
What's good to know when considering the Göttingen Minipig for Juvenile toxicology studies?

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OVERVIEW

It's good to know about:

- Animal procurement
- Study types – MTD, DRF & pivotal juvenile studies
- Animal reception and husbandry
- Littering and piglet selection criteria
- Dosing techniques
- Measurements/observations
- Advantages/disadvantages

INTRODUCTION

- Obviously the rat is the preferred and most widely used species for juvenile toxicity studies
- However, non-rodent species are required punctually
 - Pediatric only indication – no adult data
 - Rat unsuitable model or with identified concerns
 - Scientific justification (pharmacology) for a non-rodent species
- In general, one species is required
- Dog and minipig are the most likely options with non-human primate as a third species depending on the target

INTRODUCTION

- Juvenile animal studies (JAS) tend to be both complex scientifically and challenging from a practical perspective
- Whilst the thought process for establishing study designs may be the same, the planning, execution and reporting of non-rodent JAS studies are more demanding compared with those performed in rodents
- Several breeds of “miniature swine” available, principally the Göttingen Minipig, Sinclair, Hanford and Yucatan
- However, focus today is on the most used and characterized breed

GÖTTINGEN MINIPIG PROCUREMENT FOR JUVENILE STUDIES

POINTS FOR CONSIDERATION

- **Pre- and early post-weaning (less than 7 weeks of age) start of dosing**
 - Pregnant females ordered to give birth in the lab – no delivery of lactating females with litter
 - Multigravida females recommended (more expensive but important)
 - Delivery date more predictable
 - “More vigorous” piglets and litter size tends to be superior (n=6)
 - Proven nursing behaviour and milk production
- **Post-weaning start of dosing**
 - Young animals can be ordered from 6 weeks of age

GÖTTINGEN MINIPIG PROCUREMENT FOR JUVENILE STUDIES

POINTS FOR CONSIDERATION

- 3 to 6 week notification for animal order depending on the number of pregnant females required – the earlier, the better (cost!)
- Pregnant females are acclimatized for approximately 3 weeks before scheduled birth date
- Batch arrivals required for pivotal studies (up to 8 litters/batch) to optimize study management
 - Allows adjustments for last delivery batch

STUDY TYPES

Pre- and early post-weaning (less than 7 weeks of age) start of dosing

MTD/FEASIBILITY/TOLERABILITY TYPE STUDY

One or two litters to provide 1 piglet/sex/group to explore:

- Tolerability
- Dose-response (including TK/exposure evaluation)
- Method of administration (parenteral routes)
- Short dosing period
- Specific observations/parameters...

DOSE RANGE-FINDING STUDY

Three or four litters to provide 2 piglets/sex/group to explore:

- Dose-response (including TK/exposure evaluation)
- Repeated dosing (through to weaning?)
- Growth, physical development, clinical pathology, terminal examinations...

STUDY TYPES

Pre- and early post-weaning (less than 7 weeks of age) start of dosing

PIVOTAL JUVENILE TOXICITY STUDY

- Customized study design
- Group size not specified in the guidelines
- Typically consider up to double the group size used in large animal repeat dose toxicity studies: N = 6/sex (approx litter size of “selectable” piglets) + N = 3/sex for reversibility cohort
- Estimate 3 litters to provide the animals necessary for one group (with reversibility cohort)
- One control + 3 treated groups = minimum of 12 litters per study
- Other cohorts, e.g., interim sacrifice would require additional litters

MOST COMMON FACTORS IN STUDY DESIGN

Need for a range of scientific and technical expertise

- Class of drug (small molecule/biologic)
- Concerns from any adult toxicity studies
- Development of specific organ system(s)
 - Cardiovascular
 - Central Nervous System
 - Gastrointestinal
 - Pulmonary
 - Immune
 - Renal
 - Reproductive
 - Skeletal (growth)



