

Toxicological Reference Data for 6- to 9-Month Male and Female Sinclair Nanopigs®

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ABSTRACT

The use of miniature swine in safety assessment studies has grown in popularity over the past decade as the industry aims to minimize the use of canines and primates in support of the 3Rs initiatives. While there is considerable data for several breeds of miniature swine widely available to the research community, much of the data obtained from the Sinclair Nanopig® is proprietary to the organizations utilizing the model. The Sinclair Nanopig® is comparable to the well-established breeds, such as Yucatan and Göttingen minipigs®, in terms of physiology and metabolism, but is more similar in body weight to a beagle dog when maintained on a high-fiber, low-fat diet. This provides an advantage in using the Sinclair Nanopig® in that less test article is required for IND-enabling studies compared to the beagle dog or the Göttingen minipig®. The current poster presents reference data (body weight, hematology, serum chemistry, electrocardiography (ECG), ophthalmology, macroscopic tissue observations, and microscopic pathology) for the Sinclair Nanopig® at ages that correlate to the most common ages used in safety assessment studies. These data are critical for toxicologists and pharmacologists as a reference for normal variation among animal models used in nonclinical drug development. The goal of this poster is to expand upon the standard reference data for the Sinclair Nanopig® available to the entire research community.

INTRODUCTION

Miniature swine is a viable option as a non-rodent test system for studies to evaluate the safety and efficacy of new (bio)pharmaceuticals. It has long been recognized that pigs share many anatomical, physiological, and functional similarities with humans, and miniature swine are easily managed in a laboratory setting. There are several lineages of miniature swine that are purpose-bred for biomedical research. The Hormel Institute developed the Sinclair Miniature Swine in 1949. This lineage is also known as the "Minnesota Minipig" or "Hormel Minipig" and is one of the background lineages used to develop the Göttingen minipigs[®]. The Sinclair Nanopig[®] was created from the Sinclair miniature swine (Sinclair S-1) through a concerted effort that involved selective breeding for smaller size and controlled feeding of a high-fiber, low-fat minipig diet. This resulted in a miniature swine model comparable in body weight to a beagle dog, which mitigates previous challenges associated with utilizing swine models for standard pre-clinical studies, namely the amount of test articles needed for INDenabling studies. Downsizing of the Sinclair S-1 did not involve any genetic manipulations to the lineage. Thus, the background data established in the Sinclair S-1 miniature swine remains highly relevant for the downsized Sinclair Nanopig[®]. However, as the request for the Sinclair Nanopig[®] in nonclinical drug development continues to increase, it remains ever important to continue to generate a robust database of background data in the Sinclair Nanopig® itself.

METHODS

Venous blood samples were obtained from both male and female Sinclair Nanopigs® from the following age groups: 2-3, 4-5, 6-7, 8-9, 10-11, and >12 months. Standard panels for hematology and serum chemistry, consistent with those performed for toxicology studies, were generated using GLP-validated equipment. Electrocardiography (ECG) and ophthalmology examinations were conducted for male and female Sinclair Nanopigs® at 3, 4-5, 6-7, and over 9 months of age. A board-certified veterinary ophthalmologist performed the ophthalmology examinations using a portable slit-lamp biomicroscope and a binocular indirect ophthalmoscope for the posterior segment. ECG leads I, II, III, aVR, aVF, and aVL were collected, and the following variables were derived: heart rate, PR-interval, QT-interval, QRS-interval, ST-interval, P-wave, and T-wave durations. The rhythm was also carefully reviewed for irregularities and arrhythmia. The pathology data were generated from a group of 25 young adult male Sinclair Nanopigs®.

