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A CHANGING PARADIGM FOR NON-RODENT SPECIES IN NONCLINICAL SAFETY STUDIES

The miniature swine as an option in small and large molecule studies

The choice of species for nonclinical safety studies is an important step in the drug development process. The species to be used is, to a large extent, dependent on the test article type and clinical indication. Existing regulatory guidance documents, combined with careful consideration of the characteristics of the drug being developed, guided by scientific, ethical, and practical considerations, help inform this key decision.

The most common non-rodent species involved in nonclinical safety studies are nonhuman primates (NHPs) and dogs. There is, however, an increasing body of evidence that supports the use of miniature swine as a viable non-rodent option for nonclinical safety studies.

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INTRODUCTION

Drug development is a heavily regulated industry, and there are guidance documents that can help with deciding the species needed for nonclinical safety studies. The most commonly referenced guidance documents for nonclinical safety testing are ICH S6 (R1) for biologics and ICH M3 (R2) for small molecules.

For small molecules, two mammalian species are needed for the nonclinical safety studies, a rodent and a non-rodent, with the selection being primarily driven by metabolism. Historically, the dog has been the non-rodent species for most programs.

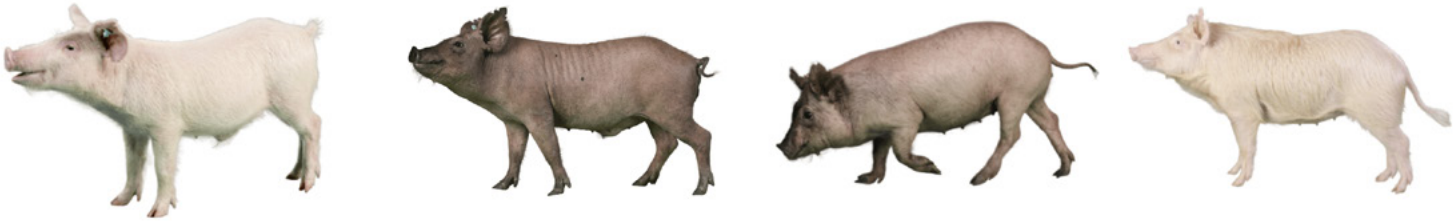
For biologics, the nonclinical safety studies should only be conducted with 'pharmacologically relevant' species, with the possibility of using a single species for certain programs. Nonclinical safety studies in non-relevant species are actively discouraged. The most commonly used non-rodent species in large molecule programs has been the NHP.

When metabolism or pharmacology do not make a clear distinction for a non-rodent species, other factors are considered to make the determination for the species to use. These factors include metabolic profile, pharmacokinetic (PK) profile, and plasma protein binding. Other considerations include data regarding potential tolerability issues, such as histamine release or emesis, adequate historical background data for different species and strains, and practical aspects, such as the route of administration or procedures that may be more practical in some species over others.

MINIATURE SWINE AS A VIABLE OPTION FOR NONCLINICAL SAFETY STUDIES

Initially used for medical research in Europe, miniature swine were introduced to the U.S. in the 1980s. Their use is now extending to many therapeutic areas due to an increasing body of knowledge supporting their appropriateness for nonclinical safety studies. The U.S. FDA Redbook recommends the use of miniature swine for different types of toxicity testing. Redbooks IV.C.3.b provides direction on short-term toxicity testing, while IV.C.4.b addresses subchronic toxicity studies. IV.C.5.b is on the topic of one-year toxicity studies. All list the miniature swine as a preferred species.

Miniature swine have been widely used in dermal and wound healing research because of the physiological similarities of miniature swine skin to human skin, including a relatively thick epidermis, distinct rete pegs, dermal papillae, and dense elastic fibers in the dermis. The use of miniature swine beyond the dermal field has been growing in recent years, for reasons we will explore.



Firstly, as mentioned, for small molecule programs, the primary driver for non-rodent selection is metabolism and absorption. The two main components for metabolism and absorption are cytochromes and transporters, and these have been found to have 70% similarity between miniature swine and humans. The GI tract has the most metabolic activity outside of the liver, and the physiology of the miniature swine GI tract is similar to human (pH of stomach, small, and large intestine; salivary amylase; rate of gastric emptying, and GI transit time).

Our understanding of the binding affinity of miniature swine Fc gamma receptors to human immunoglobulins is also increasing. Binding affinity becomes an integral part of the decision-making process when determining the non-rodent species that provides the most appropriate model for a mechanism of human-relevant toxicity associated with a particular molecule of interest.

