Introduction
There is an increased interest internationally in the use of minipigs as models in regulatory pharmacology and toxicology.\textsuperscript{1,2} This interest has come to the forefront because of the difficulties involved in procuring and using dogs and non human primates as the non rodent species. The development of minipigs has made it more practical to use this species because of the smaller size at sexual maturity. Small size makes a substantial difference in the dosage to be administered of a test substance because of the limited amount of the material that is available. When using farm pigs at sexual maturity, between 4-6 months of age, you would be handling animals >100 kg body weight. A chart of the body weights of common breeds of minipigs available for research is below for comparison.

Comparative Anatomy and Physiology
Swine in general have many anatomic and physiologic characteristics that are analogous to humans. Because pigs are true omnivores they are similar to humans in the physiology of digestion and metabolism. Many of their systems share anatomic and physiologic characteristics with humans which make them acceptable for this area of study. In general minipig breeds are considered to have immunologic and physiologic maturity at 4-6 months of age when they reach sexual maturity.\textsuperscript{1}

The cardiovascular system of the sexually mature minipig is similar in function, including hemodynamics, to humans. The Hanford pig has a human sized heart at sexual maturity and the other breeds of minipigs are proportionately smaller. The growth and development of the cardiovascular system from birth to sexual maturity is analogous to that of humans from birth until the mid-teens. Swine are well described models of atherosclerosis and vascular reactivity. Swine have right side dominant coronary arterial circulation and do not have existing collateral blood vessels. Consequently they are susceptible to both acute and chronic myocardial infarction. They can be readily induced to develop atherosclerotic lesions in specific locations by inducing endothelial damage in an artery in combination with feeding of an atherogenic diet. Wound healing in the vascular system is similar and they have a true vaso vasorum.\textsuperscript{1}

<table>
<thead>
<tr>
<th>Age</th>
<th>2 Months</th>
<th>4 Months</th>
<th>6 Months</th>
<th>8 Months</th>
<th>10 Months</th>
<th>12 Months</th>
<th>24 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinclair</td>
<td>7-9</td>
<td>14-17</td>
<td>19-23</td>
<td>26-29</td>
<td>32-36</td>
<td>37-42</td>
<td>5-70</td>
</tr>
<tr>
<td>Micro-Yucatan</td>
<td>6-8</td>
<td>13-14</td>
<td>18-20</td>
<td>23-25</td>
<td>29-31</td>
<td>35-40</td>
<td>55-70</td>
</tr>
<tr>
<td>Yucatan</td>
<td>7-9</td>
<td>16-20</td>
<td>26-30</td>
<td>36-40</td>
<td>46-50</td>
<td>55-60</td>
<td>70-80</td>
</tr>
<tr>
<td>Hanford</td>
<td>8-11</td>
<td>20-27</td>
<td>34-42</td>
<td>44-48</td>
<td>51-56</td>
<td>60-70</td>
<td>80-95</td>
</tr>
</tbody>
</table>

* Body weights are estimated for 6 months or older micro - Yucatan and Hanford *

The Sinclair and the Yucatan micropig are the smallest of these breeds and the most likely selection for test substances which have to be administered systemically.
The cardiac conduction system differs from humans in having an increased number of adrenergic and cholinergic nerve fibers in the AV node and bundle branches. Even with differences in conduction the intracardiac electrophysiologic measurements overlap those of humans. Pigs typically have an elongated QT segment. The electrophysiologic measurements of various breeds of miniature swine and typical ECGs have been published.¹

The digestive system of pigs has gross anatomic differences from humans in spite of the similar physiology. The main differences are the presence of a torus pyloricus, which is a muscular outpouching in the stomach, and the spiral colon. The spiral colon is a series of centrifugal and centripetal coils in the left upper quadrant of the abdomen which consists of the cecum, ascending and transverse colon. The gastrointestinal (GI) tract of a 12 week old pig is at approximately the maturity of a one year old human and it matures with sexual maturity at approximately 5-6 months of age. Emptying times of the various portions of the GI tract vary with maturity and the characteristics of the meal. Ranges of GI emptying times in swine are: stomach 2-8 h, small intestine 2-6 h, and large intestine 24-48 h.¹,³

The liver of the sexually mature pig is approximately the size of that of humans. Anatomically the liver is divided into six lobes. Histologically lobules are subdivided by fibrous septae. The liver is approximately 1.5% of the body weight at sexual maturity. Bile production is approximately 30-60 mg/kg/24h and similar in composition to most mammals. The bile duct enters the proximal duodenum separate from the pancreatic duct. Data on hepatic hemodynamics and metabolism of glucose, lactate, alanine and glycerol have been published and the similarities compared to humans. The cytochrome P450 system has been extensively described by Skaanild and Friis. The porcine isoenzymes CYP1A, CYP2A, CYP3A and CYP3A4 metabolize the same substrates as the human. These isoenzymes have approximately 75% identical cDNA sequence to humans. However, there are differences with CYP2B, CYP2D and CYP2E. Females have higher levels of CYP1A and CYP2E. When comparing phase II enzyme biotransformation to humans, swine have relatively high glucuro-nidation activity, low sulfation activity and high acetylation activity.¹,²,⁴-⁷

The pancreas is similar in function to humans in terms of production of insulin and glucose metabolism. Direct comparisons can be made because insulin derived from slaughterhouse porcine pancreases was used as a primary source of insulin for humans for >50 years. Porcine islet cells are the predominate candidates for xenotransplantation into human diabetics.¹

