We see brighter times ahead – the days are getting longer and our business is getting stronger. Looking back on 2009, it was a good, challenging year for our company. Some of the highlights were the completion of Barrier 3 where we were able to present our new state-of-the-art breeding facility to our customers and other business partners at the Grand Opening in June last year. Other highlights included the development of additional background information about the Göttingen Minipig to further validate this research model. The more knowledge we obtain about our Minipig, the more relevant spheres of application are revealed for this research model. Another highlight was the increasing interest shown by customers in further practical knowledge about housing and handling of our Minipigs.

More background information
As in many other businesses, sales were challenging in 2009, as this was the first year for a long time where we sold fewer Minipigs than the year before. However, we were still able to come out of the year with a positive financial result. In 2010, we will focus our sales effort on promoting the scientific rationale for using the Göttingen Minipig, especially in drug development and safety testing. Additional reports, articles and other literature will become available about the Göttingen Minipig to document its scientific value in biomedical research. The Göttingen Minipig is highly regarded and accepted by regulatory authorities.

Increased production capacity
We expect the increased amount of background information to generate higher demand. And we are capable of meeting a greater demand for Göttingen Minipigs from Ellegaard, as Barrier 3 is up and running, which gives us a very high breeding capacity.
NEW VETERINARIAN AT ELLEGAARD GÖTTINGEN MINIPIGS

We would like to welcome and introduce you to Helle Lorentsen, DVM, our new Head of Veterinary Services here at Ellegaard Göttingen Minipigs. Helle holds a DVM degree from the Royal Veterinary and Agricultural University in Copenhagen and a diploma in education and communication. Her background includes experience from veterinary practice, food inspection and teaching. In selecting our new Head of Veterinary Services, we focused on finding a veterinarian with teaching and communication skills and, of course, on finding someone with the right personality. We are convinced we have found all of this in Helle. Her teaching and communication skills are important so we can uphold the good collaboration we have with our customers and provide the best possible veterinary assistance to our minipig customers. Helle will have the important task of gathering and disseminating more information about good minipig welfare – both for in-house use in our breeding facilities but also for use in a research setting. It is important that we all strive to ensure the best animal welfare for our research Minipigs which will also lead to better welfare for the staff who work with them and ultimately to achieve the best research results.

Helle replaces Peter Gade who has worked with us for the past two and a half years. Peter did a great job here – both in-house with his veterinary qualifications and his personality, but also outwardly in assisting and collaborating with our customers. We would like to thank Peter for his great effort and we wish him all the best in his new position at Novo Nordisk. For a couple of weeks, Peter and Helle were both at Ellegaard Göttingen Minipigs, which was a good way of introducing Helle to her new job.

You will get the chance to meet Helle Lorentsen at some of the laboratory animal meetings or at relevant conferences, and we hope that you will welcome her just as we are doing now.
An increasing number of minipigs are used in research today and as a consequence the demand for minipig pens is growing. Modified dog pens are today often used to house these minipigs.

In a time when research requirements are changing rapidly, flexible solutions are the key to cost effective research and as a supplier of pens for large animals, we acknowledge the need for a combined minipig and dog pen.

This article is the result of a close collaboration between Ellegaard Göttingen Minipigs A/S as the expert on minipigs and Arrowmight as a long-standing supplier of pens for large animals. The aim of this article is to introduce a pen designed to house both dogs and minipigs. We will be focusing on the different requirements, which are to be met for dogs and minipigs respectively. We will also show you examples on how dog pens have been converted to minipig pens in-house by the users.

**Floor Area**

According to the revised Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123), June 2006, the minimum enclosure dimensions and space allowance is as follows for pigs and dogs respectively:

### TABLE G.4. Pigs and Minipigs: Minimum enclosure dimensions and space allowances

<table>
<thead>
<tr>
<th>Liveweight (kg)</th>
<th>Minimum enclosure size* (m²)</th>
<th>Minimum floor area per animal (m²/animal)</th>
<th>Minimum Lying space per animal (in thermoneutral conditions) (m²/animal)</th>
<th>Maximum nos of minipigs/pen of 2.25 m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 5</td>
<td>2.0</td>
<td>0.20</td>
<td>0.10</td>
<td>11</td>
</tr>
<tr>
<td>over 5 to 10</td>
<td>2.0</td>
<td>0.25</td>
<td>0.11</td>
<td>9</td>
</tr>
<tr>
<td>over 10 to 20</td>
<td>2.0</td>
<td>0.35</td>
<td>0.18</td>
<td>6</td>
</tr>
<tr>
<td>over 20 to 30</td>
<td>2.0</td>
<td>0.50</td>
<td>0.24</td>
<td>4</td>
</tr>
<tr>
<td>over 30 to 50</td>
<td>2.0</td>
<td>0.70</td>
<td>0.33</td>
<td>3</td>
</tr>
<tr>
<td>over 50 to 70</td>
<td>3.0</td>
<td>0.80</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>over 70 to 100</td>
<td>3.0</td>
<td>1.00</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>over 100 to 150</td>
<td>4.0</td>
<td>1.35</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>over 150</td>
<td>5.0</td>
<td>2.50</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Adult (conventional) boars</td>
<td>7.5</td>
<td>1.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table D.1. Dogs: Minimum enclosure dimensions and space allowances

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Minimum enclosure size (m²)</th>
<th>Minimum floor area for one or two animals (m²)</th>
<th>For each additional animal add a minimum of (m²)</th>
<th>Minimum height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 20</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>over 20</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

It is also worth noting that minipigs reach sexual maturity at 3 – 4 months of age when they weigh 5 – 10 kg (farm pigs when they are 7 – 8 months of age), whereas dogs reach sexual maturity at 7 – 8 months of age.