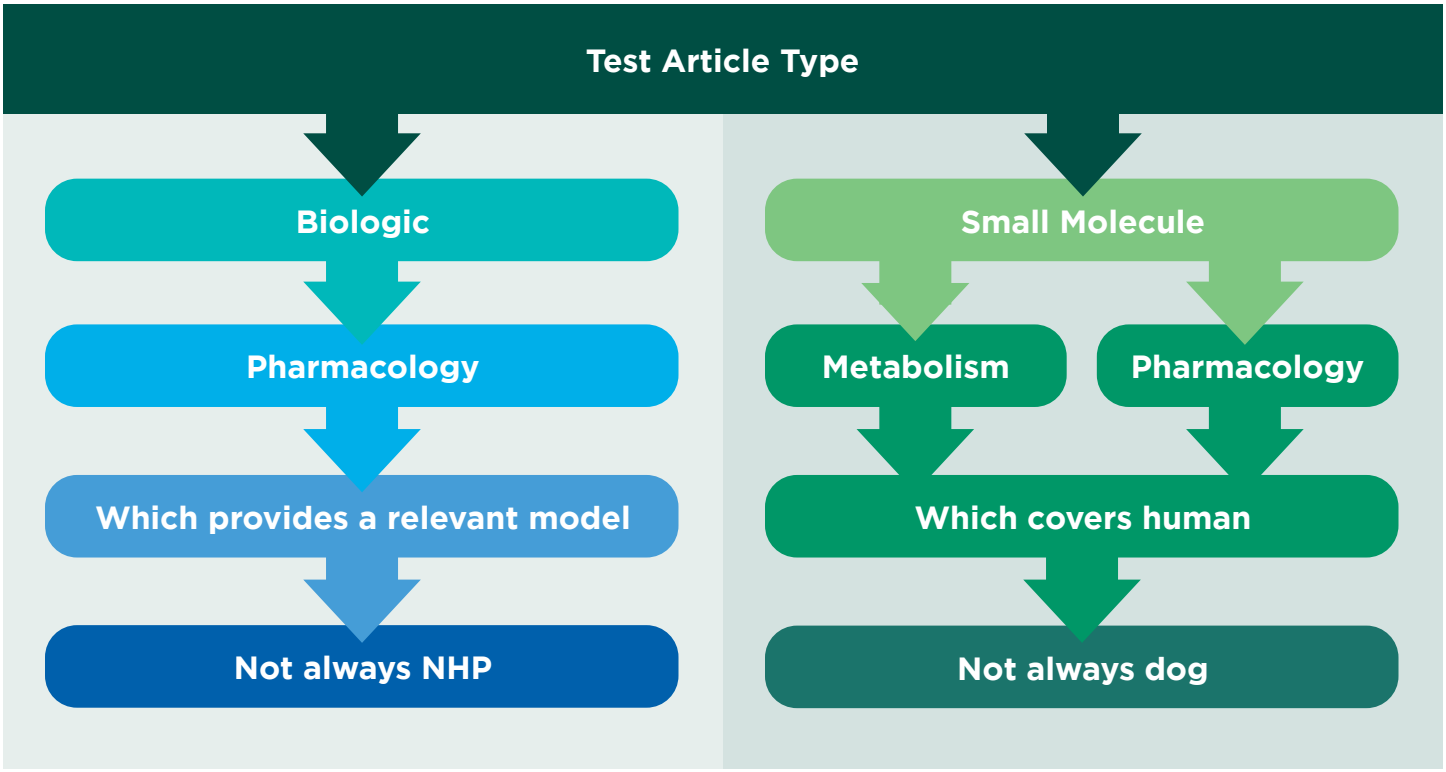
Based on this information, and the ever-growing knowledge base, adding the miniature swine to a metabolic profiling panel for a small molecule program will provide baseline data as to whether the miniature swine is an option for the program, and would add minimal cost (if any).

For programs where the test article is a biologic, there is a long-held view that NHPs are the default species, and miniature swine were not even considered as an option. However, data is emerging that challenges this belief. The first step in determining whether the miniature swine is an option for biologic programs is to understand the conservation of the target across the potential nonclinical safety species. While many programs involve specific epitopes with limited conservation across the species, there are programs where the target is highly conserved, and the miniature swine is a viable option.

