolol with varying pH in TR146 cell culture, porcine buccal mucosa and Gottingen minipigs. *Eur J Pharm Sci* 2013; 2: 117-24.
30. DESESSO JM, JACOBSON CF. Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. *Food Chem Toxicol* 2001; 3: 209-228.
31. HOLM R et al. Bile salts and their importance for drug absorption. *International Journal of Pharmaceutics* 2013; 1: 44-55.
32. LUNDELL K, WIKVALL K. Species-specific and age-dependent bile acid composition: aspects on CYP8B and CYP4A subfamilies in bile acid biosynthesis. *Curr Drug Metab* 2008; 4: 323-31.
33. ALME B et al. Analysis of Metabolic Profiles of Bile-Acids in Urine Using a Lipophilic Anion-Exchanger and Computerized Gas-Liquid Chromatography Mass Spectrometry. *Journal of Lipid Research* 1977; 3: 339-362.
34. WIETHOLTZ H et al. Stimulation of bile acid 6 alpha-hydroxylation by rifampin. *J Hepatol* 1996; 6: 713-8.
35. ARAYA Z, WIKVALL K. 6alpha-hydroxylation of taurochenodeoxycholic acid and lithocholic acid by CYP3A4 in human liver microsomes. *Biochim Biophys Acta* 1999; 1: 47-54.
36. MOSCHETTA A et al. A phylogenetic survey of biliary lipids in vertebrates. *J Lipid Res* 2005; 10: 2221-32.
37. PERSSON E et al. Simultaneous assessment of lipid classes and bile acids in human intestinal fluid by solid-phase extraction and HPLC methods. *Journal of Lipid Research* 2007; 1: 242-251.
38. ALVARO D et al. Relationships between Bile-Salts Hydrophilicity and Phospholipid-Composition in Bile of Various Animal Species. *Comp Biochem Phys B* 1986; 3: 551-554.

## Pig as a preclinical model of oral drug absorption

39. SCANFF P et al. Whole-body gamma irradiation modifies bile composition in the pig. *Radiat Res* 1997; 2: 175-80.
40. SCANFF P et al. Modified bile acid profiles in mixed neutron and gamma-irradiated pigs. *Int J Radiat Biol* 1999; 2: 209-216.
41. SUENDERHAUF C, PARROTT N. A Physiologically Based Pharmacokinetic Model of the Minipig: Data Compilation and Model Implementation. *Pharm Res* 2013; 1: 1-15.
42. ADKIN DA et al. The effects of pharmaceutical excipients on small intestinal transit. *Br J Clin Pharmacol* 1995; 4: 381-7.
43. CHRISTIANSEN ML et al. Evaluation of the Use of Gottingen Minipigs to Predict Food Effects on the Oral Absorption of Drugs in Humans. *J Pharm Sci* 2015; 1: 135-143.
44. SUENDERHAUF C et al. Pharmacokinetics of paracetamol in Gottingen minipigs: in vivo studies and modeling to elucidate physiological determinants of absorption. *Pharm Res* 2014; 10: 2696-707.
45. HENZE L.J et al. Exploring gastric emptying rate in mini-pigs: effect of food type and pre-dosing of metoclopramide. *Eur J Pharm Sci* 2018.
46. SNOECK V et al. Gastrointestinal transit time of nondisintegrating radio-opaque pellets in suckling and recently weaned piglets. *J Control Release* 2004; 1: 143-153.
47. IOANNIDES C, J. G. Hardy, S. S. Davis and G. Wilson (eds) Drug delivery to the gastrointestinal tract. *Journal of Applied Toxicology* 1990; 5: 388-388.
48. DEGEN LP, PHILLIPS SF. Variability of gastrointestinal transit in healthy women and men. *Gut* 1996; 2: 299-305.
49. AGORAM B et al. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. *Adv Drug Deliv Rev* 2001: S41-67.
50. WIENKERS LC, HEATH TG. Predicting in vivo drug interactions from in vitro drug discovery data. *Nat Rev Drug Discov* 2005; 10: 825-33.



## Pig as a preclinical model of oral drug absorption

51. DARWICH AS et al. Interplay of Metabolism and Transport in Determining Oral Drug Absorption and Gut Wall Metabolism: A Simulation Assessment Using the "Advanced Dissolution, Absorption, Metabolism (ADAM)" Model. *Current Drug Metabolism* 2010; 9: 716-729.
52. NOHR MK et al. Intestinal absorption of the antiepileptic drug substance vigabatrin in Gottingen mini-pigs is unaffected by co-administration of amino acids. *Int J Pharm* 2014; 1-2: 18-20.
53. CHEN Z et al. Structure, function and immunolocalization of a proton-coupled amino acid transporter (hPAT1) in the human intestinal cell line Caco-2. *J Physiol-London* 2003; 2: 349-361.
54. HELKE KL, SWINDLE MM. Animal models of toxicology testing: the role of pigs. *Expert Opin Drug Metab Toxicol* 2013; 2: 127-39.
55. RENDIC S. Summary of information on human CYP enzymes: human P450 metabolism data. *Drug Metab Rev* 2002; 1-2: 83-448.
56. 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; 3: 244-5.
57. PUCCINELLI E et al. Xenobiotic metabolizing cytochrome P450 in pig, a promising animal model. *Curr Drug Metab* 2011; 6: 507-25.
58. 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* 2000; 12: 1131-52.
59. ANZENBACHER P et al. Presence and activity of cytochrome P450 isoforms in minipig liver microsomes. Comparison with human liver samples. *Drug Metab Dispos* 1998; 1: 56-9.