The majority of minipigs used in research weigh less than 50 kg and most of them weigh between 7 – 25 kg. Group housing is recommended whenever possible as dogs and minipigs are social animals. Depending on how the guidelines are interpreted the space taken up by the food bowl, platforms, etc., might be deducted from the floor area and for that reason we have decided on a standard pen module size of 2.25 m².

Each pen can be connected to the adjacent pen by means of a hatch for easy extension of the floor area for housing of larger groups of animals or separation during feeding or experiments.

The height of the pen is a standard 2 metres to allow for housing of both dogs and pigs.

This set-up leaves us with a standard pen module size of 2.25 m² suitable for housing of up to 11 minipigs. Two connected pen modules provide us with a pen size of 4.50 m² suitable for housing of two dogs below 20 kg and quite a number of minipigs.
Structure and material
Reinforced glass in combination with bars and laminate panels in the colour of your choice offer a pleasant, light and open environment. This design improves the visibility and reduces the overall noise level, as the animals are calmer when they are able to observe the environment visually.

Ventilation
Good ventilation is essential to keep the facility dry and comfortable (no smell) and to avoid bacterial growth. To that end, supply ventilation should be located in the centre of the corridor and an exhaust should be positioned in the ceiling inside the pens. To avoid short cutting of air, the top part of the pen is made of solid glass panels to force the air down. The lower part of the pen is an open structure formed by stainless steel bars ensuring that the air is directed into the pen at low level.

To save energy and achieve the best possible climatic conditions, the ventilation and temperature may be CO₂ controlled, as is the case at the Ellegaard Göttingen Minipigs production facility. Air samples are taken continuously; the samples are analysed and depending on the result the air change rate is adjusted and / or the air is cooled / heated.

Low ventilation results in:
- Poor air quality (smell)
- High humidity (condensation on walls and windows)
- High concentration of disease producing bacteria and virus
- Non or low requirement of adding of heat

High ventilation results in:
- Good air quality (no smell)
- Dry air (could be too dry)
- Low concentration of disease producing bacteria and virus
- High requirement to adding of heat

It is especially important that the air in the resting / sleeping area is draft free and the air speed in this area is below 0.3 m/sec.

Hatch
A sliding hatch allows you to open up between pens increasing the floor area as required depending on whether you are housing minipigs, dogs or larger groups of either. The hatch is made of bars allowing physical contact between animals in adjacent pens. A transparent panel can be slid in place to prevent physical contact in an experimental situation though still allowing visual contact. The hatch should be positioned at the front of the pen; the front of the pen is defined as the active area closest to the corridor, where feeding and watering will be positioned. This is also where the “toilet” area will be located, whereas the rear end of the pen is for resting and privacy.

The hatches can be placed at different levels to vary the environment, though for pigs only the lower one will be used. The hatch is activated from outside the pen through an articulated arm, minimising animal disruption and stress.

Platform / Shelter
Dogs use the platform/shelter for exercise and as an observation point. The platform provides a variation in the environment. Minipigs need a dry, draft-free, warm and private sleeping and resting area, which can be provided by a shelter/platform. Therefore, the platform/shelter should be designed to meet these two different requirements. In a dog pen several platforms may be fitted in the pen at different heights to provide variation, exercise possibilities and observation points.
In a minipig pen at least one of the platforms should be placed at a height and location suitable to serve as a shelter. If the pen is deeper than it is wide, the rear of the pen lends itself well to a shelter and provides the minipig with a safe and sheltered area. To avoid draft, it is important that there are no gaps between floor and pen walls in this area. Also the floor should be well insulated and if possible bedding should be provided. An easy drop-in bedding retainer panel maintains the bedding in place and provides a safe and warm area for the pig. The internal height under the platform should be 35 cm and with the bedding retainer panel in place, the opening into the shelter is reduced to 25 cm.

**Temperature**

Pigs are highly sensitive to environmental temperature and place a high behavioural priority on thermoregulation. Within the ranges given, suitable temperatures will vary according to body weight, sexual maturity, the presence or absence of bedding, group housing and the caloric intake of the animal. If the minipig is provided with a draft-free shelter with bedding, and / or is group housed, and the room temperature is maintained at 19 – 23 degrees Celsius, extra heating will normally not be required.

Please note that the EU guidelines (table G.3. below) include both farm pigs and minipigs. The growth rate of farm pigs and minipigs are very different. Consequently the age rather than the body weight should be used as reference for room temperature. The temperature should be measured at floor level. At weaning a minipig weighs 2+ kg whereas a farm pig weighs 7+ kg! The temperatures in table 1 below are the temperatures recommended by Ellegaard Göttingen Minipigs A/S. However most importantly watch the animals, if they are piling together, it is too cold. If they are lying far apart or soiling themselves, it is too warm. As a general rule, if bedding is available the room temperature can be lowered a couple of degrees.

For young piglets it may be necessary to increase the temperature under the shelter. This may be done by floor heating or by automatic temperature controlled platforms/shelters.