There are other limitations to the miniature swine as the non-rodent species in some programs with specific parameters. If immunophenotyping, ligand binding assays, or cytokines are included as part of the nonclinical safety study design, the availability of porcine reagents may be challenging as they are lagging behind species such as rat and NHP. That said, some are available, and more are being developed.

The choice of species for nonclinical safety studies should always be driven by sound scientific rationale.

Figure 1. Algorithm for Nonclinical Species Selection



A publication from the 12th Annual Miniature Swine Research Forum (2019) stated that "there are no regulatory hurdles for using miniature swine, as the use is accepted by regulatory authorities all over the world, including Japan, Korea, China, India, the EU, and the U.S. Nobody had experience with, nor knowledge of, any instances where the miniature swine had not been accepted by regulatory agencies… the miniature swine is now so well defined and understood, it ought to be routinely included from the earliest screening stages for species selection by all companies, particularly for small molecules. Additionally, substantial consideration ought to be given for using the miniature swine for large molecule safety assessment, provided suitable screening platforms are available… It was acknowledged that, in general, technical challenges of the past are not an issue, and conduct of various safety studies is as straightforward as it is for other species."[1]

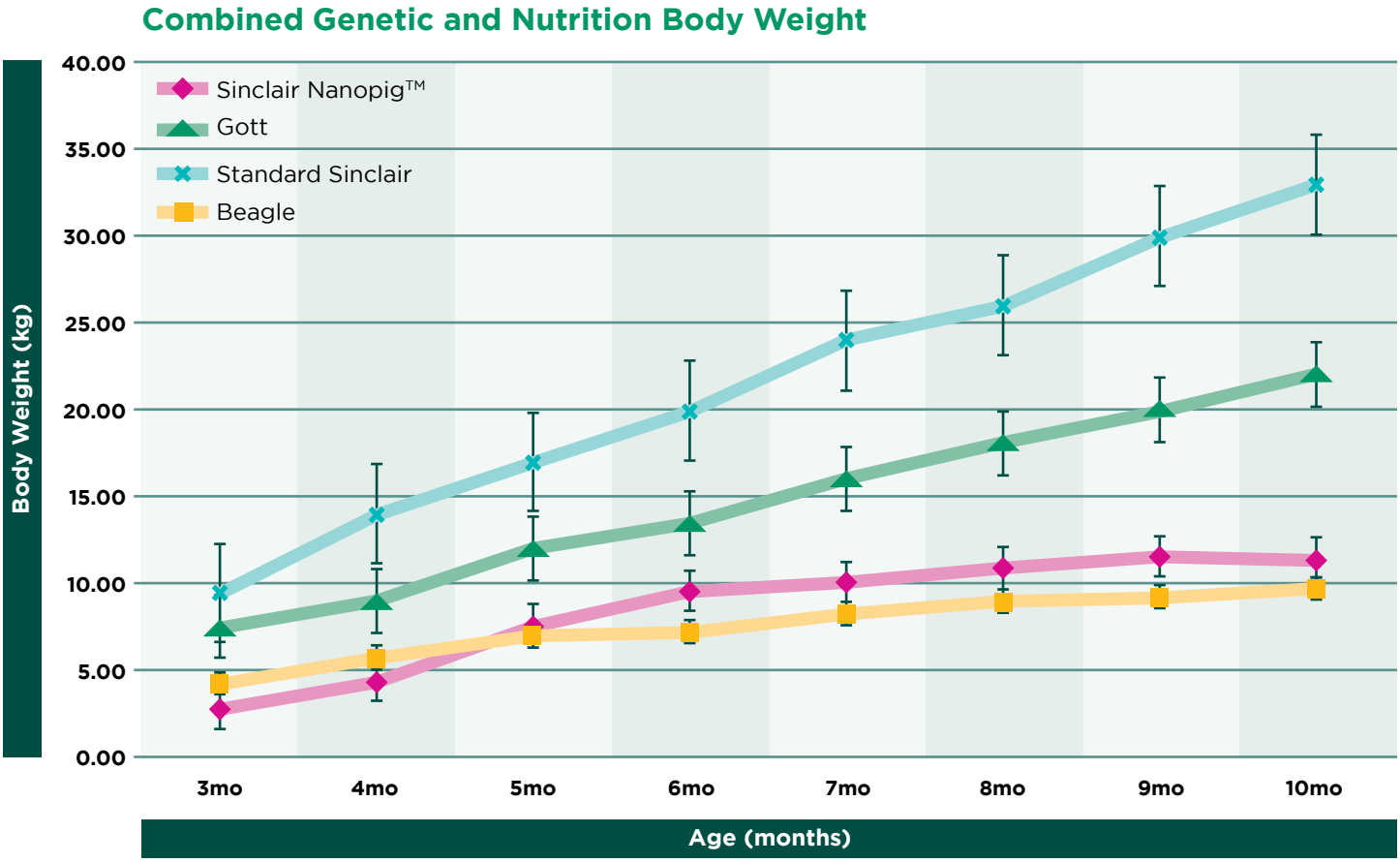
Miniature swine reach sexual maturity at an earlier age than larger agricultural pigs, which allows for a shorter breeding cycle and thus more consistent supply. Miniature swine are a genetically defined model; the majority of breeds have their entire population history documented, beginning at their early development up to present.