Pig as a preclinical model of oral drug absorption

60. THORN HA et al. Drug metabolism of CYP3A4, CYP2C9 and CYP2D6 substrates in pigs and humans. *Eur J Pharm Sci* 2011; 3: 89-98.
61. MOGI M et al. Simultaneous pharmacokinetics assessment of caffeine, warfarin, omeprazole, metoprolol, and midazolam intravenously or orally administered to Microminipigs. *J Toxicol Sci* 2012; 6: 1157-64.
62. OBERLE RL et al. Pharmacokinetics and metabolism of diclofenac sodium in Yucatan miniature pigs. *Pharm Res* 1994; 5: 698-703.
63. MUSTHER H et al. Animal versus human oral drug bioavailability: Do they correlate? *European Journal of Pharmaceutical Sciences* 2014: 280-291.
64. SIETSEMA WK. The Absolute Oral Bioavailability of Selected Drugs. *Int J Clin Pharm Th* 1989; 4: 179-211.
65. PAPICH MG, MARTINEZ MN. Applying Biopharmaceutical Classification System (BCS) Criteria to Predict Oral Absorption of Drugs in Dogs: Challenges and Pitfalls. *AAPS J* 2015; 4: 948-64.
66. SIEFERT HM et al. Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: a comparison in humans and other mammalian species. *Journal of Antimicrobial Chemotherapy* 1999: 69-76.
67. NIELSEN P, GYRDHANSEN N. Oral bioavailability of sulfadiazine and trimethoprim in fed and fasted pigs. *Research in Veterinary Science* 1994; 1: 48-52.
68. HJALMARSSON C et al. Orally Administered Trimethoprim-Sulfamethoxazole and Metronidazole as Infection Prophylaxis in Elective Colorectal Surgery. *Surg Infect (Larchmt)* 2015; 5: 604-10.
69. ZHU T et al. Comparison of the Gastrointestinal Absorption and Bioavailability of Fenofibrate and Fenofibric Acid in Humans. *Journal of Clinical Pharmacology* 2010; 8: 914-921.

## Pig as a preclinical model of oral drug absorption

70. MCCARTHY CA et al. In vitro dissolution models for the prediction of in vivo performance of an oral mesoporous silica formulation. *J Control Release* 2017; 86-95.
71. SAURON R et al. Absence of a food effect with a 145 mg nanoparticle fenofibrate tablet formulation. *Int J Clin Pharm Th* 2006; 2: 64-70.
72. GEISLINGER G et al. Pharmacokinetics of ketoprofen enantiomers after different doses of the racemate. *Br J Clin Pharmacol* 1995; 1: 73-5.
73. NEIRINCKX E et al. Species comparison of enantioselective oral bioavailability and pharmacokinetics of ketoprofen. *Research in Veterinary Science* 2011; 3: 415-421.
74. REGARDH CG et al. Pharmacokinetics of metoprolol and its metabolite alpha-OH-metoprolol in healthy, non-smoking, elderly individuals. *Eur J Clin Pharmacol* 1983; 2: 221-6.
75. ABBOT EL et al. Vigabatrin transport across the human intestinal epithelial (Caco-2) brush-border membrane is via the H<sup>+</sup>-coupled amino-acid transporter hPAT1. *Br J Pharmacol* 2006; 3: 298-306.
76. DIVOLL M et al. Absolute bioavailability of oral and intramuscular diazepam- effects of age and sex. *Anesthesia and Analgesia* 1983; 1: 1-8.
77. MENG-LUND E et al. Buccal absorption of diazepam is improved when administered in bioadhesive tablets-An in vivo study in conscious Gottingen mini-pigs. *International Journal of Pharmaceutics* 2016; 1-2: 125-131.
78. WESTPHAL JF et al. Nifedipine enhances amoxicillin absorption kinetics and bioavailability in humans. *Journal of Pharmacology and Experimental Therapeutics* 1990; 1: 312-317.
79. AGERSO H, FRIIS C. Bioavailability of amoxycillin in pigs. *J Vet Pharmacol Ther* 1998; 1: 41-6.
80. MUELLER EA et al. Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. *Pharm Res* 1994; 2: 301-304.

Pig as a preclinical model of oral drug absorption

81. PRIMMETT DRN et al. Cyclosporine monitoring in patients with renal transplants: Two- or three-point methods that estimate area under the curve are superior to trough levels in predicting drug exposure. *Ther Drug Monit* 1998; 3: 276-283.
82. KEOHANE K et al. Enhanced colonic delivery of ciclosporin A self-emulsifying drug delivery system encapsulated in coated minispheres. *Drug Dev Ind Pharm* 2016; 2: 245-253.
83. RAWLINS MD et al. Pharmacokinetics of paracetamol (acetaminophen) after intravenous and oral administration. *Eur J Clin Pharmacol* 1977; 4: 283-6.
84. UNO T et al. Absolute bioavailability and metabolism of omeprazole in relation to CYP2C19 genotypes following single intravenous and oral administrations. *Eur J Clin Pharmacol* 2007; 2: 143-9.
85. ALLONEN H et al. Midazolam kinetics. *Clin Pharmacol Ther* 1981; 5: 653-61.
86. LIGNET F et al. Characterization of Pharmacokinetics in the Gottingen Minipig with Reference Human Drugs: An In Vitro and In Vivo Approach. *Pharm Res* 2016; 10: 2565-79.
87. JONKMAN JH et al. Disposition and clinical pharmacokinetics of microcrystalline theophylline. *Eur J Clin Pharmacol* 1980; 5: 379-84.
88. WEBSTER J et al. Cimetidine- a clinical and pharmacokinetic study. *British Journal of Clinical Pharmacology* 1981; 4: 333-338.
89. MASON WD et al. Kinetics and absolute bioavailability of atenolol. *Clin Pharmacol Ther* 1979; 4: 408-415.
90. BRACHTEL D, RICHTER E. Absolute bioavailability of caffeine from a tablet formulation. *J Hepatol* 1992; 3: 385-385.
91. PORTER CJ, CHARMAN WN. In vitro assessment of oral lipid based formulations. *Adv Drug Deliv Rev* 2001: S127-47.