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn piglets</td>
<td>0.4 – 0.5 kg</td>
<td>34</td>
</tr>
<tr>
<td>4 weeks *)</td>
<td>2 – 3 kg</td>
<td>28</td>
</tr>
<tr>
<td>6 weeks</td>
<td>3 – 5 kg</td>
<td>26</td>
</tr>
<tr>
<td>3 months</td>
<td>6 – 7 kg</td>
<td>22-24</td>
</tr>
<tr>
<td>6 months</td>
<td>13 – 14 kg</td>
<td>20-22</td>
</tr>
<tr>
<td>More than 6 months</td>
<td>&gt;13 kg</td>
<td>18-20</td>
</tr>
</tbody>
</table>

Source: Ellegaard Göttingen Minipigs A/S
*) Local temperature under the shelter, should be gradually lowered until weaning at 4 weeks.

<table>
<thead>
<tr>
<th>Liveweight</th>
<th>Recommended temperature range °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 3 kg</td>
<td>30 to 36</td>
</tr>
<tr>
<td>from 3 to 8 kg</td>
<td>26 to 30</td>
</tr>
<tr>
<td>over 8 to 30 kg</td>
<td>22 to 26</td>
</tr>
<tr>
<td>over 30 to 100 kg</td>
<td>18 to 22</td>
</tr>
<tr>
<td>over 100 kg</td>
<td>15 to 20</td>
</tr>
</tbody>
</table>

Source: Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123)
Minipigs are particularly sensitive to water deprivation. Therefore group-housed pigs should have access to at least two drinking points. Two types of drinking valves are currently used in pens:

We have seen both chewing valves and licking valves used for both minipigs and dogs. Minipigs like to chew on chains and bars and providing chewing valves for the minipigs would be an additional enrichment.

To keep the pen dry and clean the drinking valve should be placed right outside the pens and not at the centre of the corridor, to keep the corridor and pens dry at all times. In some facilities mainly production facilities slatted floors are used for ease of cleaning. In research facilities it is normally not possible to have a slatted floor.

Solid flooring is often preferred as it facilitates observation of faeces. Minipigs quickly learn where to defecate and if the floor is slatted they will press the faeces through the slats with their hooves. This will not be the case if you are using the pens for dogs.

If you are considering slatted flooring, the slats should be 10 – 20 mm and the openings between the slats should be 10 – 11 mm. Housing of pigs younger than 4 weeks require smaller openings, which can be achieved by covering the slats with a rubber mat with smaller openings. The height of the drinking valve needs to be adjustable 10 – 50 cm; always located at the upper shoulder height of the pig. This way the pig will have to lift its head to drink and the water will run directly into its mouth instead of out of its mouth. Also the size of the opening through which the pig is drinking should be adjustable to accommodate the snout of the pig’s head.

The water valve must provide 1 – 2 litres of water per minute at a water pressure of 1 – 2 bar.

### TABLE G.6. Pigs and minipigs: Minimum drinking point allowances

<table>
<thead>
<tr>
<th>Drinker type</th>
<th>No. of pigs per drinking point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nipple or bite drinkers</td>
<td>10</td>
</tr>
<tr>
<td>Large bowl drinkers (which allow at least two pigs to drink at the same time)</td>
<td>20</td>
</tr>
</tbody>
</table>

### TABLE G.7. Pigs and minipigs: Minimum drinking water flow rates for pigs

<table>
<thead>
<tr>
<th>Type of pig</th>
<th>Minimum water flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaners</td>
<td>500</td>
</tr>
<tr>
<td>Growers</td>
<td>700</td>
</tr>
<tr>
<td>Dry sows and boars</td>
<td>1000</td>
</tr>
<tr>
<td>Lactating sows</td>
<td>1500</td>
</tr>
</tbody>
</table>

Source: Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123)
Cleaning
It is important to keep the pen as clean and dry as possible to minimise bacterial growth. The mini pigs will normally use the “wet” area as the toilet. The pigs are clean animals and they will find a place which they will use as a toilet, normally where there is a bit of water, for example by the water valve. A good trick is to wet with water the area that you want the pigs to use as a toilet - before introducing the pigs into the pen.
To reduce the consumption of water and to maintain a dry, healthy environment and to minimise bacterial growth, it is worthwhile considering to spot clean daily and only deep clean weekly. A foam cleaning system like the Dosatron system lends itself well for this purpose. Apply the foam on the surface, leave it to work for five minutes, rinse with water and clean off with a squeegee. The foam loosens the dirt and as a result the amount of water required is reduced and the relative humidity is maintained at a reasonable level. High pressure cleaning requires a large amount of water and leaves you with a high relative humidity.

Flooring
The floor should be sloping 3% towards the pen front / corridor. It should be made of non-slip material e.g. epoxy with sand. This type of flooring also allows for natural wear of the hooves and offers a good non-slip play area for dogs.

Feeding
The food bowl should be height adjustable to ensure that also small mini pigs can eat from it. It should be firmly secured to ensure that it does not come off when the pigs/dogs eat and play. For minipigs the bowl should be adjustable from 7 – 15 cm from floor to the edge of the bowl.

Enrichment
Group housing whenever possible as both minipigs and dogs are social animals. For dogs, platforms should be placed at different heights to provide good exercise possibilities and excellent observation points. For minipigs the provision of straw and hay will be welcomed. The straw is excellent as bedding material and it keeps the animals occupied with rooting and chewing activities. If straw cannot be used for experimental reasons, a stainless steel chain is a good alternative. There should be one chain per pig and they should be hanging 5 – 10 cm from floor level. A plastic chew stick is also a good supplement. Plastic balls with holes (ferret balls) can also be used as enrichment. Make sure the holes are not too big; the pig should not be able to get its head through, only its snout.

General pen arrangement
Special requirements
This article does not cover special requirements for housing off newly born piglets and juvenile studies.

