The use of minipigs in juvenile toxicity testing

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Introduction

- Obviously the rat is the preferred and most widely used species for juvenile toxicity studies for small molecules
- In general, one species is required
- However, non-rodent species are sometimes required:
  - Pediatric only/first indications
  - No adult data
  - Rat unsuitable model or with identified concerns
  - For dermal formulation (minipig)
- Dog (and minipig) most likely options

Species selection

- Ontogeny of the pharmacological or toxicological target in animals versus intended pediatric population
- Preference for species in which adult data are available to allow comparison between juvenile and adult animals
- Toxicological target organs
- The technical and practical feasibility
- Similarity of ADME characteristics

Guidelines

- ICH S11
  - Selected species should be justified
  - Alternative species include minipig
  - Table included with age-dependant development of different organ systems in the minipig
    - Integument: similarities in development to human
    - CV: similarities in development to human
    - GI: model for human stomach development
    - Model for human immune development
    - Brain development in neonatal pig similar to the human neonate

Postnatal age categories

Category	Human	Minipig	Dog
Neonate	0-28 days	0-15 days	0-21 days
Infant	1-23 months	2-4 weeks	3-6 weeks
Child	2-12 years	4-14 weeks	6-20 weeks
Adolescent	12-16 years	4-6 months	5-7 months

Physical development

- Epitheliochorial placenta
- Passive uptake of IgA, G, M on PND1 via colostrum
- Born with eyes open, able to walk, some teeth erupted
- No thermoregulation at birth
- Require Fe supplementation to maintain blood Hgb level
  - Poor iron storage in liver
  - Little Fe in milk
  - Lab piglets have no access to Iron from rooting in soil
- Weaned at PND 28
- Rapid postnatal growth
- Puberty at ± 6 months
- Epiphyseal growth plates close at 18 months

Advantages

- Similarities to man
  - Skin
  - Cardiovascular system: physiological and morphological similarities, sensitive to cardiotoxic agents
  - Brain for the general anatomy, growth and development
  - Gastro-intestinal system
  - Metabolism (CYP enzymes)
  - Sensitive to human teratogens

References:

- The minipig in biomedical research. CRC Press, Taylor & Francis Group
- Pediatric non-clinical testing: principles, requirements, and practices. Wiley
- The RETHINK project on minipigs in the toxicity testing of new medicines and chemicals: Conclusions and recommendations. R Forster and all, Journal of Pharmacological and Toxicological Methods, 62 (2010) 236–242,

Advantages

- Large litter size
  - Pig: 8-10 (multiparous sows)
  - Dog: 5-6
  - Monkey: 1
- Easy cross fostering
  - Helpful due to the high number of piglets
  - Allow harmonizing number of piglets in litters
- No neonatal pathology
  - SPF Ellegaard Göttingen sow
  - Prophylaxis necessary for dog at birth (vaccination, antiparasite)
  - Whereas only iron injection for piglets

Advantages

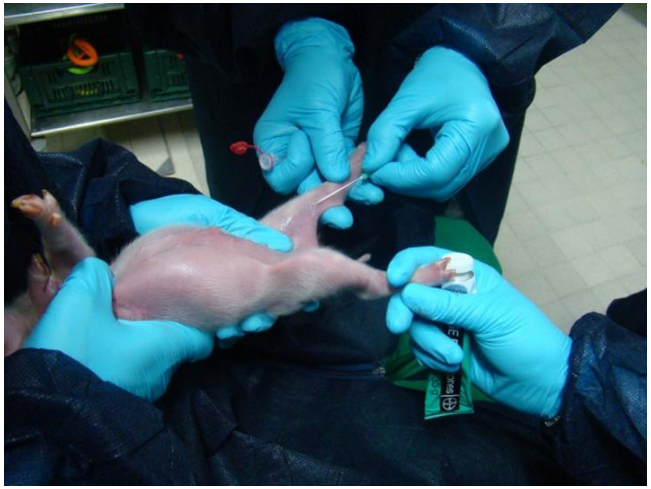
- Easy piglet selection at birth
  - Selection criteria are based on:
    - Body weight (generally >300g)
    - Physical/functional development parameters
  - Immediately after birth, selected piglets
    - Stand up
    - Walk
    - Suckle
    - Opened eyes (a plus for ocular administration/examination)
  - Eliminated piglets : weak piglets that cannot stand up, thus cannot suckle, with closed eyes