## Pig as a preclinical model of oral drug absorption

92. NIELSEN FS et al. Bioavailability of probucol from lipid and surfactant based formulations in minipigs: influence of droplet size and dietary state. *Eur J Pharm Biopharm* 2008; 2: 553-62.
93. CHARMAN WNA, STELLA VJ. Estimating the Maximal Potential for Intestinal Lymphatic Transport of Lipophilic Drug Molecules. *International Journal of Pharmaceutics* 1986; 1-2: 175-178.
94. CALIPH SM et al. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J Pharm Sci* 2000; 8: 1073-84.
95. HOLM R, HOEST J. Successful in silico predicting of intestinal lymphatic transfer. *Int J Pharm* 2004; 1-2: 189-93.
96. GRIFFIN BT et al. Comparison of in vitro tests at various levels of complexity for the prediction of in vivo performance of lipid-based formulations: Case studies with fenofibrate. *European Journal of Pharmaceutics and Biopharmaceutics* 2014; 3: 427-437.
97. FEI Y et al. Analysis of the enhanced oral bioavailability of fenofibrate lipid formulations in fasted humans using an in vitro-in silico-in vivo approach. *Eur J Pharm Biopharm* 2013; 3 Pt B: 1274-84.
98. STILLHART C et al. Biopharmaceutical modeling of drug supersaturation during lipid-based formulation digestion considering an absorption sink. *Pharmaceutical research* 2014; 12: 3426-44.
99. O'SHEA JP et al. Lipidic dispersion to reduce food dependent oral bioavailability of fenofibrate: In vitro, in vivo and in silico assessments. *European Journal of Pharmaceutics and Biopharmaceutics* 2015: 207-216.

## Pig as a preclinical model of oral drug absorption

100. THOMAS N et al. In vitro lipolysis data does not adequately predict the in vivo performance of lipid-based drug delivery systems containing fenofibrate. *AAPS J* 2014; 3: 539-49.
101. GUICHARD JP, SAURON RLP. A Comparison of the Bioavailability of Standard or Micronized Formulations of Fenofibrate. *Curr Ther Res Clin E* 1993; 5: 610-614.
102. KULKARNI R et al. Comparative pharmacokinetics studies of immediate- and modified-release formulations of glipizide in pigs and dogs. *J Pharm Sci* 2012; 11: 4327-4336.
103. IKEGAMI K et al. Bioavailability and in vivo release behavior of controlled-release multiple-unit theophylline dosage forms in beagle dogs, cynomolgus monkeys, and gottingen minipigs. *J Pharm Sci* 2006; 9: 1888-95.
104. KOSTEWICZ E et al. Examination of two sustained release nifedipine preparations in humans and in pigs. *European Journal of Pharmaceutical Sciences* 1996; 6: 351-357.
105. KESISOGLOU F et al. Suitability of a Minipig Model in Assessing Clinical Bioperformance of Matrix and Multiparticulate Extended-Release Formulations for a BCS Class III Drug Development Candidate. *J Pharm Sci* 2014; 2: 636-642.
106. CHAMBLISS WG et al. Development and evaluation of enteric-coated penicillamine tablets. *J Pharm Sci* 1984; 9: 1215-1219.
107. DORKOOSH FA et al. Peroral absorption of octreotide in pigs formulated in delivery systems on the basis of superporous hydrogel polymers. *Pharm Res* 2002; 10: 1532-1536.
108. JONES HM et al. A novel strategy for physiologically based predictions of human pharmacokinetics. *Clinical pharmacokinetics* 2006; 5: 511-42.
109. DRESSMAN JB et al. An update on computational oral absorption simulation. *Expert Opinion on Drug Metabolism & Toxicology* 2011; 11: 1345-1364.

## Pig as a preclinical model of oral drug absorption

110. GRIGNARD E et al. Considerations for the development of in vitro dissolution tests to reduce or replace preclinical oral absorption studies. *Eur J Pharm Sci* 2017; 193-201.
111. REPPAS C, VERTZONI M. Biorelevant in-vitro performance testing of orally administered dosage forms. *J Pharm Pharmacol* 2012; 7: 919-30.
112. ARNDT M et al. Dissolution media simulating the proximal canine gastrointestinal tract in the fasted state. *Eur J Pharm Biopharm* 2013; 3: 633-41.
113. GOVENDER M et al. A Dual-Biotic System for the Concurrent Delivery of Antibiotics and Probiotics: In Vitro, Ex Vivo, In Vivo and In Silico Evaluation and Correlation. *Pharmaceutical research* 2016; 12: 3057-3071.
114. KOSTEWICZ ES et al. PBPK models for the prediction of in vivo performance of oral dosage forms. *European Journal of Pharmaceutical Sciences* 2014: 300-321.
115. BUUR JL et al. Estimating meat withdrawal times in pigs exposed to melamine contaminated feed using a physiologically based pharmacokinetic model. *Regulatory toxicology and pharmacology : RTP* 2008; 3: 324-31.
116. SHIN BS et al. Physiologically based pharmacokinetics of zearalenone. *Journal of toxicology and environmental health Part A* 2009; 21-22: 1395-405.
117. YUAN LG et al. A physiologically based pharmacokinetic model for valnemulin in rats and extrapolation to pigs. *Journal of veterinary pharmacology and therapeutics* 2011; 3: 224-31.
118. YANG B et al. A physiologically based pharmacokinetic model for the prediction of the depletion of methyl-3-quinoxaline-2-carboxylic acid, the marker residue of olaquinox, in the edible tissues of pigs. *Journal of veterinary pharmacology and therapeutics* 2014; 1: 66-82.
119. YANG X et al. A physiologically based pharmacokinetic model for quinoxaline-2-carboxylic acid in rats, extrapolation to pigs. *Journal of veterinary pharmacology and therapeutics* 2015; 1: 55-64.