Acknowledgement
Arrowmight has been a leader in the design, fabrication and installation of large-scale bespoke animal housing facilities for over 25 years. We would like to thank Ellegaard Göttingen Minipigs A/S for inviting us to write this article and for sharing their knowledge and experience on minipigs with us. Together our knowledge and understanding of these animals will contribute to the ongoing development of our minipig / dog pens, ensuring that we continue to provide the best environment for the animals’ welfare.

The hatch can be formed from either solid panels, stainless steel bars or glass for no contact, physical contact or visual contact only as required by the experimental situation.

To keep the rear of the pen dry and clean, drinking valves and feeding bowls are placed at the rear of the pen.

Sliding hatch for social interaction between pens

Adjustable food bowl. Lockable in closed position
The platform is placed at the rear of the pen and it provides a secure and warm resting / sleeping area for the minipig. At the same time it serves as a raised area and observation point for the dog. A low panel is fitted to retain bedding under the platform.

Hinged platform for ease of cleaning and removal if required

Adjustable drinking valve to accommodate pigs and dogs at different age
The Göttingen minipig is a widely used large animal model in preclinical dermatological drug safety studies. Using standard restraint techniques (slings, etc.), applying topical compounds to the backs of minipigs can be challenging because minipigs are often fractious. This can lead to injuries to animals or staff, as well as inaccuracies in dosing and even contamination.

Operant conditioning behaviour analysis (“clicker training”) is used with many species for general care and research, and has been used with pigs in discrimination research, animal welfare research, cognitive science, and animal science and husbandry. At Pfizer Global Research and Development - Drug Safety Laboratories and Comparative Medicine in Ann Arbor, Michigan, USA, the goal was to use this training method to train minipigs to voluntarily cooperate with husbandry and experimental procedures, primarily for dermal toxicology studies. By pairing the sound of a click with food, animals learned that they would be rewarded for performing specific behaviors.

Each animal’s food rations for the day were placed in a small plastic bucket attached to its kennel (Figure 1); each “click” earned the animal a few food pellets. At the end of the day, any remaining food was fed out to the pig, ensuring that the animals received only their normally allotted rations. Enrichment foods (bananas, etc.) were given to the minipigs at the end of the training session as a "jackpot".

We utilized the “protected contact” model of physically handling the animals as little as possible; this was advantageous for handler safety and non-contamination during experimental procedures. For example, minipigs received their food pellets from a small frying pan rather than from the trainer’s hand.

Animals were trained to touch and then follow a target stick (large plastic spoon) (Figure 2). When comfortable with this exercise, minipigs were then trained to walk into the bottom half of a standard plastic shipping crate, equipped with a piece of matting on the bottom for improved traction. A target (plastic disk with a hole drilled through it) was installed at the end of the crate, along with a ladle (Figure 3). The animals easily generalized from touching the target stick to touching the installed target and receiving the pellets in the ladle rather than the frying pan, freeing the technicians’ hands for study procedures. All study procedures could be done in the animal’s home pen, further reducing chances of contamination, as crates, target sticks, etc., could all go through cagewash. Minipigs could also be moved out of the home pen by following a target stick into a shipping crate on a scale (Figure 4) for body weight determination, or onto a cart for transport within the institution.

Table 1 shows the shaping plans used for to train the desired behaviours. The trainer graded each animal’s proficiency/fluency for each step of each behavior and the results were documented. Progression to the next step or behavior was dependent upon the animal’s competence. Training lasted approximately ten minutes per business day.
per pig over a 2.5-week period. An approximate training schedule is shown in Table 2. Using this protocol, behaviors were strong enough to maintain excellent behavior through a thirty-day study period. Some animals kept in house for up to 6 months retained the behaviors even without regular maintenance training. Age made little difference in the trainability of the minipigs. When designing training plans for novel behaviors, extra time will be required to develop appropriate and efficient shaping plans.

Setting up the bargain (Click = Treat) with the animal is generally called “charging the clicker”. The minipig indicates understanding of the “bargain” by looking at the trainer for the click. Every time the trainer clicks, the animal must receive a treat, even if the click was made by mistake. As training progresses, the click itself can be given on a variable schedule for a particular behaviour, requiring the animal to do several repetitions of the behaviour to earn the click, or the timing can be delayed to shape the behaviour for increased duration.

Head turns are an essential but often overlooked tool in “clicker training”. As soon as the animal understands that Click = Treat, teaching headturns shows the minipig that it can modify its behaviour and physically do something to earn the reward. It also serves to improve the trainer’s technique and timing. When the animal looks to one side (arbitrary, but consistent within a training session), even just a glance, it is rewarded with a click. The motivation for the movement is not important. When the animal is consistently rewarded for glancing or moving its head toward the chosen side, it quickly learns how to “make” the trainer click. Head turns are also useful as a “backup” behaviour: if an animal is frustrated with an exercise, it will often start to offer head turns instead of jumping on, or biting the trainer. No commands are necessary at this level of training – the position of the trainer and equipment act as cues.

Integral to the success of this program was a core group of technicians using well defined protocols. Five technicians who expressed special interest received advanced training in operant conditioning behaviour analysis, and helped train and supervise the numerous other technicians involved either pre- or on-study. One of these core trainers was study monitor for each minipig study. Although some were initially dubious, staff bought in to the program once they saw how easy (and fun) it was, and the major difference in overall minipig behaviour.

Defined shaping plans were used to train each behaviour, and records were kept for every training session for each animal. We tracked the amount of time spent on each behaviour and step of the training, monitored the progress of individual animals, and made changes to protocols as necessary. This also gave us the metrics necessary to demonstrate the progress and success of the programme to senior staff.