PROJECT SCHEDULING

Essential for success

	Month 1		Month 2			Month 3				Month 4				Month 5				Month 6				Month 7				Month 8				Month 9				Month 10				Month 11							
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3	4	5	6	7	8	9	10	11	12	13

MTD VO juvenile

AO

AA



DRF juvenile

AO

AA



Main juvenile

AO

AA



 In life or experimental phase

 Reporting phase

AO: Final Animal Order

AA: Animal Arrival (pregnant dams)

AD: Audited draft

D: Draft report

STUDY SCHEDULING - PIVOTAL STUDY

Preparation/Organization/Execution

phase	grp 1 untreated		grp 2 water		grp 3 vehicle		grp 4 5 mg/kg/day		grp 5 10 mg/kg/day		grp 6 25 mg/kg/day			
	sex and pup no.	litter no.	sex and pup no.	litter no.	sex and pup no.	litter no.	sex and pup no.	litter no.	sex and pup no.	litter no.	sex and pup no.	litter no.		
reversibility			M 21	307	M 41	308	M 71	305	M 101	303	M 147	311 ^b		
			M 22		M 42		M 72		M 102		M 148			
			M 23		M 43		M 73		M 103		M 149			
			M 24		M 44		F 75		F 105		F 150			
			M 25		M 45		F 76		F 106		F 151			
			M 26		F 46		F 77		F 107		F 154			
main	F 1	306 ^a	M 27	302	M 47	309	M 74	314	M 104	315 ^d	M 141	313		
	F 2		M 28		M 48		F 78		M 108		M 142			
	F 3		M 29		F 49		M 79		M 109		M 143			
	F 4	M 30	F 50		M 80		M 110		F 144					
	M 6	312	F 31		F 51		M 81		M 111		F 145			
	M 7		F 32		M 52		M 82		F 112		F 146			
	M 8	F 33	M 53		F 83		F 113		M 155					
	M 9	F 34	M 54		F 84		F 114		M 156					
	M 10	322	M 35		F 56		M 85		M 115		F 157			
	M 11		F 36		F 57		F 86		M 116		F 158			
	M 12		F 37		F 58		F 87		F 119		F 159			
	F 13		F 38		F 59		F 88		F 120		F 160			
	F 14		F 125								F 317			
	F 15										M 162			
	interim killed	F 16							M 55		M 89		M 117	M 163
		F 17							M 60		M 90		M 118	M 164
									M 61		M 91		F 121	F 165
					M 62	F 92	F 122	F 166						
					F 63	F 93	M 123	F 167						
					F 64	F 94	F 124	F 168						

Animals sampled for TK

MB 04/02/2015

MB 06/02/2015

MB 07/02/2015

MB 10/02/2015

MB 12/02/2015

MB 13/02/2015

MB 14/02/2015

MB 12/03/2015

MB 13/03/2015

MB 14/03/2015

MB 16/03/2015

- Several cohorts likely (main study, interim euthanasia, reversibility...)
- Multiple birth dates for start of dosing (formulations!)
- Various litter sizes and sex ratio

ANIMAL RECEPTION AND HUSBANDRY

ELLEGAARD ••
GÖTTINGEN MINIPIGS

CERTIFICATE

Wii Research Europe-Lyon
329 Impasse du Domiane Rozier
Les Oncins
Saint-Germain-Nuelles, F-69210
FRANCE

Livré au: 19-08-14

Numéro: 214145 Né date: 15-01-12 Barrière: B2 Sexe: Female Mère: 206007 Père: 206640

Poids à la naissance: 0,47 Poids (kg): 57,60 Date: 12-08-14

Mating Date: 19-05-14 Expected Farrowing: 10-09-14 Litter: 5


D.V.M. Head of Veterinary Services
Définés en production, genétique et santé

Minipig Farrowing

- Duration of gestation: 3 months, 3 weeks and 3 days... and then there is reality
 - Expected delivery date provided by Ellegaard
 - Our experience with non-induced delivery is +/- 4 days the expected date
 - Female status – nursing, milk production
 - Litter size, sex ratio and selection