## Pig as a preclinical model of oral drug absorption

120. BUUR J et al. Use of probabilistic modeling within a physiologically based pharmacokinetic model to predict sulfamethazine residue withdrawal times in edible tissues in swine. *Antimicrobial agents and chemotherapy* 2006; 7: 2344-51.
121. YANG F et al. Use of a Monte Carlo analysis within a physiologically based pharmacokinetic model to predict doxycycline residue withdrawal time in edible tissues in swine. *Food additives & contaminants Part A, Chemistry, analysis, control, exposure & risk assessment* 2012; 1: 73-84.
122. 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. *American journal of veterinary research* 2005; 10: 1686-93.
123. BUUR JL et al. A physiologically based pharmacokinetic model linking plasma protein binding interactions with drug disposition. *Research in Veterinary Science* 2009; 2: 293-301.
124. KESISOGLOU F et al. Utility of PBPK Absorption Modeling to Guide Modified Release Formulation Development of Gaboxadol, a Highly Soluble Compound with Region-Dependent Absorption. *Journal of pharmaceutical sciences* 2016; 105: 722-728.
125. SJÖGREN E et al. The pharmacokinetics and hepatic disposition of repaglinide in pigs: mechanistic modeling of metabolism and transport. *Molecular pharmaceutics* 2012; 4: 823-841.
126. SJÖGREN E et al. Effects of verapamil on the pharmacokinetics and hepatobiliary disposition of fexofenadine in pigs. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* 2014; 214-23.



Pig as a preclinical model of oral drug absorption

127. DUBBELBOER IR et al. A Model-Based Approach To Assessing the Importance of Intracellular Binding Sites in Doxorubicin Disposition. *Molecular Pharmaceutics* 2017; 3: 686-698.

Pig as a preclinical model of oral drug absorption

Table 1 Comparison of the pH in the GI tract of pigs versus human

Segment	Human		Minipig <sup>1)</sup>		Landrace pig	
	Fasted pH	Fed pH	Fasted pH	Fed pH	Fasted pH	Fed pH
	[23; 24]	[24; 4]	[25]	[25]	[21; 4] 2)	[26]
Stomach	1.0-3.5	3.0-6.0	0.3-1.7	3.6	1.2-4.0	4.4
Duodenum	6.0-7.0	5.0-5.5	7-8		6.7	6.1-6.5
Jejunum	6.0-7.7	5.0-6.5		n.a.	6.8	6.3-6.6
Ileum	6.5-8.0	6.5-8.0			6.9	6.5-6.7
Colon	5.5-8.0	6.0-7.5	n.a.	n.a.	6.1-6.6	6.5-6.6

n.a.: not available; <sup>1)</sup> Yucatan minipig <sup>2)</sup> in house data (12 pigs)

Pig as a preclinical model of oral drug absorption

Table 2 Total small intestine surface areas of pig and human, adapted from DeSesso et. al, <sup>[27]</sup>

	<b>Pig</b>	<b>Human</b>
Body weight [kg]	47	70
Smooth luminal surface area [m <sup>2</sup> ]	1.4	0.42
Fold-increase factors		
Plicae	1	3
Villi	6	10
Micro-villi	20-25	20
Combined multiplication factor	120	600
Estimated total surface area [m <sup>2</sup> ]	168-210	252

Pig as a preclinical model of oral drug absorption

Table 3 Major bile components in pigs and humans, based on published data <sup>[36]</sup>: \*primary, \*\*secondary bile acids

	<b>Pig</b>	<b>Human</b>
Conjugation	Taurine, Glycine	Taurine, Glycine
Major bile acids	Cholate (C)*, Hyocholate (HC)*, Hyodeoxycholate (HDC)**	Cholate (C)*, Chenodeoxycholate (CDC)*, Deoxycholate (DC)**

Pig as a preclinical model of oral drug absorption

Table 4 Comparison of oral in vivo data from pigs and humans from various pharmacokinetic studies.

Compound Name	PK parameters pigs					PK parameters human					Ref.
	Pig F abs [%]	t <sub>1/2</sub> [h]	V <sub>d</sub> [L/kg]	Cl [L/(h*kg)]	T max [h]	Human F abs [%]	t <sub>1/2</sub> [h]	V <sub>d</sub> [L/kg]	Cl [L/(h*kg)]	T max [h]	
Moxifloxacin <sup>1)</sup>	54 ± 103	11.0 ± 1.1	3.8 ± 1.2	0.7 ± 1.39	4.0 ± 1.01	82 ± 19.7	12.0	2.2	0.1	2.0 ± 1.90	[66]
Trimethoprim <sup>2)</sup>	90 ± 11	n.r.	1.8	0.6	2.1 ± 1.2	98 ± 22.4	10.9	1.6	0.1	1.8 ± 0.7	[67; 68]
Fenofibrate <sup>2)</sup>	71 ± 26	n.r.	n.r.	n.r.	5.0 ± 2.4	69 ± 10.8	20.0 ± 7.7	n.r.	n.r.	2.33 ± 0.73**	[69-71]
Ketoprofen <sup>2)</sup>	86 ± 20	0.8 ± 0.2	0.4 ± 0.2	0.7 ± 0.2	3.85 ± 1.13	85 ± 20.1	1.3 ± 0.3	n.r.	n.r.	0.75	[72; 73]
Metoprolol <sup>1)</sup>	3 ± 1	n.r.	7.7 ± 0.7	4.4 ± 0.6	0.88 ± 0.38	50 ± 7.0	3.5 ± 0.2	4.0 ± 0.3	n.r.	n.r.	[74; 29]
Vigabatrin <sup>1)</sup>	75 ± 4	n.r.	n.r.	0.2 ± 0.1	0.5	85 ± 5*	n.r.	n.r.	n.r.	n.r.	[75; 52]
Diazepam <sup>1)</sup>	31 ± 6	n.r.	0.05	0.1.	4.8 ± 0.8	94	n.r.	n.r.	n.r.	2.5	[76; 77]
Amoxicillin <sup>2)</sup>	33 ± 14	9.9 ± 4.3	0.6 ± 0.2	0.5 ± 0.1	1.9 ± 0.9	65 ± 11.4	1.2 ± 0.1	0.5 ± 0.1	0.3	2.06 ± 0.43	[78; 79]
Cyclosporine <sup>2)</sup>	58 ± 33	n.r.	n.r.	n.r.	2.17 ± 0.15	60	n.r.	n.r.	n.r.	n.r.	[80-82]
Paracetamol <sup>1)</sup>	83 ± 29	3.8 ± 0.3	0.7 ± 0.1	5.3 ± 1.1	1.38	89 ± 4	n.r.	n.r.	n.r.	0.73 ± 0.42	[83; 44]
Omeprazole <sup>3)</sup>	11 ± 7	0.6 ± 0.1	n.r.	n.r.	0.6 ± 0.3	41 ± 1.5*	n.r.	n.r.	n.r.	2.5	[84; 61]
Midazolam <sup>1)</sup>	12	1.0 ± 0.4	1.3	22.3 ± 8.6	3.0 ± 3.4	44 ± 17	n.r.	n.r.	n.r.	1.5 ± 0.33	[85; 64; 86]
Theophylline <sup>1)</sup>	108	11.9 ± 4.5	0.9 ± 0.1	1.1 ± 0.4	2.6 ± 1.2	103 ± 10.2	0.3 ± 0.2	n.r.	n.r.	n.r.	[87; 86]
Cimetidine <sup>1)</sup>	33	0.8 ± 0.1	1.5 ± 0.2	37.4 ± 8.2	1.7 ± 1.0	78	1.7 ± 0.4	n.r.	n.r.	0.83	[88; 86]