Table 1. Hematology Data

	Male 2-3 Months (n=54)		Male 2-3 Months (n=54) Female 2-3 Months (n=46)			46)	Male 4-5 Months (n=42) Female 4-5 Months (n=40)					Male	6-7 Mo	onths (n	=50)	Fem	ale 6-7 N	/lonths (r	n=50)	Male 8-9 Months (n=50)				Female 8-9 Months (n=51)								
Hematology Parameters	Mean	SD	98 Refe	5% rence nge	Mean	SD	95% Ref	erence	Mean	SD	95% Re	eference nge	Mean	SD	95% Re	ference		SD	9: Refe	5% rence nge	Mean	SD	95% Re	ference nge	Mean	SD	95% Re	eference nge	Mean	SD	95% Re	eference nge
WBC (10 ³ /μL)	14.5	2.9	9	19.8	14.2	2.4	10.2	18.3	13.7	2.7	9.5	19.9	13.77	2.5	9.3	18.8	14.3	4.1	6.2	22.4	11.4	2.5	6.3	16.4	13.9	3.0	7.9	20.0	11.6	2.5	6.6	16.5
RBC (10 ⁶ /μL)	7.7	0.8	6.2	9.1	7.9	0.7	6.5	9.2	7.7	0.9	6.7	9.8	7.57	0.5	6.8	8.5	7.5	0.6	6.3	8.7	7.7	0.6	6.5	8.9	7.8	0.9	6.0	9.5	7.4	0.5	6.5	8.4
Hgb (g/dL)	14.3	1.3	11.8	16.6	14.5	1	12.5	16	14.7	1.9	11.4	17.9	14.54	0.9	12.9	16.2	14.4	1.2	12.0	16.8	15.2	1.1	12.9	17.4	14.6	1.3	12.0	17.3	14.9	0.8	13.2	16.6
HCT (%)	43.3	3.8	36.2	50.7	43.3	3.2	37.1	48.9	43.2	4.4	35.1	50.3	41.25	3.2	35.8	47.7	43.2	3.6	36.1	50.4	45.5	2.7	40.1	50.9	45.1	4.3	36.5	53.8	45.0	2.7	39.7	50.3
MCV (fL)	56.4	3.4	49.9	62.1	55.5	1.9	52.3	59.3	56.7	3.1	51.5	62.3	55.01	3.7	49	61.2	57.6	1.8	54.1	61.2	59.6	3.2	53.3	66.0	58.4	3.3	51.9	65.0	60.7	3.7	53.4	68.1
MCH (pg)	18.5	1	16.4	20.1	18.5	0.8	16.8	19.9	19.3	1.4	16.8	22.1	19.2	1	17.6	20.8	19.2	0.7	17.8	20.6	19.9	1.0	18.0	21.8	19.0	1.1	16.8	21.1	20.0	0.8	18.4	21.7
MCHC (g/dL)	32.9	0.8	31.4	34.4	33.3	0.8	31.8	34.6	33.8	1.7	30.7	36.8	34.94	1.2	32.4	36.5	33.4	1.2	30.9	35.8	33.4	1.4	30.6	36.1	32.5	1.1	30.2	34.7	33.0	1.2	30.6	35.5
PLT (10 ³ /μL)	564.4	128.3	351.4	826.5	562.6	113.4	335.4	771.5	470.6	101.9	324.3	634.2	441.12	80.3	259.7	588.7	460.7	155.7	149.3	772.2	395.8	131.7	132.3	659.3	465.2	138.9	187.4	742.9	386.0	122.1	141.8	630.1
NEU (10 ³ /μL)	3.4	1.5	0.5	6.2	2.8	1.3	0.1	5.3	3.7	1.5	1.6	6.4	3.19	1	1.4	5.1	5.2	3.6	0.0	12.3	2.5	1.3	0.0	5.1	4.7	2.6	0.0	9.9	2.7	1.7	0.0	6.1
NEU (%)	24.2	9.8	3.9	42.7	21.3	6.8	10.9	36	28.6	10.4	11.9	47.8	22.68	6	11.2	36.3	32.9	15.9	1.0	64.7	21.9	8.6	4.8	39.0	33.8	13.5	6.8	60.7	23.3	9.5	4.4	42.3
LYM (10 ³ /µL)	9.4	2.5	4.1	13.9	10.1	2	7	13	8.8	1.7	5.7	11.3	9.85	2.3	5.8	14.4	7.7	2.4	2.8	12.6	8.1	1.9	4.3	11.8	7.9	2.0	3.9	11.8	7.4	1.8	3.9	11.0
LYM (%)	65.7	10.8	39.8	80.2	72.3	5.1	61.9	80.1	64.8	9.5	46.6	79.4	71.65	4.8	64.8	79.8	54.3	10.7	33.0	75.7	71.2	9.0	53.2	89.2	57.1	12.0	33.1	81.0	65.6	10.6	44.5	86.7
MONO (10 ³ /μL)	0.63	0.18	0.37	1.13	0.85	0.38	0.25	1.7	0.49	0.15	0.28	0.75	0.53	0.17	0.31	0.88	1.0	0.9	0.0	2.9	0.4	0.3	0.0	0.9	0.6	0.5	0.0	1.5	0.5	0.3	0.0	1.2
MONO (%)	4.69	1.53	2.42	7.7	5.45	1.99	2.8	10.09	3.68	1.19	1.69	6.3	3.9	0.98	2.59	5.75	7.5	8.4	0.0	24.4	3.7	2.3	0.0	8.4	4.1	2.8	0.0	9.8	4.5	2.1	0.4	8.6
EOS (10 ³ /μL)	0.19	0.13	0.03	0.45	0.15	0.07	0.04	0.3	0.14	0.07	0.05	0.29	0.18	0.07	0.09	0.3	0.2	0.1	0.0	0.4	0.2	0.1	0.0	0.5	0.2	0.1	0.0	0.4	0.3	0.2	0.0	0.7
EOS(%)	1.4	0.92	0.27	3.44	1.07	0.58	0.41	2.49	0.95	0.41	0.39	1.91	1.26	0.45	0.59	2.31	1.3	0.9	0.0	3.0	1.8	1.0	0.0	3.9	1.2	0.6	0.0	2.5	2.2	1.7	0.0	5.6
BAS (10 ³ /µL)	0.06	0.05	0.01	0.15	0.04	0.01	0.02	0.07	0.05	0.04	0.01	0.12	0.03	0.01	0.01	0.06	0.1	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.2	0.0	0.0	0.0	0.1
BAS (%)	0.38	0.27	0.1	1	0.28	0.08	0.2	0.4	0.35	0.22	0.1	0.7	0.22	0.07	0.1	0.31	0.4	0.2	0.0	0.9	0.4	0.2	0.0	0.7	0.5	0.4	0.0	1.3	0.4	0.1	0.1	0.6
LUC (10 ³ /µL)	0.16	0.09	0.03	0.34	0.13	0.09	0.03	0.37	0.11	0.08	0	0.29	0.13	0.03	0.09	0.17	0.5	1.0	0.0	2.4	0.1	0.1	0.0	0.4	0.3	1.2	0.0	2.7	0.4	0.9	0.0	2.2
LUC(%)	1.05	0.57	0.3	2.3	0.98	0.75	0.2	2.82	0.74	0.46	0	1.66	1.09	0.38	0.7	1.8	3.5	7.3	0.0	18.1	1.0	0.9	0.0	2.8	2.3	7.4	0.0	17.1	3.7	6.5	0.0	16.7
RET (10 ⁹ /L)	84.1	43.8	19	177	95.2	45.5	28.6	177.2	77.4	25.1	38.1	128.2	65.63	27.2	25.2	124.1	91.5	43.5	4.5	178.5	90.0	30.9	28.2	151.8	90.4	39.3	11.8	169.0	82.7	44.8	0.0	172.4
RET (%)	1.1	0.6	0.3	2.5	1.2	0.6	0.4	2.7	1	0.3	0.5	1.7	0.86	0.3	0.3	1.6	1.2	0.6	0.1	2.3	1.2	0.4	0.3	2.0	1.2	0.5	0.1	2.2	1.1	0.6	0.0	2.3