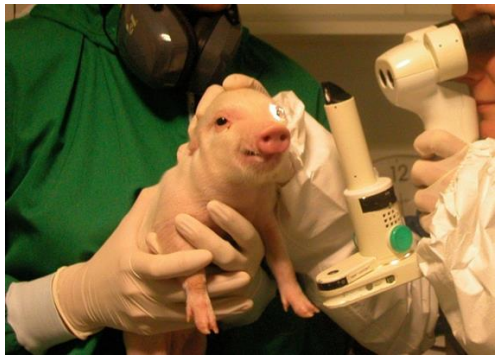
Advantages

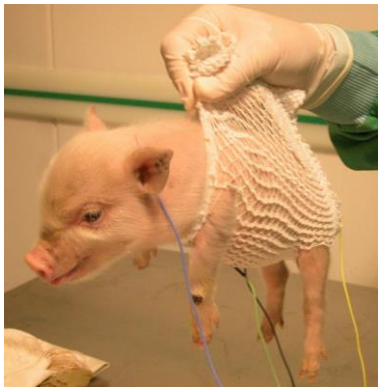
- Sows are amendable for human interventions

- Thus piglets are very practical for technical procedures, possible as early as PND1:

  - Administration (oral, IV bolus, dermal)
  - Measure of growth parameters
  - Blood sampling (microsampling)
  - ECG recordings from PND5
  - Ophtalmoscopy from PND7







Advantages

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- Early developmental stages for the piglet:
  - Weaning
    - Pig: 4 weeks
    - Dog: 6 weeks
  - Sexual maturation
    - Pig: 6-7 months
    - Dog: 9-12 months

=> Allow the evaluation of multiple developmental stages into a short juvenile study

=> Thus the quantity of compound necessary for a juvenile toxicity study in minipig no more than in dog

Disadvantages

- Less extensive knowledge on DT/DME ontogeny compared with the rat

- Limited background data
  - Especially for neonatal period
  - Few studies conducted within each age range
  - Thus might be a concern for interpretation of results

References

- Feyen et al., "all pigs are equal" Does the background data from juvenile Gottingen minipigs support this? Reproductive Toxicology 64 (2016)105-115.
- Black grossi A. https://minipigs.dk/fileadmin/filer/Pictures/News_2017/Reference_data_of_clinical_chemistry_and_hematology_in_juvenile_G%C3%B6ttingen_Minipigs.pdf
- Makin et al. Haematology and blood biochemistry changes related to age in Gottingen minipigs. Abstract of the society of Toxicology 2012.
- Van Groen et al. Ontogeny of hepatic Transporters and Drug-Metabolizing Enzymes in humans and in non clinical species. Pharmacological Reviews April 2021, 73 (2) 597-678