## Pig as a preclinical model of oral drug absorption

Hydrochlorothiazide <sup>1)</sup>	62	5.2 ± 1.2	3.2 ± 0.8	11.8 ± 1.6	2.3 ± 1.5	72 ± 17	n.r.	n.r.	n.r.	n.r.	[64; 86]
Atenolol <sup>1)</sup>	34	4.4 ± 1.5	1.5 ± 0.1	7.9 ± 2.9	1.6 ± 0.5	58 ± 16	6.7 ± 2.6	n.r.	9.3 ± 1.2	2.5 ± 1.1	[89; 86]
Phenazone <sup>1)</sup>	36 ± 10	9.3 ± 5.8	0.8 ± 0.3	1.5 ± 0.9	2.4 ± 1.3	91 ± 10*	n.r.	n.r.	n.r.	n.r.	[64; 86]
Warfarin <sup>3)</sup>	84 ± 38	17 ± 12	n.r.	n.r.	11 ± 9	93 ± 8	n.r.	n.r.	n.r.	n.r.	[64; 61]
Caffeine <sup>3)</sup>	79 ± 28	11 ± 4	n.r.	n.r.	3.3 ± 2.2	80 ± 16	n.r.	n.r.	n.r.	n.r.	[90; 61]
Diclofenac <sup>4)</sup>	107 ± 5	n.r.	n.r.	n.r.	0.75	42 ± 27	n.r.	n.r.	n.r.	0.25	[62]

n.r.= not reported. Including absolute bioavailability ( $F_{abs}$ ), half-life ( $t_{1/2}$ ), volume of distribution ( $V_d$ ), clearance (Cl) and time of peak plasma

concentration ( $t_{max}$ ) for 20 compounds. Different pig breeds were included: <sup>1)</sup>Göttingen Minipig. <sup>2)</sup> LR pigs. <sup>3)</sup>Microminipig. <sup>4)</sup>Yucatan Minipig

\*Range. \*\* different PK study <sup>[71]</sup>

Pig as a preclinical model of oral drug absorption

Table 5 Summary of in vivo studies in pigs involving assessment of in vivo performance of various formulation technologies(MP=minipig; LR=landrace pig)

Formulation technology	Drug	Pig species	Dosage [mg/kg]	Formulation type		<i>in vivo</i> performance	Formulation components	Ref.
Bio-enabling formulation	Fenofibrate	LR	3.72	Mesoporous Silica Formulation	SBA-15	slower rate of drug absorption due to a slower release (compare to Lipantil Supra)	Fenofibrtae loading onto SBA-15 251.3 mg drug/g silica	[69; 70]
			3.72	Nanosized Formulation	Reference formulation	F <sub>abs</sub> 71% not significant higher than micronized commercial products, it is similar to reported human oral bioavailability of 69%	Lipantil <sup>®</sup> Supra 145 mg film coated tablets	
Bio-enabling formulation	Fenofibrate	LR	5.48	Lipid based formulations	LFCS Type IIIA	Bioavailability is similar to the LFCS Type IIIB/IV (nearly 70% F <sub>abs</sub> )	40% Miglyol, 20% Cremophor RH, 40%, Tween 85	[96]
			5.84	Lipid based formulations	LFCS Type IIIB/IV	No major differences within t <sub>max</sub> and c <sub>max</sub> values due to the other LBF of Fenofibrate	33% Cremophor RH, 67% Tween 85	

Pig as a preclinical model of oral drug absorption

Formulation technology	Drug	Pig species	Dosage [mg/kg]	Formulation type		<i>in vivo</i> performance	Formulation components	Ref.
			4.32	Micronized Formulation	Reference Formulation	The absolute bioavailability was 66% a little bit lower than the LBF above.	Lipantil® Micro 67 mg hard Capsules (Abbott)	[99]
Bio-enabling formulation	Fenofibrate	MP	16.06	Micronized Formulation	Reference Formulation	No apparent difference in the overall extent of bioavailability between Lipanthyl and the SNEDDs below, $t_{max}$ performance: 1:3 (Lipanthyl : SNEDDs <sub>below</sub> )	Lipanthyl 200 M capsules containing micronized Fenofibrate (Abbot AG)	[100]
Bio-enabling formulation	Fenofibrate	MP	16.06	Lipid based formulations	SNEDDS	Very similar <i>in vivo</i> performance of the various SNEDDs formulation	24 % soybean, 32.2 % Maisine 35-1, 30% Kolliphor RH 40, 13.8% Ethanol (75 % drug load)	[100]