DETAILS - PENS ADAPTED FOR NEONATAL MINIPIGS



MINIPIG FARROWING

- Pregnant females received 3 weeks before parturition
- 1 sow & litter in double pens (2 x 2 m²) with autoclaved sawdust bedding
- Infra-red lamp in each box in order to maintain a temperature of approximately 25°C



Healthy piglets: open eyes, rapidly moving around the pen and suckling

MINIPIG NEONATAL PIGLET SELECTION CRITERIA

ADVANTAGES COMPARED WITH THE DOG

- Piglets pre-selected shortly after birth based on weight and physical/functional development parameters:
 - Selected piglets: standing with open eyes, suckling and walking
 - “Doubtful” piglets: « runts » less than 300 g (fetus weight!) not meeting one of the above criteria (open eyes or walking)
 - Eliminated piglets: weak pups that cannot stand/move without stimulation with closed eyes

PIGLET SELECTION CRITERIA

Eliminated piglet - soft tissue examination - diaphragmatic hernia



PIGLET CARE AND FEEDING REGIME

Piglet care:

- Colostrum transfer
- Clinical examination: daily from birth
- Iron intramuscular injection 24 to 48 hours after birth

Feeding:

- From PND 10, yogurt is provided for the piglets which is progressively mixed with diet as the piglets get older
- Yogurt/diet mix provided until one week after weaning
- From 7 weeks of age, pelleted diet only is provided twice daily

DOSING TECHNIQUES - ROUTES OF ADMINISTRATION

Earliest day possible post partum

DOSE ROUTE	SPECIES	
	Dog	Minipig
Oral gavage	1	1
Intravenous bolus	1	1*
Intravenous infusion	5**	Not tested
Inhalation	10	Not tested
Parenteral (IM/SC)	1	1
Dermal	42	1

* Peripheral vein PND 1 to PND 4 and then with indwelling catheter

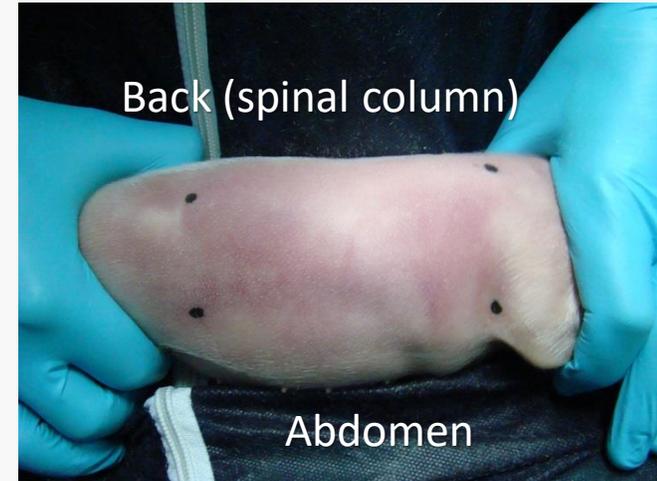
** Implanted iPrecio micro-pump for potent drugs requiring a small volume (max 900µL)

DOSING TECHNIQUES

Innovative Dermal Application

- Application sites: both flanks, defined with anatomic landmarks (established in validation study):
 - Rectangular between scapulae and hipbone
 - Treated area = at least 10% of total BSA ⁽¹⁾
 - Application sites shaved if necessary
 - Animals socialized and kept with the mother during daily application period

(1) Regulatory guideline requirements (OECD 410 Repeated Dose Dermal Toxicity Study ; EPA-Health Effects Test Guidelines-OPPTS 870,3200)



DOSING TECHNIQUES

Innovative Dermal Application

- Application method: dressing depends on the weight of the piglet:
 - Piglets < 0,5 kg (birth)
 - Piglets > 0,5 kg
 - From 4 weeks of age (2-2,5 kg),



WHEN TO DOSE

Postnatal age categories compared with human

Category	Human	Minipig	Dog
Premature	Less than term	/	0 to 4/10 days
Neonate	0-28 days	0-15 days	5/11 to 21 days
Infant	1-23 months	2-4 weeks	3-6 weeks
Child	2-12 years	4-14 weeks	6-26 weeks
Adolescent	12-16 years	4-6 months ¹	5-9 months

¹ Howroyd, P. C., B. Peter and E. de Rijk (2016). Review of Sexual Maturity in the Minipig. *Toxicol Pathol* 44(4): 607-611.