MINIATURE SWINE SIZE CONSIDERATIONS

A drawback for miniature swine has been their size, as larger animals are more costly to maintain and manage, and require larger amounts of test article in nonclinical safety studies. Miniature swine, specifically developed for research purposes, are significantly smaller than agricultural swine, and typically weigh between 30 and 70 kilograms at maturity.

Recently, Sinclair Bio Resources, a U.S.-based breeder of research swine, has developed a downsized miniature swine, referred to as a nanopig, that is very similar in size to a beagle (see Figure 2 below). This provides an opportunity to use the miniature swine and keep the test article usage within reason. When conditions are appropriate (see case studies in this issue), an excellent opportunity exists to work with miniature swine as a non-rodent species.

Figure 2. Downsizing of the Sinclair Miniature Swine



REAL-WORLD EXAMPLES FOR MINIATURE SWINE USE IN CLINICAL DEVELOPMENT

An article published in Toxicology Research in 2020 included highly relevant commentary from Richard Haworth of GSK. Titled "When is the miniature swine the relevant non-rodent toxicology species?"[2], the publication included three case studies for activities that GSK undertakes when determining if the miniature swine is an appropriate model for a given study.

According to the author, GSK considers the dog and miniature swine as the appropriate non-rodent species for toxicology studies early in development, for every small molecule program. The decision-making process is supported by an objective and thorough assessment of the human relevance of possible animal models.

There is no default species assigned. Rather, selection of the non-rodent species for each molecule is scientifically investigated, measured, and documented. A science-based, data-driven approach using factors listed below is applied, and NHPs are only used if the dog and the miniature swine do not sufficiently meet concordance requirements.

Species Selection Criteria and Processes[2]

1 **Conservation of the drug target.** The homology of the human target is compared across the nonclinical species. Literature searches, in silico, in vitro, transcriptomic and proteomic analysis, and quantitative immunostaining are options for determining the similarity of the target and its distribution in the nonclinical species, relative to humans. This is more important for biologic drugs, and also applies to small molecule drugs.

2 **PD responsiveness.** It is important to demonstrate that not only is the target conserved, but the drug is active at the target. This is commonly accomplished through the use of cultured cells known to possess the target of interest with some type of measurable pharmacodynamic output.

3 **Drug exposure.** Once a drug is administered, it is vital to understand and quantify systemic circulation. This is important for drugs that are administered through a route other than intravenous. Non-GLP PK studies are conducted to determine the bioavailability of the drug following administration in miniature swine. The goal of the analysis is to understand if there is a difference between species, and which species is more likely to attain sufficient drug exposure to meet study objectives.

4 **Metabolite profile.** An in vitro metabolite cross-species is used to compare patterns of major metabolites (animal to human). The purpose is to provide safety data that covers the metabolites that are predicted to occur in humans, and to be sure the nonclinical species provides that coverage.

5 **Physiological and toxicological human relevance.** Used where, for example, a species has demonstrated proven over-sensitivity to a drug modality, molecular class, formulation, or target, such as compounds likely to cause acute histamine release in dogs, that species is not considered.

6 **Study logistics and animal welfare considerations.** Species have differing practical implications, constraints, and impact on study-specific scientific objectives. The technical feasibility, tolerability, or comparative stress associated with special techniques—such as collection of lymph or cerebrospinal fluid—must be considered for each species.

