

Minipigs in Toxicological Research at Janssen: Practical Aspects

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Introduction

In recent years, minipigs have become more popular as an alternative non-rodent species for toxicity testing and are considered an acceptable alternative when the dog is not a suitable species. At Janssen the first toxicity study using minipigs was performed in 2006. Important considerations when performing toxicity testing in minipigs include the extent of experience with minipigs in toxicity studies, including background data and the level of training of animal caretakers and technicians. Improper handling can stress the animals, which can have consequences for the study. Stress can be minimized in animals that are well-socialized and acclimated to research procedures. The scope of this paper is to give a brief overview of the practical modifications that were implemented to adequately perform toxicity studies in the minipig.

Type of study

Over the past four years, studies using minipigs have been performed for several compounds at Drug Safety Sciences of Janssen, the majority of which were local tolerance studies (IM

dosing, GLP and non-GLP studies). The studies were carried out using minipigs because dogs are too sensitive. Due to the many similarities between human skin and minipig skin, these types of studies using minipigs can better predict the adverse effects in humans than local tolerance studies using dogs. In addition, a number of oral gavage tolerance studies were performed using both minipigs and dogs but due to the dogs' excessive vomiting and a better TK profile for minipigs, minipigs were chosen as the non-rodent species for the First in human package including a one-month GLP study.

Practical aspects

Before the first studies were conducted in our department, the animal technicians were extensively trained by Ellegaard of Denmark. In spite of this training, some difficulties were experienced during the studies, mainly in procedures related to animal handling, housing and feeding. Recently, an additional training course was taken at Ellegaard during which it became clear that a different approach had to be taken for handling minipigs than





for handling dogs. The training focused more on understanding minipig behaviour and how to apply this to the different procedures and techniques. After the visit, several changes were made to the housing procedures (ranking, cage enrichment, environmental conditions, feeding procedure) and the handling of the animals including experimental procedures (approaching and picking them up, putting them in a sling, blood sampling and dosing). Furthermore, theoretical and practical training was foreseen for the biotechnicians, TK biotechnicians and other employees in contact with the animals.

1.1 Housing and feeding

Minipigs at our facility are housed in dog pens which provide enough floor area and resting space. The walls of the cages are composed of Trespa® plates and glass and the floors are solid and covered with Epoxie®. In the first studies performed at our facility, minipigs were housed individually but, as they are social creatures, group-housing is preferable. In order to group-house the animals without hierarchy problems or aggression, the animals are ranked on the day of arrival based on their body weight and sibling relations. In addition, the housing conditions at the supplier Ellegaard are also taken into consideration. In recent studies, male minipigs aged 3 to 8 months on arrival were group-housed without significant problems. Some fighting was observed among older animals in the first days after arrival until the hierarchy was established. In general, however, no serious problems with aggression in male minipigs have occurred.

Minipigs are diurnal animals, meaning that the provision of 12h of light (200 lux) and 12h of dark complies with their activity pattern. The room temperature is age dependent. For animals aged 3 to 6 months, temperatures should range from 21 to 25°C, whereas for animals older than 6 months, temperatures between 19 and 23°C are suitable. Although light intensity and room temperature can easily be modified at our facility, the temperature was not originally modified for minipig studies and ranged from 19 to 23°C. This was adjusted, however, following the observation of occasional shivering, which could be confused with tremors. An overview of the environmental conditions is given in Table 1.

Table 1: Environmental conditions

	Previous practice	Current practice
Light intensity	300 lux	200 lux
Room temperature	All ages: 19–23°C	Age 3–6 months: 21–25°C Age >6 months: 19–23°C
Relative humidity	40–70%	40–70%

Minipigs should be offered environmental enrichment in line with their natural behaviour. While 70–80% of their time is spent sleeping or lying down, the remainder is spent rooting, chewing and exploring. They are provided with bedding material and different toys for rooting and chewing which are replaced daily to maintain their interest. In addition, a stainless steel chain is affixed to their cage to allow additional chewing behaviour.