When beginning a training session, the technician refers to the animal’s most recent training logs to decide what behaviour and step of the shaping plan to begin with. Training logs were made as simple as possible: the behaviour and step in the shaping plan, number of minutes and trials of each step, and animal’s proficiency at that step could all be either circled from a list or written as an abbreviation, followed by a short comment as necessary.
**Table 1:** Shaping plans for each of the desired behaviours. Each training session is documented; a new step is not attempted until the animal is confident with the previous step.

<table>
<thead>
<tr>
<th>“Charge Clicker”</th>
<th>Click and put pellets in pan (Click and treat; C&amp;T); repeat Continue until animal is confident and looks for the food after the click Move pan around to acclimate animal to being in different areas relative to the trainer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Turns</td>
<td>• C&amp;T when the animal looks to the left or the right (pick one) • Initial motivation for the head turn is irrelevant • C&amp;T for a larger head turn • C&amp;T for a longer head turn</td>
</tr>
<tr>
<td>Target Stick</td>
<td>• C&amp;T for interest in target stick (plastic spoon) • C&amp;T for closer proximity to the target stick • C&amp;T for touching target stick on flat end • C&amp;T for touching target stick longer</td>
</tr>
<tr>
<td>Crate Training</td>
<td>• Use target stick to lure minipig into crate, feed from the pan • Advance into crate at ~1 foot intervals. Do not advance until minipig is confident at each step (1 foot in crate, 2 feet in crate, etc.) • As minipig approaches the end of the crate, place the food into the installed ladle rather than the pan • Guide the minipig out of the crate with target stick (back up or turn around, depending on the size of the animal) • Feed from the pan in the pen outside of the crate</td>
</tr>
<tr>
<td>Fixed Target in Crate</td>
<td>• When minipig is confident standing in the crate touching target stick, place the target stick over the installed target • C&amp;T for touching the installed target instead of the target stick • Crate Transfer • Set up the crate outside the pen (on cart, scale, etc.) • Use target stick to guide minipig into crate, C&amp;T for touching stick • C&amp;T for touching stationary target • Guide out of crate with target stick • C&amp;T when back in pen</td>
</tr>
<tr>
<td>Dermal Dose Training (use two trainers – one to dose, one to C&amp;T)</td>
<td>• C&amp;T for touching the target during the following: • Start with hand resting on crate • Move the hand into air over the animal • Put hand over animals back • Let hand rest on animals back • Gently stroke animals back • Rub animals back • Acclimate animal to dosing rod as with hand • Acclimate animal to dosing procedure using water</td>
</tr>
</tbody>
</table>

**Table 2:** Example of a training schedule with goal training activities for each day. Some animals may progress more quickly than others; new behaviours should not be attempted until previous behaviours are fluent. Each day’s training session averages 10-15 minutes per pig. In order to facilitate technicians’ schedules, no training is done on weekends. New step is not attempted until the animal is confident with the previous step.

<table>
<thead>
<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training begins</td>
<td>• Head turns</td>
<td>• Head turns</td>
<td>• Target stick</td>
<td>No training</td>
<td>No training</td>
<td>No training</td>
</tr>
<tr>
<td>• Charge clicker</td>
<td>• Target stick</td>
<td>• Target stick</td>
<td>• C&amp;T for a larger head turn</td>
<td>• Target stick</td>
<td>• C&amp;T for a longer head turn</td>
<td></td>
</tr>
<tr>
<td>• Target stick</td>
<td>• Crate training</td>
<td>• Crate training</td>
<td>• C&amp;T for a larger head turn</td>
<td>• C&amp;T for a longer head turn</td>
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<tr>
<td>• Crate transfer</td>
<td>• Dermal dose training</td>
<td>• Dermal dose training</td>
<td>• C&amp;T for a larger head turn</td>
<td>• C&amp;T for a longer head turn</td>
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**Study Initiation** Study procedures include: clipping, dermal dosing, electrocardiograms, body weight collection, physical examinations, etc.
If a problem or question arose in a training session, the technician was advised to leave the pen, problem solve and revise the shaping plan appropriately before reengaging with the animal. Trainers were able to help each other identify timing or position issues. Experiencing how quickly the training sessions translated into observable behavioural changes was rewarding for the technicians, and a team atmosphere developed among the trainers.

The equipment used was simple and functional. The clicker (iClick models were preferred, as they can be touched from any angle) could be taped to the handle of the target stick or the pan for easy access. Feeding out of the pan reduced the need for the animal to move around the floor to ensure that all pieces were eaten, which both sped things up and kept the pig engaged in the activity. The ladle served the same function in the crate. The bucket containing food was hung from the pen wall at a place that was accessible to the trainer without being distracting to the pig.

To efficiently train an exercise, and reduce frustration for the trainer and the animal, it was helpful to physically practice the series of movements outside of the pen before beginning the exercise. Occasionally, having another trainer “act” as the pig would help with the timing or movement issues. For most activities, only one trainer was needed; however, for dermal dose training, two trainers were involved. One technician would click and treat, and the other would practice perform the dosing procedures (using water instead of compound). This could be theoretically done using one technician and an automatic feeder, our study protocols require two technicians to be present during dosing, which worked well for our training needs.