All animal studies in the following case studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.[2]

Case study 1[2]

A small molecule, innate immune system agonist was evaluated in the dog, miniature swine, and NHP. The pharmacological responses are induction of interferon (IFN)-α as the clinical marker of efficacy and tumor necrosis factor (TNF)-α as the clinical marker of safety. Evaluations were conducted in miniature swine and NHP using in vitro assays. In previous PK studies evaluating other small molecules directed at the same target, the dog did not tolerate the test molecule well, demonstrating lethargy, subdued behavior and emesis, and was discounted from future studies in this area. Very similar relative potency between human and cynomolgus monkey was demonstrated through comparison of TNF-α and IFN-α responses in whole blood or peripheral blood mononuclear cells (PBMC), cultured in vitro with the test article (unpublished data). The miniature swine also demonstrated comparable TNF-α results with human; however, levels of IFN-α were significantly lower. There was poor selectivity for IFN-α compared to TNF-α, which precluded the miniature swine as a suitable non-rodent test species. The cynomolgus monkey had been shown to produce comparable cytokine profiles for both IFN-α and TNF-α, with similar selectivity for IFN-α over TNF-α.

In this comparison, the cynomolgus monkey exhibited a more similar pharmacological response than the miniature swine relative to humans, and thus was selected as the non-rodent toxicology species. In this example, the PD responsiveness was a key factor in the rational selection of the non-rodent species.

Case study 2[2]

A small molecule for an undisclosed indication, delivered orally, against a drug target which is well conserved across the non-rodent toxicology species, was evaluated. A previous compound in the same pharmacological class had used the dog as the test species, and was also evaluated in this case to ensure compatibility. Emesis has been identified as a potential risk from knowledge of the compound class and pharmacology; a three-day tolerability study in the dog was conducted and emesis was confirmed. This led to non-linear TK at higher doses, and the dog was deemed unsuitable as the toxicology species.

The miniature swine tolerated the same dose, and provided sufficient drug exposure relative to the anticipated clinical dose to be selected as the non-rodent toxicology species. In this example, tolerability and secondary effects on toxicokinetics were decisive in the species selection.

Case study 3[2]

A small molecule was in development for a subcutaneous clinical indication, requiring dosing in the toxicology program by the same route. The miniature swine was selected as the non-rodent species and provided satisfactory PK. The clinical route subsequently changed to intravenous infusion, and toxicology studies via that route (infusion via ear vein) encountered procedural dosing issues and poor tolerability in miniature swine. When the dog was used, the intravenous infusion dosing was tolerated, and the dog was substituted as the species of choice.

In this example, application of study-specific and animal welfare considerations ensure the appropriate species was chosen to enable successful dosing.

It is not always necessary to evaluate data on all factors, particularly if there is a well-documented and defining difference between the toxicological or physiological relevance of dog and miniature swine. Where no clear direction is apparent, determining if the miniature swine is the most relevant non-rodent toxicology species for a particular project requires careful and thorough evaluation of the relevant factors. If the miniature swine is determined to be the appropriate species for a program, there is value in further examining the different strains, as described in Altasciences' case study below.

Case study 4

Downsized Sinclair (Nanopig™) vs Göttingen Miniature Swine: Similarities and Differences of Toxicological Reference Range Data in Preclinical Safety Studies

Yafei Chen, Adam Martin, Zoe Patenaude, Derek Brocksmith, Dr. Wendell Davis

Miniature swine are recognized as offering advantages over other established non-rodent models such as beagle dogs based upon substantial evidence of similarities to humans with regard to anatomy, physiology, and biochemistry. Miniature swine are used increasingly in nonclinical CROs and (bio)pharmaceutical industry to support IND-enabling toxicology studies. However, similarities and differences in toxicological reference data between the commonly used Göttingen and Sinclair breeds have not been reported.

To provide scientific justification for selection of the most appropriate strain of miniature swine for sponsors' drug development program, and as part of the Altasciences Historical Control Database initiative, this study was performed to compare reference baseline/background data for a battery of standard toxicological parameters obtained from Sinclair and Göttingen miniature swine studies conducted at Altasciences' Columbia site.

Data for Göttingen miniature swine was extracted from electronic data capture system (Pristima®) and compared with the reference data of downsized Sinclair miniature swine (nanopig™) (Book of Normals 2021; SBR), including body weight, clinical pathology (hematology, serum chemistry, coagulation, urinalysis), organ weights and histopathology background lesions of a panel of tissues from nine physiological organ systems. Multiple statistical analyses, including mean and Standard Deviation (SD), range (min, max), fold difference of average, quartile, interquartile range (IQR), and Tukey fence (upper and lower limit) were used for data comparison.