Minipigs at our facility are fed “Ssniff MPig”, and they receive fixed amounts primarily according to their age and sex and secondarily

according to their body weight. Female minipigs receive less than males and animals younger than 6 months are fed twice a day. An overview of the feeding regimen is presented in Table 2. In the first studies conducted at Janssen, the minipigs were fed according to their weight, similar to normal practice in dog studies. However, minipigs tend to eat everything they are given resulting in heavy animals gaining more weight than lighter animals and exacerbating the disparity. Females were routinely fed the same amount as males resulting in a marked weight difference between males and the much heavier females particularly in long-term studies.

Table 2: Feeding Practice

Age (months)	Weight (kg)	Males (g)	Females (g)
2–4	5–8.9	225*	200*
4–6	9–12.9	270*	240*
6–8	13–16.9	360	310
8–10	17–20.9	405	360
10–12	21–24.9	450	360
>12	>25	500 or more	410 or more

*Total amount of food/day, fed in 2 portions

Practically, the protocol details the date from which minipigs are fed an amount of food based on their age. In consultation with the study director, the amount of food of a certain animal can be modified if the age and weight categories no longer correspond.

1.2 Socialization, handling and experimental procedures

The following paragraph describes how procedures are currently performed and their practicalities:

Acclimatization period

The purpose of the acclimatization period is to allow animals to socialize and gradually become accustomed to the experimental conditions to minimize stress, not only for the animals but also for the staff. It is deemed important to create a pleasant, undisturbed atmosphere for the good conduct of the study. This period is somewhat labour intensive but can ultimately save time during the study. The socialization currently consists of touching, hand feeding and picking up the animals and training them to lie in a sling for experimental procedures. Socialization lasting approximately 3 hours per day for 3 weeks is required for a one-month GLP study. In the initial studies, less time was spent on training which resulted in more vocalization from and struggling with the animals.

Approaching and picking up

In the initial studies, minipigs were picked up in their cage in the same way one would pick up a dog, which resulted in a lot of vocalization from them, signifying stress. When approaching a minipig, it is important to be aware that you are dealing with a prey animal. First, a hand is offered for the animal to sniff and then food is thrown on the floor between the minipig and handler to encourage the animal to come closer. To pick them up, they are allowed to come out of their cage, a hind leg is grasped with one hand and the other hand is placed under the thorax and the pig is lifted. It is important to hold the animal loosely and to always pick them up in the same way.



Dosing

As oral dosing is a stressful procedure for the minipig, training is not considered beneficial. Ellegaard has designed a special chair on which the biotechnician sits with the minipig during the oral gavage procedure. This procedure is more labour intensive than in dogs but a level of acceptance can be achieved and the procedure takes less time and effort during a study.

Blood collection via the jugular vein

In the initial studies, blood was taken while the minipig was held steady on a biotechnician's lap with the help of one or two other biotechnicians to take the sample. This procedure caused vocalization, struggling and stress. The current practice for young minipigs is to roll the animal onto its back into a V-trough.¹ For older and heavier animals, blood can be collected using a sling with an opening in the sling to access the blood sampling site. This procedure does not require more time than in dogs, although an extra person may be needed to hold the minipig, particularly in younger animals.



Eye examination

Initially, animals were held in the arms to perform the eye examination although this has been modified so that now minipigs are placed in a sling. Before the start of the study, animals need to be trained for the sling, as detailed above. Putting eye drops into the eye and the eye examination itself are slightly more time consuming than in dogs because it is more difficult to correctly position the head and examine the eyes, as the pig's eyes are more deeply set and have long eyelashes.

ECG

Other than the time required to train the animals for the sling during the pre-study period, this procedure does not require more time or personnel than in a dog study. Standard ECG leads are placed similar to those in the dog (I, II, III, aVR, aVL and aVF). For interpreting the ECG signal, the spontaneous changing of the T-wave's polarity and a longer QT interval (than for the dog) are taken into account. The postprandial heart rate increase is surpassed by not feeding the animals during the 4 hours prior to ECG recordings.

Clinical Pathology

Haematology and clinical chemistry analysis were validated in 2007. As regards the haematological analysis, blood-sample re-analysis is required more frequently than when working with dog samples. This is probably related to the occasional difficulty of blood sampling in some minipigs, sometimes resulting in a low-quality sample (e.g. a clothed sample).

¹ Device designed to roll the minipig onto its back and restrain it to collect blood.