Pig as a preclinical model of oral drug absorption

Formulation technology	Drug	Pig species	Dosage [mg/kg]	Formulation type		<i>in vivo</i> performance	Formulation components	Ref.
			16.06	Lipid based formulations	Supersaturated SNEDDS	All SNEDDSs showed an increase rate of drug absorption (higher $C_{max}$ ) compared to Lipanthyl	same LBF (150 % drug load)	
			16.06	Lipid based formulations	SNEDDS-suspension	In vivo performance of the SNEDDS was significantly enhanced compared to commercial Lipanthyl	same LBF (100+ 50% drug load)	
Modified Release Formulations	Glipizide	LR	0.42	Sustained Release	Commercial product	F 92% in pig compare to only 21% bioavailability in beagle dogs. Hence the pig is a better model to predict MR formulation performance (human F is 95%)	Glucotrol XL®	[102]
	Theophylline	MP	9.09	Sustained Release	Capsule (300 mg of EC-coated beads)	$F_{rel}$ was low, close to 50% compared to an oral solution	34% Theophylline, 20% Sucrose, 34% Nonpareil-103,	[103]

Pig as a preclinical model of oral drug absorption

Formulation technology	Drug	Pig species	Dosage [mg/kg]	Formulation type		<i>in vivo</i> performance	Formulation components	Ref.
							9% Sucrose, 3% Ethyl-cellulose	
	Nifedipine	LR	1.58	Sustained Release	Pellets in gelatine capsule	Difference in the absorption characteristics of the two formulations (experimental formulation and Procardia XL)	n.a.	[104]
	Nifedipine	LR	1.58	Sustained Release	oral osmotic pump	The mean Cmax is considerably higher for the experimental formulation when compared against Procardia XL	Procardia XL tablet (Pfizer)	[105]
Modified Release Formulations	MK-0941 (BCS class III)	Yucatan MP	0.08	Sustained Release	Matrix tablet	The pharmacokinetic performance of the matrix table and multiparticulate formulation was comparable	Hydroxypropyl methylcellulose based matrix tablets	[105]

Pig as a preclinical model of oral drug absorption

Formulation technology	Drug	Pig species	Dosage [mg/kg]	Formulation type		<i>in vivo</i> performance	Formulation components	Ref.
	MK-0941 (BCS class III)	Yucatan MP	0.08	Sustained Release	Multiparticulate formulation	The <i>in vivo</i> performance in pigs appeared to reasonably reflect the clinical performance	Multiparticulate formulation consisted of a drug-containing core with a EC-based coating, filled in a capsules	
	Penicillamine	LR	10.95	Enteric coated	Coated tablet	The shape of the plasma concentration curve was similar to that in human. The $F_{rel}$ 66.5% compared with the uncoated table	5 layers of cellulose acetate phthalate formulation	[106]
	Octreotide	LR	0.64	Enteric coated	Coated Capsule	Different composition were tested, combination with Chitosan as an extra absorption enhancer demonstrate the best bioavailability (16.1%)	1) core: ocreotide 2) conveyor system: super-porous hydrogel (SPH) 3) placed in enteric coated capsule (Eudragit S100)	[107]

Pig as a preclinical model of oral drug absorption

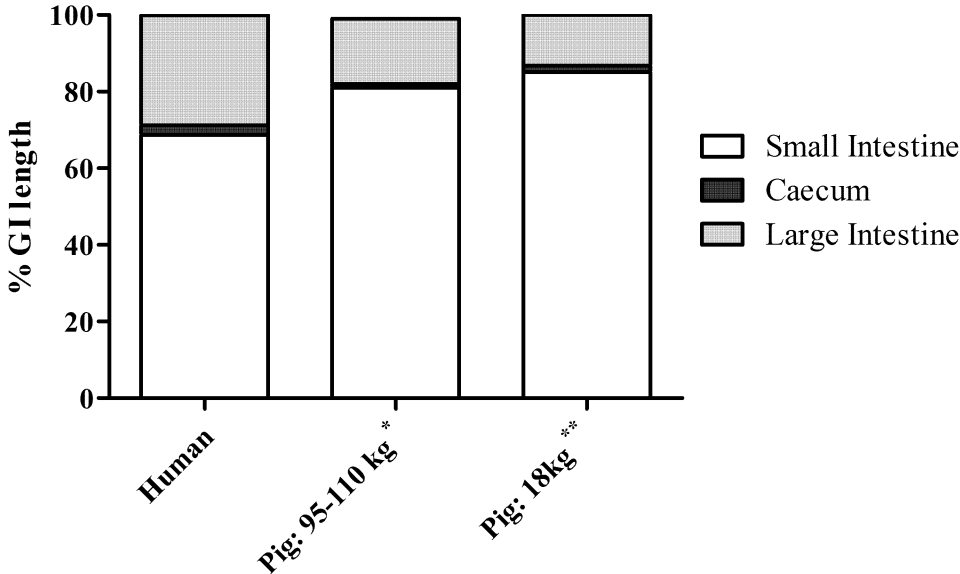


Figure 1 Relative gastrointestinal (GI) length [%] of sections of the GI tract from: published pig (crossbreed of large white and landrace)<sup>[26]</sup> and human data <sup>[4; 27]</sup>; in comparison to LR (unpublished data) <sup>\*\*</sup>.