Disadvantages

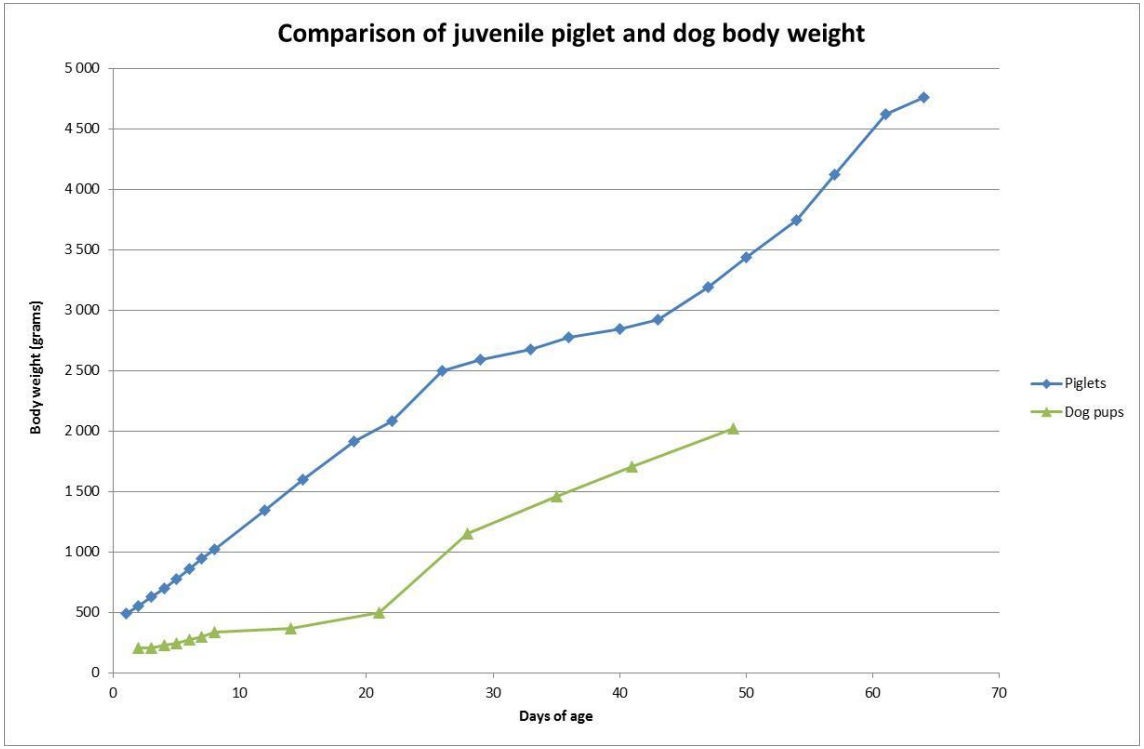
- Long gestation duration and variability in delivery day
  - Rat: 22 days +/- 1 day
  - Dog: 63 days +/-3 days
  - Minipig: 114 days (3 months, 3 weeks, 3 days) +/- 4 days (non induced delivery)

→ However easier to obtain pregnant sows from Ellegaard than pregnant dogs

- At birth, piglets risk being crushed by sow
  - Adaptation of box to decrease risk
  - Iron bar around the wall
  - Only 1/132 (0,76 % incidence) in our study

Disadvantages

- High variability in BW at birth
  - 550g +/- 100g (mean +/-SD)
  - Min/max: 300/800g



- Rapid growth

- Thus rapid increase in compound has to be estimated

Background data

- Study design
  - Group size not specified in guidelines
    - Aim: N=6/sex/group + N=3/sex/group for recovery
  - Estimate 3 litters to provide necessary animals for one group
  - 3 deliveries of pregnant 7-8 sows
  - Treatment period
    - Dosing from PND1-35 to cover pediatric age range (weaning +1 week)
    - Interim group added: dosing PND1-14 to cover dosing period in the clinic
  - Number of dose groups: 6 including 3 control groups
    - Untreated control
    - Treated control
    - Vehicle control
  - Rationale for adding 3 control groups
    - Evaluation of the vehicle (similar vehicle as in pediatric trials)
    - Evaluation of stomach lesions observed in the pilot study in all dose groups including the vehicle group
    - Generation of background data