Necropsy and pathology

Before necropsy, fasting minipigs are weighed and anaesthetised by intramuscular injection with a mixture of 1 flacon of Zoletil® 100 (250 mg tiletamine + 250 mg zolazepam, dry substance only), 12.5 ml xylazine (20 mg/ml), 2.5 ml Ketamine (100 mg/ml) and 5 ml butorphanol (10mg/ml) at an appropriate dosage volume of 0.1 ml/kg body weight. Stress should be avoided as much as possible especially to avoid malignant hyperthermia and muscle degeneration. Dogs are administered with IV heparin to prevent coagulation, but as it is more difficult to give IV injections to minipigs and they are very sensitive to stress, no anti-coagulant is given. The deeply anaesthetized minipig exsanguinates through the front or hind leg veins and arteries. Cervical veins and arteries are not cut because this further complicates the preparation of cervical tissues (thymus, thyroids, salivary glands). To accelerate exsanguination, a pulley is installed from which to vertically suspend the pig.

At our facility, necropsies of non-rodents (dogs, minipigs) are organized so that one animal is dissected by several people in "a chain": the animal is passed from one technician to the next. When minipigs are to be necropsied, additional assistance is required as minipig necropsy is more laborious. The anatomy of the minipig (heavier, larger, more compact than dogs complicates the tissue dissection, where the neck region and gastrointestinal tract are particularly challenging. In the neck region, the separate thymus lobes need to be taken and the thyroid glands are not easy to localize. The parathyroid glands are embedded in the thymus during necropsy and are separated from the thymus after fixation. Yet even after careful dissection, the parathyroid glands are not always found. Salivary glands are more compact. The gastrointestinal tract is enormous compared to the dog and additional samples (compared to the dog) are taken (e.g. 4 tissue samples of the stomach versus 2 in the dog). Male genital tissue, such as testes and seminal vesicles, are very large in mature minipigs and there is a clear need for additional training of technicians and pathologists to be consistent in taking representative samples.

Bone decalcification of the distal femur takes longer due to a thicker cortex. Continuous professional training is needed to discriminate between common background lesions in the minipig from drug-related histopathological lesions. Focal accumulation of mononuclear inflammatory cells (lymphocytes, macrophages and plasma cells) in various organs (generally in perivascular and interstitial locations) is the most common histopathological background lesion noted in the minipig. These mononuclear cell infiltrates can be seen in adrenal glands, brain, kidneys, liver, parotid gland, oesophagus, stomach, etc. The interlobular fibrous connective tissue in the minipig liver is another common example of a physiologic 'pig-specific' finding.

Apart from these anatomical variations, there are other specifics which pathologists and toxicologists should be aware of, including the sensitivity of pigs to acute heart failure due to stress and especially viral and bacterial infections. In rodents and dogs, these infections have been almost completely eradicated, but in minipigs these risks need to be kept in mind (Aujeszky's disease, Swine Fever, Salmonella, Haemolytic *E. coli*, etc.), since employees can potentially come in contact with farm pigs and be a source of infection for experimental pigs. Not only should the pathology of these diseases be known and understood, but also the implications for public regulations for legally contested illnesses and the possibility of zoonoses occurring during toxicity studies.

Conclusion

The additional training both at Ellegaard and at our facility organized by the biotechnicians of the minipig expertise team led to a change in the handling approach. In the light of the important fact that pigs are more sensitive to stress, this change has obviously been beneficial. This new approach, together with modified and new techniques, led to a more optimal environment for carrying out studies with significantly less vocalization from and struggling of the animals. This is not only beneficial for the animals but also for the biotechnicians working with them – and most importantly for the study results. In addition, good knowledge of background data and histopathological background lesions is very important for the interpretation study results.

Although techniques and handling procedures have been optimized, more personnel and/or more time are still required for performing certain phases of a minipig study as compared to a dog study, not only while the animals are alive but also during necropsy. The largest difference between the two species is the high workload during acclimatization. Training and socializing minipigs require more time because minipigs are animals of prey which makes them more wary and shy, thus requiring a greater effort to gain their trust. Once this is established, minipigs can be accustomed and trained to participate in a study.

Animal caretakers who usually work with dogs can still apply their basic animal interaction skills but they should be aware that dealing with minipigs requires a different approach altogether. As biotechnicians become more accustomed to working with minipigs, they will acquire more experience, and the training and handling of the animals will flow more smoothly and the workload should be alleviated.