Fig as a preclinical model of oral drug absorption

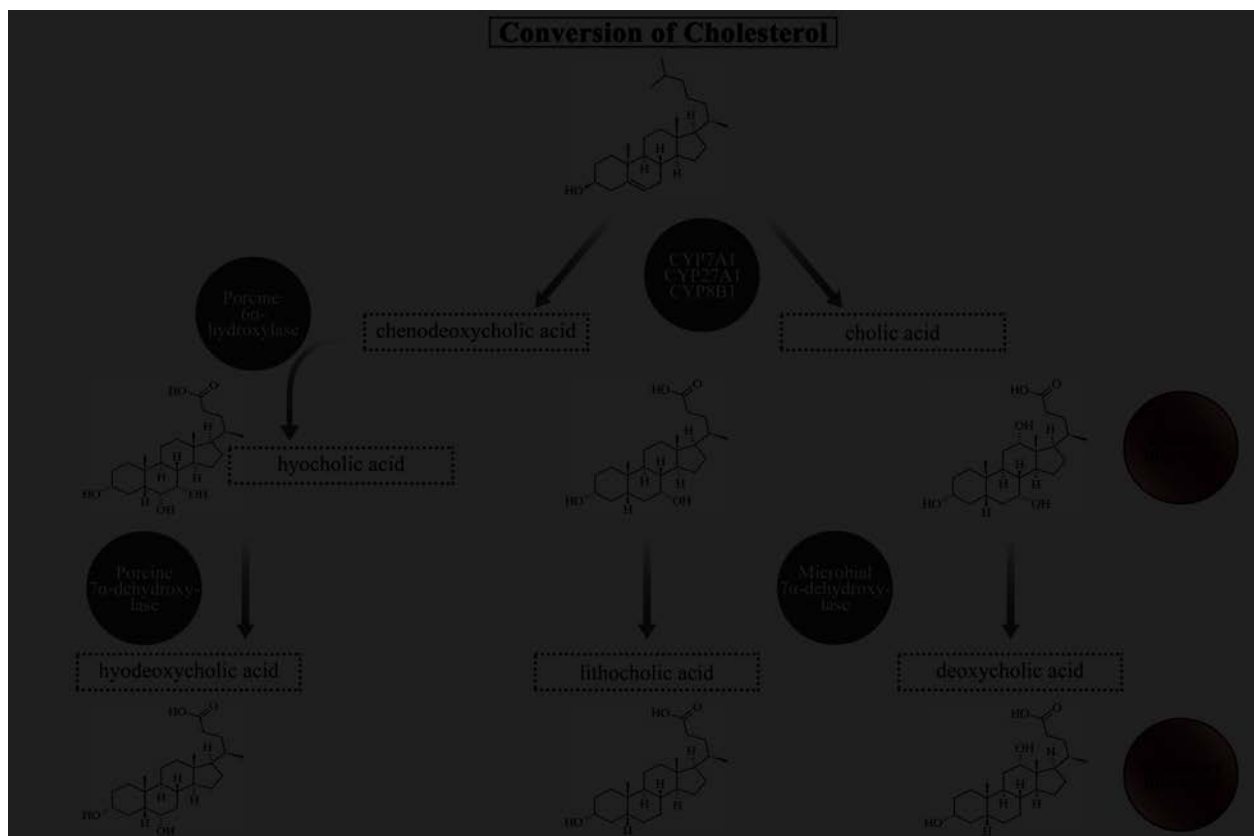


Figure 2 Conversion of Cholesterol into primary and secondary bile acids, adapted scheme from Ref. [32]; .

Pig as a preclinical model of oral drug absorption

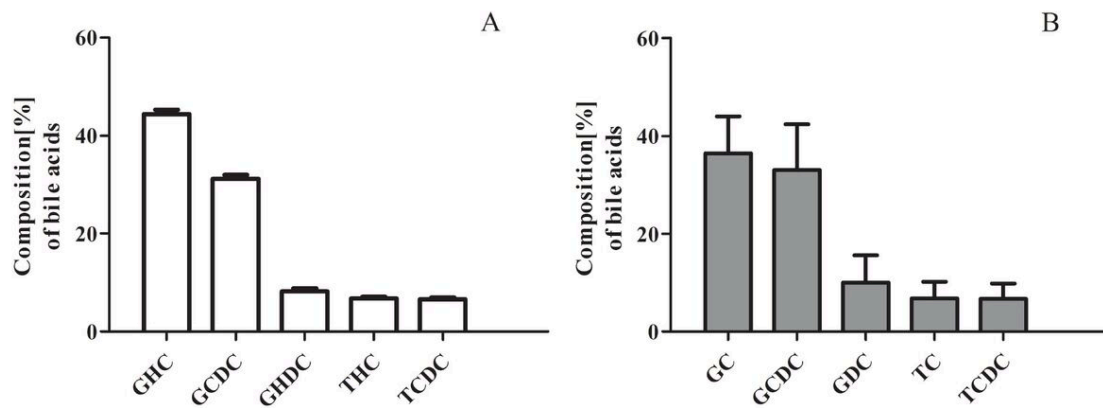


Figure 3 Rank order of prevalence of bile salts (A) Pigs, GHC= Glycohyocholate; GCDC= Glycochenodeoxycholate; GHDC= Glycohyodeoxycholate; THC= Taurohyocholate; TCDC= Taurochenodeoxycholate; based on the reported data from Scanff *et al.* <sup>[40]</sup>, (B) human bile salts, GC= Glycocholate; GCDC= Glycochenodeoxycholate; GDC= Glyco-deoxycholate; TC= Tauro-cholate; TCDC= Tauro-chendeoxycholate. <sup>[38]</sup>

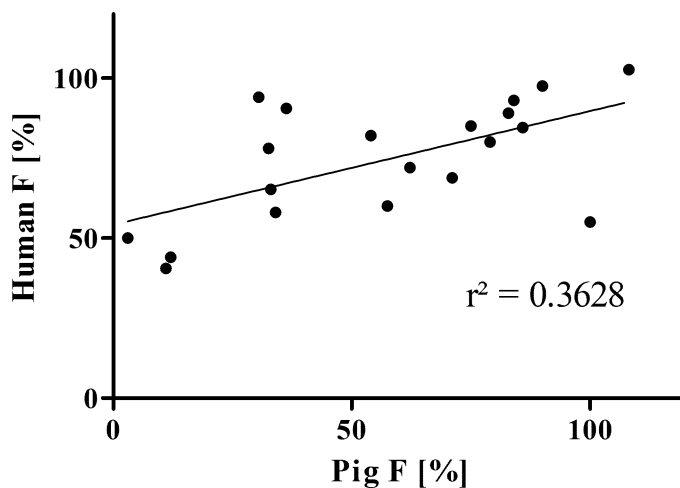


Figure 4 Direct correlation between the oral bioavailability in human and pig of the compounds presented in Table 4.