In the past, minipigs were brought into the facility four weeks prior to study initiation. A technician spent 15 minutes per animal per day in the pen with the minipig to acclimate it to the presence of humans and allowing touching. Rather than taking longer than the traditional procedures, operant conditioning protocols allowed an unanticipated time savings. Documenting and timing every training session allowed us to quantitate the actual time required to run this training programme. As detailed in Table 3, a 24 pig study saved an average of 72 technician hours during the pre-study period. Once on-study for 7 days, 28 technician hours were saved. Thus, for a 7 day, 24 minipig study, there was an average time savings of 100 technician hours. This did not include the time saved on physical examinations, electrocardiograms, body weight determinations, clipping, etc. The significant time savings frees technicians for other studies, and also allows a greater number minipig studies to be run each year.

Using operant conditioning to train minipigs in our facility resulted in significant reductions in a) injuries to animals and staff, b) stress levels of animals and staff, c) the number of staff required for study procedures, and d) the number of technician hours required compared to previous protocols. This training enriches the animals’ environment and their interactions with handlers. Technicians trained in the techniques of operant conditioning behaviour analysis gain a valuable skill set which can be used with any research species. Operant conditioning is a cost-effective way to provide a positive environment for animals and staff, and has ergonomic and economic benefits.

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**TABLE 3:** Documentation of every training session allowed us to quantitate the time required to run this training programme. Operant conditioning protocols resulted in significant and unanticipated time savings.

<table>
<thead>
<tr>
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<th>Traditional Acclimation</th>
<th>Operant Conditioning</th>
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<tbody>
<tr>
<td>Pig arrival time required pre-study</td>
<td>4 weeks (20 training days)</td>
<td>2.5 weeks (12 training days)</td>
</tr>
<tr>
<td>Total average acclimation/training time required (per pig)</td>
<td>5 hours (15 min / day)</td>
<td>2 hours (10 min / day)</td>
</tr>
<tr>
<td>Average preparation time for a 24 pig study</td>
<td>120 technician hours</td>
<td>48 technician hours</td>
</tr>
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<tr>
<th></th>
<th>Staff required per pig for dermal dosing</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>On Study</td>
<td>3 technicians</td>
<td>84 technician hours</td>
</tr>
<tr>
<td>7 day, 24 pig study, once daily, 10 min dosing</td>
<td>2 technicians</td>
<td>56 technician hours</td>
</tr>
<tr>
<td>Overall</td>
<td>204 technician hours</td>
<td>104 technician hours</td>
</tr>
</tbody>
</table>

*Time savings on one 7 day study* 100 technician hours

* Not including time savings on physical examinations, electrocardiograms, body weight determination, clipping, etc.
At LAB Research (Scantox), we have a long history of using the Göttingen Minipig in preclinical research. Various experimental procedures, such as dosing, weighing, physical examinations etc., are the parameters usually included in preclinical studies, and the majority of these procedures require minipigs to be restrained to complete the procedures successfully. However, this stresses the animals, which has the potential to impact the study results. In addition, it does not provide optimal working conditions for technical staff.

To eliminate the stress experienced by the animals, to increase the animals’ well-being and to optimise working conditions for our staff, we sought to find a method that would facilitate various study-related procedures. Positive Reinforcement Training (PRT), which uses a marker for behaviour that earns positive reinforcement, soon caught our interest. PRT has been used for many years in training pet and zoo animals, but has been used to only a limited extent in training laboratory animals.

We decided to implement this method in a repeat dose toxicity study with intranasal dosing several times daily. The first step was to educate a dedicated team of trainers to undertake all minipig training. The minipig training was performed using clickers as a marker for the desired behaviour and GLP-certified dietary pellets as rewards. The principle of “shaping” was used in the training sessions, gradually transforming specific behaviour into desired behaviour. First, the sound of the clicker was associated with the reward, followed by the acceptance of approximation of the trainer to the animal. This was followed by further successive training steps until the complete desired behaviour was achieved. The fully trained animal voluntarily came forward, stepped onto a box (to elevate the animal) and accepted approximation of the intranasal device and the subsequent dosing in one nostril in a “freeze” position.

All animals learned the complete behaviour sequence prior to the start of the study, although differences in ease of learning were noticed among the animals. The study was successfully completed using PRT throughout the study, increasing animal welfare and working conditions significantly. Subsequent to this first study, we have successfully implemented PRT in connection with other study-related procedures, such as weighing procedures and dermal and subcutaneous dosing procedures. Our staff has adopted PRT with significant enthusiasm and they are continuously creative in finding new areas where it can be used. We therefore consider PRT to have great potential in relation to other experimental procedures used with the Göttingen Minipig.

**Using positive reinforcement in Göttingen minipigs**

Peter Glerup, Chief Veterinary Surgeon, LAB Research (Scantox), Denmark

Clicker Training at Ellegaard Göttingen Minipigs

Adrian Zeltner, Laboratory Technician

Clicker Training, Positive Reinforcement Training and Operant Conditioning are all terms which describe the same process: training an animal to intentionally perform behaviour that will bring about a desired consequence. In other words, getting a trained animal to actively participate in the daily routines for the benefit of everyone involved. Although this does not happen overnight, it is considered the fastest way to train an animal, and the method can be learned by anybody with a keen interest. All it takes – apart from a clicker, standard dietary pellets and perhaps a target stick – is commitment. Commitment not only from the staff for training but also from management for investing the time required to, firstly, educate trainers and, then, train the Minipigs.

As Peter Glerup and Melissa Cox has indicated in their articles on clicker training in this newsletter, the investment in clicker training of the minipigs will result in savings because fewer staff members and less technician hours are required. Clicker training makes the work with the minipigs easier and improves the welfare of both the minipigs and the staff.