Body weights were similar between these two miniature swine strains up to approximately three months of age. There was considerable overlap of mean and statistical clinical pathology values between Sinclair nanopig™ and Göttingen miniature swine, except that globulin, lymphocyte, and monocyte values were significantly higher (two to threefold) in Sinclair nanopig™ as compared to Göttingen miniature swine. In addition, brain and thymus weights for Sinclair nanopigs™ were higher than Göttingen miniature swine in both males (1.4-fold and 2.5-fold, respectively) and females (1.3-fold and 1.4-fold, respectively). The sex differences of organ weight to brain or body weight ratio were also

observed, as the heart and adrenal weights to brain ratio were lower, while thymus weight to body weight ratio was higher in male (only) Sinclair nanopigs™ as compared to Göttingen miniature swine.

The most common microscopic finding noted in Sinclair nanopigs™ was multifocal lymphohistiocytic infiltration in various tissues. The detailed descriptions of differences as well as similarities of spontaneous histopathological findings and incidence rate will be available in 2023.

Based on data comparison in this study, all apparent differences among clinical pathology, organ weight, and background microscopic findings between Sinclair nanopigs™ and Göttingen miniature swine were considered minor in magnitude and biological significance.

CONCLUSION

This study, for the first time, provides assessment criteria for miniature swine strain selection, data quality control and interpretation of results in preclinical toxicity studies using downsized Sinclair nanopigs™, which have similar toxicological reference data versus Göttingen miniature swine, are more cost effective, and readily available at different ages for customized study design for sponsors' drug development strategy.

HOW ALTASCIENCES CAN HELP

When all else is equal, other considerations gain importance in influencing the species selection. If global supply issues affect the availability of dogs or cost of NHPs, the greater availability and reasonable cost of the miniature swine can be the deciding factor (when the miniature swine has proven to be scientifically appropriate). Although not as readily available as rodents, the supply of miniature swine does not show any signs of being a limiting factor moving forward, as currently large litter sizes and rapid sexual development ensure a steady supply can be achieved, with appropriate lead time.

Species selection for your toxicology program must always be driven by sound scientific rationale. The experts at Altasciences will evaluate all available data, and conduct any additional species selection studies that may be needed to inform a final, rational, and appropriate decision for the specifics of your program. We have experience with all the major species and routes of administration to support the best decisions for your program.

We have vast experience with miniature swine and nanopig models, including any in vitro species comparison studies that may be necessary. Our experts can help you make the most appropriate species selection to deliver robust data for your development program.

ALTASCIENCES' EXPERIENCE WITH MINIATURE SWINE STUDIES (2018 – 2022)

We conducted 303 total studies with miniature swine, involving well over 4,000 animals. Thirty-five of the studies were for surgical indications, the remainder for various routes of administration, as below:

Routes of administration

Topical	131
Subcutaneous	58
Intravenous	37
Oral	33
Intramuscular	9

Contact our experts to see how the advantages of including miniature swine in the species selection for nonclinical safety studies can be applied to your upcoming programs.

ALTASCIENCES' RESOURCES

Webinars/Videos/Podcasts:

The Miniature Swine as a Model for Juvenile Toxicity Studies

Choosing the Right Model: Miniature Swine Model Selection Criteria for Toxicology and Pharmacology Studies. Includes in-depth comparisons to human for many relevant parameters.

How do I Select the Right Species for My Toxicology Program?

REFERENCES

1 Jones K, Harding J, Makin A et al. Perspectives From the 12th Annual Miniature swine Research Forum: Early Inclusion of the Miniature swine in Safety Assessment Species Selection Should be the Standard Approach. Toxicologic Pathology 2019, Vol. 47(7) 891-895

2 Prior H, Harworth R, Labram B et al. Justification for Species Selection for Pharmaceutical Toxicity Studies. Toxicology Research, 2020, 9, 758–770

ABOUT ALTASCIENCES

Altasciences is an integrated drug development solution company offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 25 years, Altasciences has been partnering with sponsors to help support educated, faster, and more complete early drug development decisions. Altasciences' integrated, full-service solutions include preclinical safety testing, clinical pharmacology and proof of concept, bioanalysis, program management, medical writing, biostatistics, clinical monitoring, and data management, all customizable to specific sponsor requirements. Altasciences helps sponsors get better drugs to the people who need them, faster.