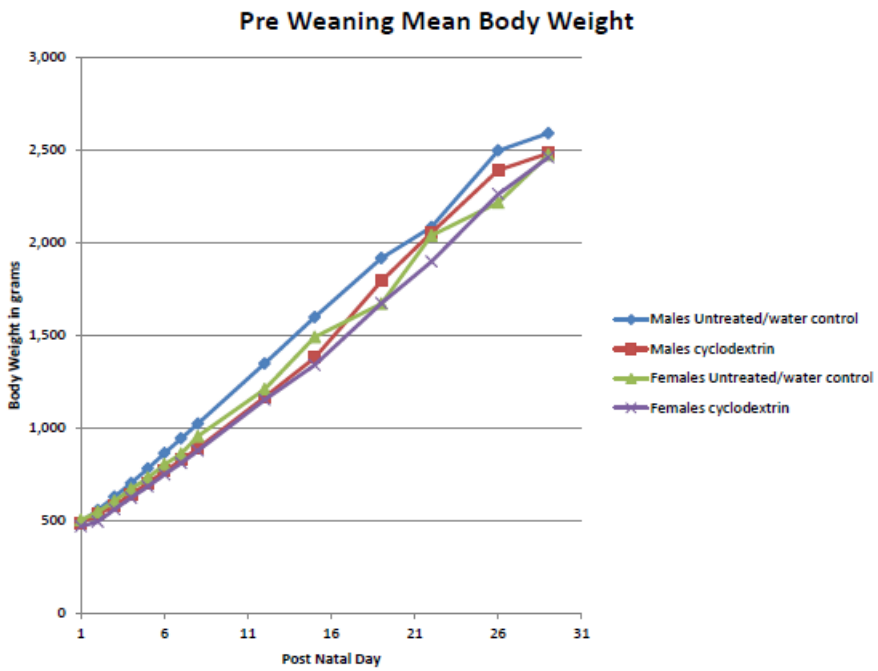
A practical example: Study design

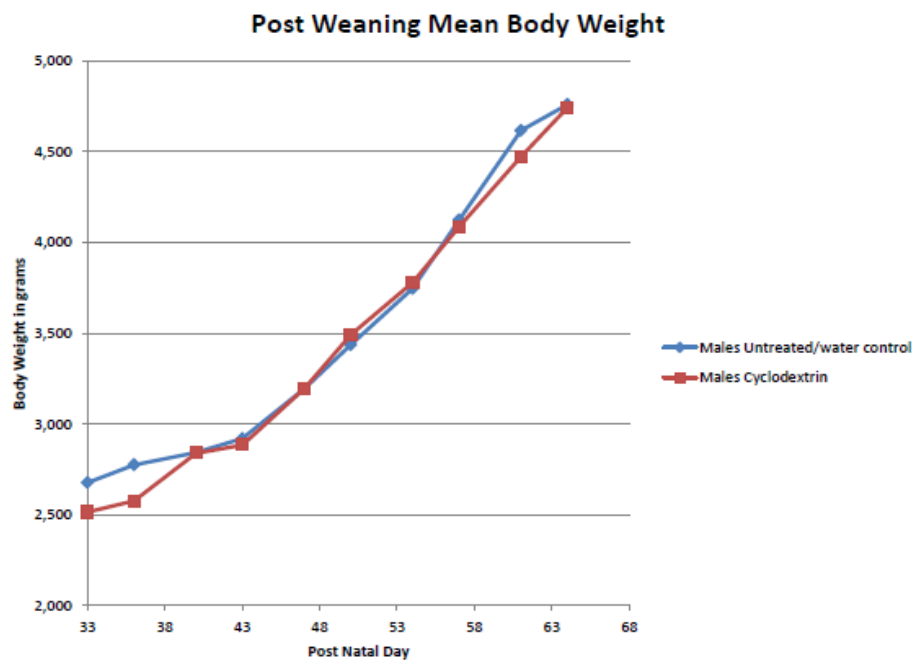
phase	grp 1 untreated			grp 2 water			grp 3 vehicle			grp 4 5 mg/kg/day			grp 5 10 mg/kg/day			grp 6 25 mg/kg/day		
	sex and pup no.		litter no.	sex and pup no.		litter no.	sex and pup no.		litter no.	sex and pup no.		litter no.	sex and pup no.		litter no.	sex and pup no.		litter no.
reversibility				**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	301	M	74		M	104		**M**	**141**	313
	F	2		M	28		M	48		F	78		F	108		M	142	
	F	3		M	29		F	49		**M**	**79**	314	**M**	**109**	315 [d]	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**	309	M	82		**F**	**112**		F	146	
	M	8		F	33		M	53		**F**	**83**		F	113		M	155	321 [c]
	M	9		F	34		M	54		F	84		F	114		M	156	
	M	10	322	M	35	312	**F**	**56**		M	85	310	M	115	316	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											M	125	317			
	F	15														M	162	319
interim killed	F	16					M	55	318	M	89	320	M	117	316	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	317	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