Taberner, E., Navratil, N., Jasmin, B., Salerno, M., Grambo, B., Althouse, G. C. (2016). Pubertal age based on testicular and epididymal histology in Göttingen minipigs. *Theriogenology*. 2091-2095.

STANDARD MEASUREMENTS/OBSERVATIONS

- Growth parameters (body weight & tibia length) – as of PND 1
- Ophthalmology
- Clinical pathology (microvette or vacutainer)
- Toxicokinetics (microsampling when possible)
- Cardiovascular examinations (ECG)
 - measurement of heart rate
 - analysis of rhythm
 - measurement of QRS complex duration, PR and QT intervals.
- Organ weights and histopathology

POSTNATAL ENDPOINTS

Examples: ECG recording and ophthalmology

ECG from PND 5



Ophthalmology from PND 7



KEY ACTS (CURRENT FEASIBILITY)

Juvenile minipig

PROCEDURE	MINIPIG
Blood “microsampling” (60-80µL)	From PND 1 (saphenous and ear vein non-anaesthetized animal)
Blood sampling for clinical pathology	PND 14 (hematology only), PND 21 (jugular vein non- anaesthetized animal)

ADDITIONAL INVESTIGATIONS

- Developmental immunotoxicity (specific cohort)
- Reproductive development
- Hormone evaluations
- Neurobehavioural tests (neurological examinations and FOB)
- Specific biomarkers
- ...

ADVANTAGES/DISADVANTAGES

- Easier to select vigorous healthy piglets (compared with puppies) to the study and perform technical procedures/physical examinations etc as of PND 1
- The developmental periods are shorter in the minipig compared with the dog to consider long-term studies (including reversibility)
- Physical size of the Göttingen Minipig during developmental “windows” is very conducive for technical procedures/assessments
- Limited HCD/references in juvenile minipig compared with the dog but progressing well in the Göttingen in many areas (metabolism, PK, morphology, sexual maturation, immune function, histopathology... check it out in ICH S11 and on PubMed!)

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“All pigs are equal” Does the background data from juvenile Göttingen minipigs support this?

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Original Article

Specificities of the Skin Morphology in Juvenile Minipigs

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Abstract
The Göttingen minipig is recognized by the scientific community and by health authorities as the animal model of choice to evaluate dermally applied drugs under development. Young adults of approximately 4 months of age are most generally chosen to participate in dermal pharmacology and toxicology studies, and recently, minipigs have been proved to be also suitable for juvenile studies. A complete anatomical cartography of the skin morphology of juvenile male and female minipigs from postnatal day 1 (PND1) to twelve weeks of age was performed measuring the thickness of skin layers for each ana-

ADVANTAGES/DISADVANTAGES

- Venous access gets difficult for blood sampling and IV dosing as minipigs get older
 - Catheter implantation
- Advanced growth rate and maturity of the piglet at birth (eg., neuromuscular and respiratory systems) may lead to the dog being a more appropriate model for a number of compounds/target organ systems
- Skeletal growth continues post puberty for a longer time in the minipig
- Do I mention test compound!?

CONCLUSION

- There are many logistical and scientific considerations when developing a juvenile tox program, in particular for a large animal species
- Select a CRO that has the expertise and resources necessary to do the job
- The minipig should be considered when selecting the most pertinent animal model for pre-clinical development
- **Select a well characterized breed that has regulatory acceptance!**

Thank You for Your Attention