At Ellegaard Göttingen Minipigs we have acquired expertise in training our Minipigs and are happy to share this with you. We also offer introductory courses here at our facility, perhaps in combination with one of our free handling and dosing courses. If you already possess Göttingen Minipigs, you have the option of holding a course at your facility.

Please contact us if you would like more information about how to go about training a Minipig or to make arrangements for an introductory course.
The minipig in PBPK modeling

PK-Sim® from Bayer Technology Services is a commercially available tool for physiologically-based pharmacokinetic (PBPK) modeling of drugs in laboratory animals and humans. The aim of PBPK modeling is to describe mathematically all physical and physiological processes that determine the uptake, distribution, and elimination of a compound in an organism in as much detail as possible. PBPK modeling can be used as a tool for an early in silico prediction of ADME properties of new compounds.

In short, PK-Sim® is a generic PBPK model with 17 organs and tissues. Represented organs/tissues include arterial and venous blood, adipose tissue (separable adipose, excluding yellow marrow), brain, bone (including yellow marrow), gonads, heart, kidneys, large intestine, liver, muscle, portal vein, pancreas, skin, small intestine, spleen and stomach. Each organ further consists of four sub-compartments namely the plasma, red blood cells (which together build the vascular space), interstitial space, and cellular space. PK-Sim® estimates model parameters (organ partition coefficients, permeabilities) from physico-chemical properties of compounds and from the composition of tissues in terms of lipids, water and protein. The physico-chemical properties needed as input information are lipophilicity, plasma protein binding constant or alternatively the fraction unbound in plasma, and molecular weight. In addition to physico-chemical inputs, compound specific clearance information (either in vivo plasma or blood clearances or intrinsic clearances determined from in vitro experiments) are required. As output information, PBPK modeling allows to predict the pharmacokinetic behavior of a compound on the basis of these parameters. Moreover it is a powerful tool for investigating the sensitivity of pharmacokinetics with respect to them.

In light of the increasing interest in the minipig as a favorable model for pharmacokinetic studies, a minipig module has been developed and implemented in PK-Sim®. Thus, besides the established human module and the animal models of the monkey, dog, rat, and mouse, PK-Sim® now allows the simulation of pharmacokinetics in the minipig. To evaluate how suitable the new module is for the prediction of pharmacokinetics in the minipig, a PBPK model of the nonsteroidal anti-inflammatory drug diclofenac administered intravenously and orally was first set-up for humans and then extrapolated to the minipig.

Detailed information on methods and the results of the diclofenac study is given in the below-mentioned poster presented at the 2nd Pharmaceutical Sciences Fair and Exhibition in Nice, France (June 8-12th 2009).

For further information on the PBPK software, PK-Sim®, please visit the webpage www.systems-biology.com/products/pk-sim.html or contact info@systems-biology.com.

REFERENCES

S. Willmann, J. Lippert, and W. Schmitt. From physicochemistry to absorption and distribution: predictive mechanistic modelling and computational tools. Expert Opinion on Drug Metabolism and Toxicology. 1. 159-168. 2005
The miniPIG in PBPK modeling

K. Thelen1,2, J.B. Dressman1, M. Sevestre3, J. Solodenco3, S. Willmann2, J. Lippert2

(1) Institute of Pharmaceutical Technology, Goethe University, Frankfurt am Main, Germany
(2) Competence Center Systems Biology, Bayer Technology Services GmbH, Leverkusen, Germany
(3) Competence Center Computational Solutions, Bayer Technology Services GmbH, Leverkusen, Germany

Introduction
• Due to similarities between man and pig with regard to physiology and biotransformation, pigs are valuable non-rodent research models.
• Among pigs, miniature breeds were developed for the special demands of preclinical experiments.
• The miniPIG can be used successfully for intravenous (i.v.), oral, and dermal studies.

Purpose
• Development of a miniPIG module and implementation into the commercial physiologically-based pharmacokinetic (PBPK) software tool PK-Sim®

Methods
1. Implementation of physiological information into the model database of PK-Sim®
2. Evaluation of the new module: Extrapolation of diclofenac pharmacokinetics (PK) from human to the miniPIG

Results and Discussion
• Using the identical model previously established in humans the plasma concentration-time profiles following i.v. administration of 25 and 50 mg diclofenac sodium to miniPIGs could be very well predicted (Fig. 3).
• Following oral administration of different solution types (50 mg diclofenac sodium in a solution of 50 mL phosphate buffer pH 7.4, 60 mL water or 200 mL water), the differences in diclofenac absorption were perfectly predicted by the software (Fig. 4).
• Obviously, in cases of a 50 mL unbuffered solution, the drug precipitates in the GI tract, as predicted by the software considering pH-dependent solubility. By contrast, both buffering and increased intake volume could increase the absorption rate of diclofenac, which could be better predicted assuming pH-independent solubility.