Background data: growth



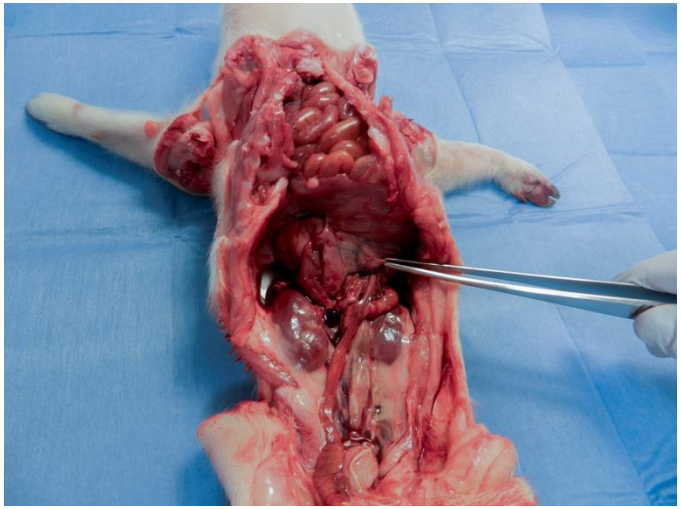


- Rapid growth
- No difference in body weights between males and females
- Slightly lower bodyweights in vehicle treated pigs from PND8-15

Background data: Clinical pathology

- Haematology
  - No sex related differences
  - No treatment related differences
  - Age related differences:
    - lower rbc, Hgb, Hct at the age of 2 weeks versus older minipigs
    - higher reticulocytes, MCV and MCH at the age of 2 weeks versus older minipigs
- Clinical chemistry
  - Sporadic differences between sexes
    - Higher cholesterol and triglycerides in females
  - Sporadic differences between treatments
    - Higher ureum in vehicle treated minipigs
  - Age related differences:
    - Decrease in ureum, bilirubin, globulin, ALP and triglycerides with increasing age
    - Increase in albumin with increasing age
- The age related differences in several parameters described above, underline the importance of having adequate background data