The kidneys of the pig have an analogous intrarenal anatomy to humans. The kidney is multirenulate and multipapillate with a true calyceal system. Maximum urinary concentration in the pig is approximately 1080 mOsm/kg and
urine output is 5-40 ml/kg which compare favourably to humans. The calyx contracts approximately 15 times/min and the renal pelvis 3-6 time/min. Metabolic functions of the kidney also compare favourably to humans.1

The reproductive system of the pig is similar to most other quadriped mammals with an estrous rather than a menstrual cycle. The female has a bicornuate uterus and an epithelochorical placenta. Minipigs have a gestation period of 111-114 days and depending upon the breed produce 4-8 fetuses. Males have all of the accessory sex glands that humans have, however, the prostate is rudimentary and the bulbourethral glands are greatly enlarged. Background data on fetal development and reproductive parameters of the various breeds have been published. Swine have been used in a variety of fertility and vaginal irritation studies.1, 8, 9

The lungs have seven lobes. The pulmonary tissues are friable and subject to rupture and development of emphysematous bullae if traumatized. Pulmonary function matures at 2 weeks of age but growth and remodelling continue into adulthood. The lungs are the shock organ in swine as demonstrated by their use in endotoxic shock studies.1

Regional angiography, MR and PET/CT images of the central nervous system have been published on a CD-ROM. The brain weighs approximately 0.35% of the body weight in adults. Intracranial pressure is <10 mmHg and brain blood flow is approximately 1 ml min-1g-1.

The pig brain is gyrencephalic with similar distribution of gray and white matter when compared to humans. They also share similar dopanergic functions. Swine have a rete mirabile at the base of the brain. There is an increasing interest in the development of stroke and brain trauma models in swine.1

Similarities of the integumentary system of swine have made them a primary model of transdermal toxicology, transdermal drug delivery, thermal injury, wound healing and phototoxicity. The pig is relatively hairless with a fixed skin and similarities in histology. There are a limited number of eccrine sweat glands but extensive apocrine glands in contrast to humans. Blood flow to the skin and skin thickness vary depending upon the area of the body. In general the skin is thickest over the neck and dorsum of the body and thinnest over the ventral abdomen. Blood flow varies from 3-12 ml/min/100g depending upon the area.1, 10

Testing and Monitoring Methodologies
Swine may be administered test compounds by oral, injectable, inhalation and transdermal routes and the various methods have been extensively described. Swine can be trained and socialized to allow manipulation and handling without the use of anesthetics for some procedures. Swine can be trained to remain in restraint slings for hours for continuous monitoring procedures (Figure 1). Non-invasive monitoring can be
performed after surgically implanting telemetry units (Data Sciences International) which can be used to measure a combination of parameters including ECG, EEG, EMG, blood pressure and pressure from any body cavity.

Respiratory data and ECG can be collected using an ambulatory jacket that has been modified from humans for swine (LifeShirt System®, Figure 2). Jackets are also available with side pockets for carrying ambulatory infusion pumps or other monitoring equipment (AKCMA, Inc, Figure 3).1, 2, 11

Oral medications can be readily administered in food and water if the compound is palatable. If it is not palatable it can be hidden in meat balls made with dog or cat food, sweet syrups (chocolate, strawberry), fruit or pastries. Swine may be taught to tolerate the passage of stomach tube por os but it is frequently necessary to use a mouth gag to prevent them from chewing the tube if they are not sedated (Figure 4). An esophagostomy tube or a gastric fistula may be implanted surgically as an alternative. Systems for implantation of catheters into various segments of the GI tract for infusion of liquid compounds have been described.1

Figure 2. Pig wearing an ambulatory jacket for monitoring ECG and respiratory data

Transdermal patches may be utilized for administration of compounds. Absorption will depend upon the thickness of the skin and other environmental parameters. The skin behind the ear and on the ventral part of the abdomen is the thinnest and provides areas for faster permeation. It may be necessary to apply a bandage around the body to prevent the pig from rubbing off a patch.1
Injections may be given IM, SC, IV or IP. The IM route is painful in swine if large volumes or caustic compounds are administered. The SC route in the neck using a butterfly catheter is the preferred route of parenteral administration. IV injections may be given in vessels in the ear, neck, abdomen and leg. If repeated injections and chronic blood sampling is required then vascular access ports or external catheters should be surgically implanted. The techniques for surgical implantation of intravascular catheters have been described. Continuous infusion protocols can also be conducted in swine using these methods.\(^1\)

Fetal and juvenile studies may be performed in swine to address the regulatory issue of pediatric response vs. adult response to drugs and teratogenicity. Study directors should be aware of the litter effect where animals may be closely related which could skew the data. This requires that multiple unrelated litters be utilized or a single sow can be used to cross foster piglets from multiple litters if neonates are required in the study. Fetal and maternal infusion systems have been described (Figure 5).

**Conclusions**

Swine have been gaining regulatory acceptance as a non rodent species in pharmacology and toxicology.\(^2,12\) In particular they have been demonstrated to be viable alternatives to dogs for many compounds. Regulatory acceptance for their use has in particular been growing in the US, EU and Japan. Background data and tables of normal values for the various species of minipigs have been published. Other manuscripts on the Sinclair website (http://www.sinclairresearch.com/) are complementary to this manuscript.
Selected References