Input parameters and model parameterization
• Physico-chemical data of diclofenac was taken from the literature (Table 1)
• Species-specific data and application parameters were adjusted to values reported in each of the two studies used for comparison (Fig. 1)
• Distribution model: 4 subcompartments per organ
• Adjustment of the epithelial permeability between plasma and interstitium: 0.1 cm/s for all organs with the exception of the liver (10 cm/s) → the high protein binding of diclofenac (89.5%) is expected to restrict the passage of the compound from the plasma into the interstitium. The liver sinusoidal wall, however, contains open pores (fenestrations) which facilitate permeability into this organ
• Diclofenac plasma clearance was implemented according to the reported values (253 mL/h/kg in humans and 52 mL/h/kg in the miniPIG (3,4))
• For the oral study in humans, a lag time of 30 min and the in vitro dissolution profile of Veltaren® enteric-coated (EC) tablets at pH 7 was included in the model to account for the delay in diclofenac release from the orally administered EC tablets (5,6)
• Simulation of two different cases: 1. in consideration of charge-dependent distribution and solubility (----) and 2. without taking charge-dependent distribution and solubility into account (-----)
• The two cases show almost identical distribution behavior (Fig. 2) whereas there is a considerable difference in their predicted absorption kinetics (Fig. 2)

Conclusions
• The established human diclofenac model could be easily extrapolated to the miniPIG
• Differences in diclofenac absorption due to pH-dependent solubility in the GI tract were simulated well with the PBPK model
• The possibility of predicting PK in the miniPIG and/or extrapolating data from one species to another is a valuable tool for pre-clinical development, facilitating dose adjustments for species, enabling efficient blood sampling protocols and potentially resulting in a reduction of the number of animals/subjects required for the study

References

Bayer Technology Services
MINIPIG RESEARCH FORUM

For the first time ever, the Minipig Research Forum held meetings in both Europe and North America with a total of 104 attendees. The meetings offered minipig users the opportunity to learn from one another and to share knowledge, ideas and experiences – which is also the underlying idea of the MRF.

European 2009 MRF meeting
The European meeting was held on 28–29 October 2009 in Cannes. The surroundings were beautiful and the Hotel Gray D’Albion was an excellent venue for the meeting.
51 people took part, helping to make it a fantastic event. The scientific programme included presentations on anaesthesia; surgery; prevention of pain, distress and suffering; sampling techniques; non-traditional dosing routes; telemetry; the cardiovascular system; clinical pathology; and case studies. The presentations were very interesting and showed a common focus on developing the scientific use of the minipigs and ensuring and improving minipig welfare.

North American 2009 MRF meeting
The North American MRF meeting was held at the National Conference Center in Virginia.
The meeting had 53 attendees and the scientific programme included the following interesting presentations:

- Conceptual history of the minipig
- The minipig as a model for chronic heart failure
- ECGs in minipigs in the preclinical safety evaluation process and its comparison to other commonly used species
- Göttingen minipig skin in organ culture and monolayer cell culture: Relevance to drug safety testing
- Minipig as a model in dermal toxicity testing
- Accelerating insulin pharmacokinetics using recombinant human hyaluronidase (rHuPH20) in a Yucatan minipig model
- The minipig as a model for juvenile toxicity testing
- Use of minipigs for FDA-regulated products
- Basic husbandry and housing of the minipig
- Cooperating in their own care: The use of operant conditioning to train minipigs
- Principles of anaesthesia and pain management in minipigs
- Surgery and telemetry implantation in minipigs
- Continuous intravenous infusion in Göttingen minipigs

2010 MRF meetings
The next North American MRF meeting will be held on 25–26 June 2010 in Chicago, Illinois.
The next European MRF meeting will be held on 29–30 November 2010 in Cannes, France.

You can register for the MRF and MRF meetings on the website www.minipigresearchforum.org.


We offer various types of equipment that can make your work with minipigs easier. You are welcome to contact us if you need any auxiliary equipment, and if you would like a demonstration of the equipment, you can sign up for one of our Handling and dosing courses.
COURSES

At Ellegaard Göttingen Minipigs, we offer free training courses in handling and dosing at our breeding facility in Dalmose, Denmark or at your own facility. The courses include theory and practice, and the content of the courses can be adjusted to the needs and interests of the participants. The handling and dosing courses are organised by Adrian Zeltner, our experienced Laboratory Technician, and you are welcome to contact us if you are interested in learning more about the courses.

Ellegaard Göttingen Minipigs also offers regular courses in surgery and anaesthesia. We have a good working relationship with Mr Tony Webb who provides consultancy services associated with experimental surgery involving Göttingen Minipigs. You can register for one of the following planned courses or contact us if you are interested in further information on the courses. Additional courses can be arranged, and the courses are tailored to the specific needs of the participants.

### Surgery and anaesthesia courses scheduled for 2010

2–3 June
A course will be scheduled this autumn. (www.minipigs.com)

For further information, please contact Ellegaard Göttingen Minipigs
Email: ellegaard@minipigs.dk
Tel.: +45 58 18 58 18
Fax: +45 58 18 58 80

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MEETING CALENDAR

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<th>PLACE</th>
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<tr>
<td>SOT and ToxExpo</td>
<td>8-10 March</td>
<td>Salt Lake City, Utah</td>
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<tr>
<td>IAT</td>
<td>16-18 March</td>
<td>UK</td>
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<tr>
<td>BTS</td>
<td>29 March</td>
<td>Edinburgh, UK</td>
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<tr>
<td>FELASA / Scand-LAS</td>
<td>14-16 June</td>
<td>Helsinki, Finland</td>
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<td>MRF North America</td>
<td>25-26 June</td>
<td>Chicago, Illinois</td>
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<tr>
<td>IUTOX</td>
<td>19-22 July</td>
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<td>ETS</td>
<td>5-8 September</td>
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<td>SPS</td>
<td>20-22 September</td>
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<td>26-28 September</td>
<td>Toulouse, France</td>
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<td>GV-SOLAS IGtP</td>
<td>30 September-1 October</td>
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<td>Nice, France</td>
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<td>MRF Europe</td>
<td>29-30 November</td>
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