Background data: post mortem examination



diaphragmatic hernia

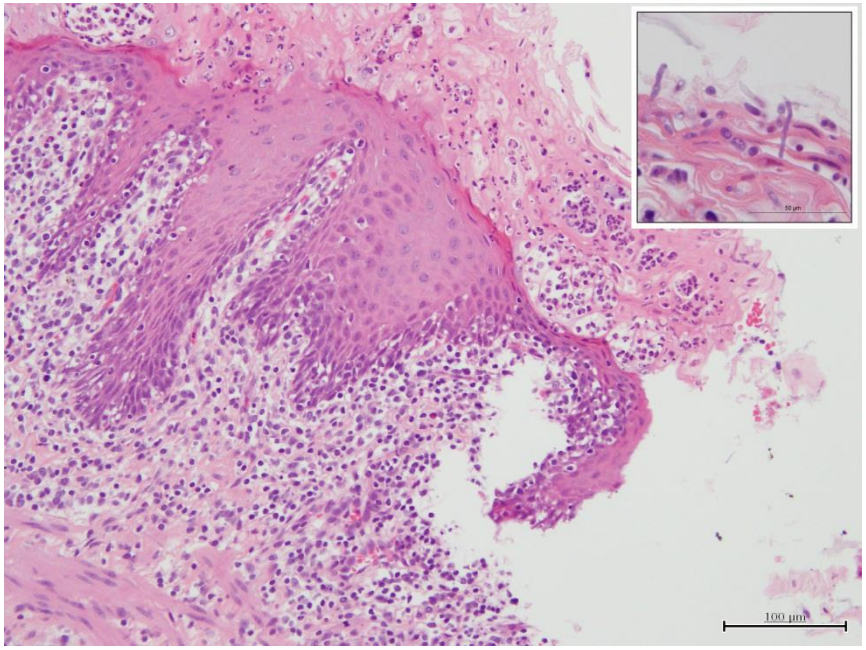
- Litter incidence: 2/25 (8%)
- Piglet incidence was 3/167 (1.80%)
- Incidence in literature: 0.24%
- Clinical observations:
  - laboured/irregular breathing,
  - death or moribund condition between birth and PND 12
  - occasional fainting when put on its back
- Considered congenital as supported by
  - the incidence of 2 in a single litter,
  - the absence of any signs of trauma at necropsy
  - observations generally occurring shortly after birth
- Care should be taken that symptoms are not attributed to the test article.
- In case of suspicion: examination of the entire litter is recommended, for example by ultrasound.

Background data: Histopathology

Stomach:

Focal ulceration of the non-glandular stomach with chronic inflammation. Mycotic colonies at the surface of the ulcerated mucosa (insert) in 5w old treated control minipig.

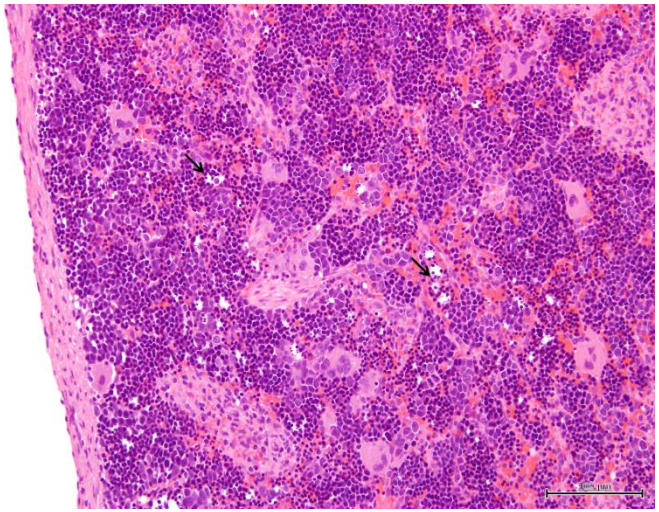
- In control and vehicle pigs
- Only post-weaning