Pig as a preclinical model of oral drug absorption

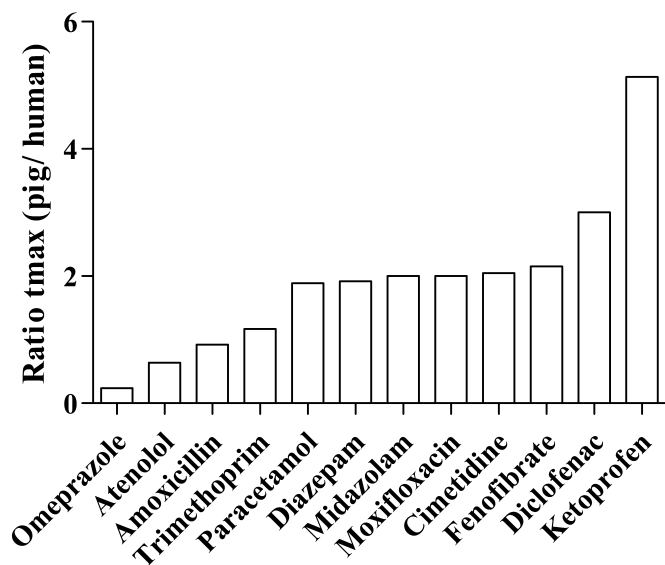


Figure 5 T<sub>max</sub> ratios of pig/human values for 12 drugs of Table 4.

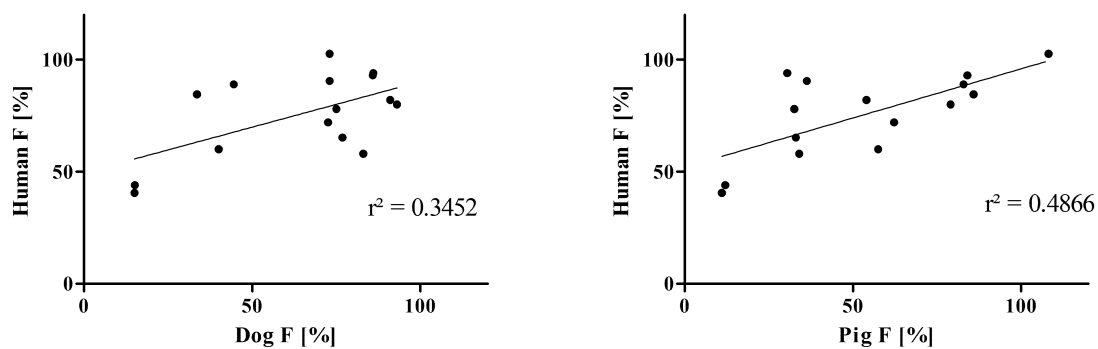
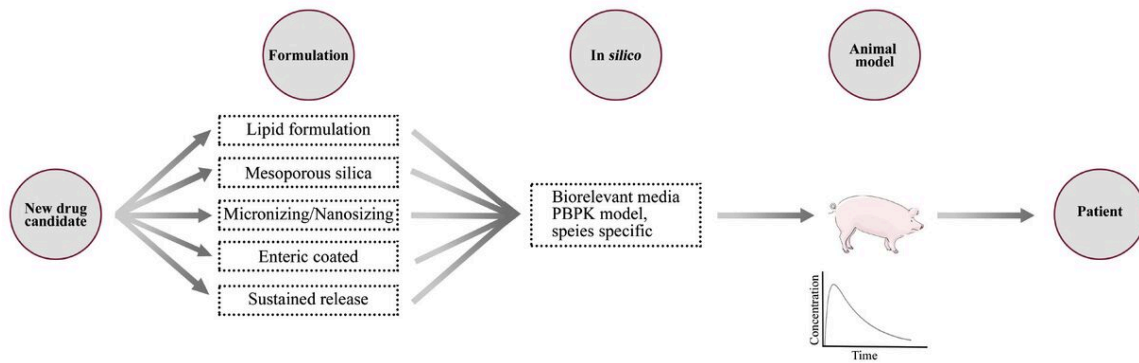


Figure 6 Direct correlation of oral bioavailability: human versus dog and human versus pig, for 15 compounds presented in Table 4.

Pig as a preclinical model of oral drug absorption



**Reduce risk in preclinical testing**

Figure 7 Role of the pig model in formulation development

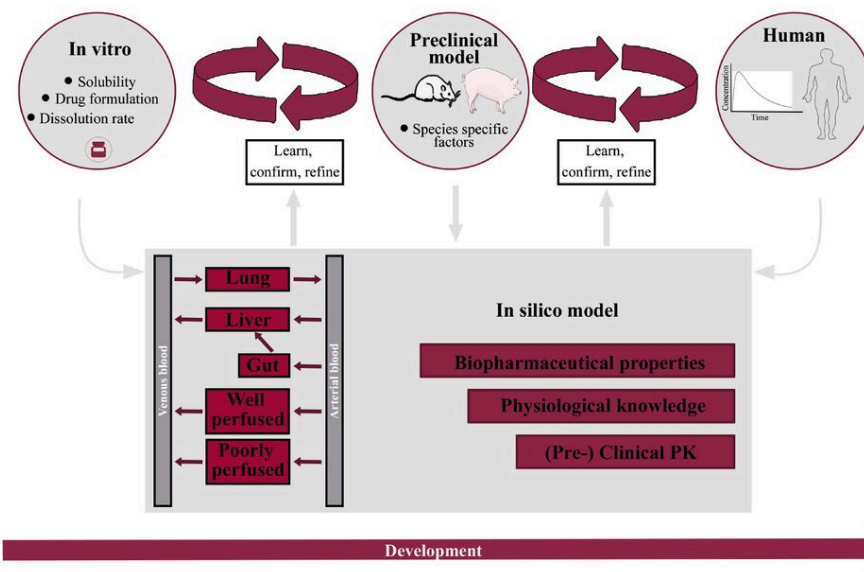


Figure 8 A schematic presentation of the compound selection and formulation optimisation process and the placement of in silico model in the processes