Inflammation with erosion of the epithelium in the non-glandular stomach

- In control and vehicle pigs
- Also seen pre-weaning

Background data: Histopathology

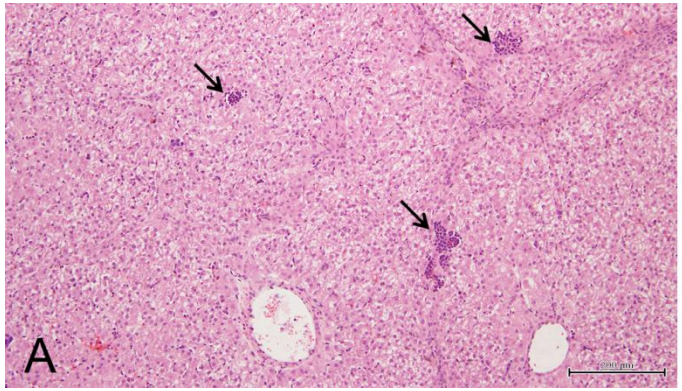


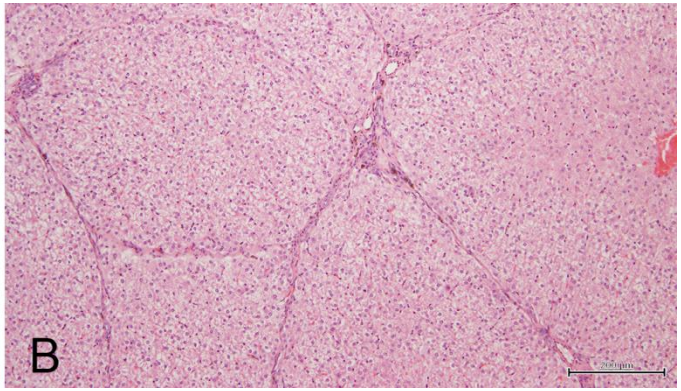
Spleen:

Extramedullary hematopoiesis and tingible body macrophages (black arrows) in spleen of a 2 week old minipig.

Liver:

A. Residual sites of fetal hematopoiesis (extramedullary hematopoiesis) in the liver of 2-week old vehicle dosed minipig.
B. Extramedullary hematopoiesis disappeared in 5-week old treated control minipig.

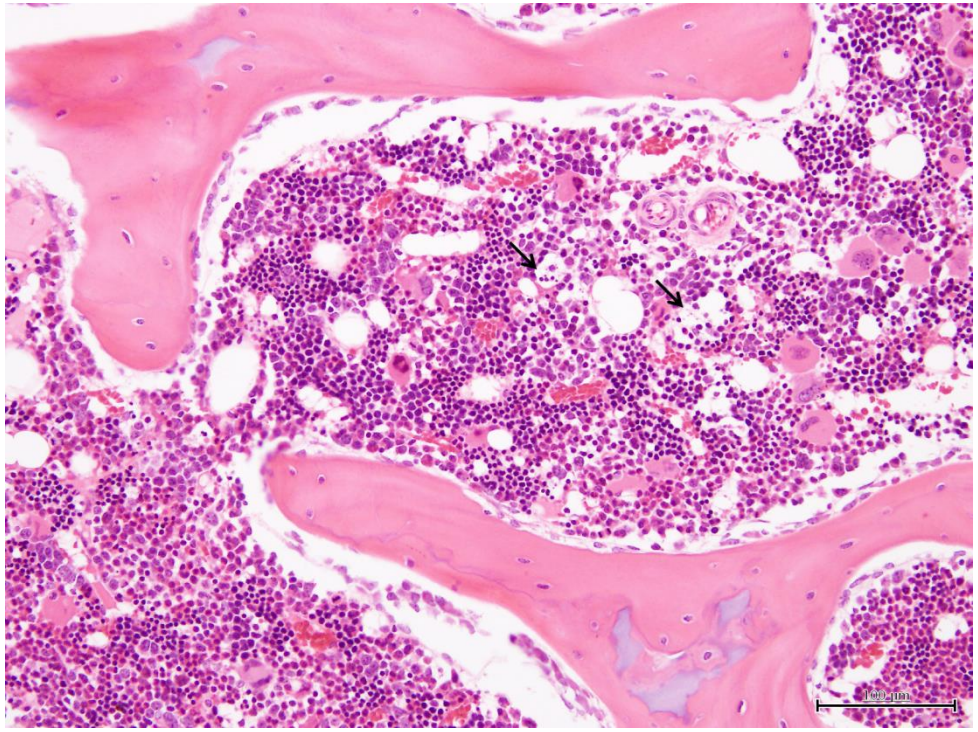




Background data: Histopathology

Bone marrow

Scattered tingible body macrophages in bone marrow (black arrows) in 5w old treated control minipig.



Conclusion

- The Göttingen Minipig is a suitable model for juvenile toxicity studies
- The minipig has many advantages compared with other non-rodent species
- Dosing can be performed from PND 1 onwards when applying proper piglet selection criteria
- It is important to include an adequate control group in view of the age related differences for several parameters

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