Table 2. Serum Chemistry Data

Serum	Mal	e 2-3 M	onths (n	=54)	Fema	ale 2-3 I	Months ((n=46)	Male	e 4-5 Mo	onths (n	=42)	Fema	ale 4-5 N	<i>l</i> lonths ((n=40)	Mal	le 6-7 M	onths (n	=50)	Femal	le 6-7 N		<u> </u>	Mal	e 8-9 Mo			Fema	le 8-9 N	lonths (ı	n=50)
Chemistry Parameters	Mean	SD		ference nge	Mean	SD		eference nge	Mean	SD		eference nge	Mean	SD		eference ange	Mean	SD		eference ange	Mean	SD	Refe	5% erence inge	Mean	SD	Refe	5% erence inge	Mean	SD	95% Ref Rar	
BUN (mg/dL)	15.2	5.3	6.1	22.9	18.2	3.2	13	25	13.9	3.9	8	22	14.8	2.7	9.9	19	13.6	3.3	6.9	20.2	14.3	3.4	7.5	21.1	13.7	4.9	3.9	23.4	16.1	2.4	11.3	20.9
CRE (mg/dL)	0.9	0.2	0.5	1.3	0.9	0.2	0.5	1.3	1.1	0.2	0.8	1.5	1.1	0.1	0.9	1.3	1.1	0.1	0.8	1.4	1.1	0.2	0.6	1.5	1.1	0.2	0.6	1.6	1.3	0.2	0.9	1.6
GLU (mg/dL)	97.8	16.5	71.4	137.6	88.3	10.2	70.5	108.8	84.5	12.5	65.9	108.5	76.9	15.6	56	102.2	102.0	29.0	43.9	160.1	78.5	16.3	46.0	111.1	96.0	23.5	49.1	142.9	78.7	16.4	45.8	111.6
Na (mEq/L)	144.3	2.7	139	149.1	143.6	4.3	136.1	151	143.4	3.1	138.9	150	144	1.9	141	147.1	143.7	9.7	124.2	163.2	138.6	13.8	111.0	166.2	141.2	5.4	130.3	152.0	144.1	3.1	138.0	150.2
K (mEq/L)	5.9	0.7	4.6	7.1	6.4	0.7	5	7.8	5.8	8.0	4.3	7.1	6	0.8	4.8	7.3	5.6	0.8	4.1	7.2	5.7	0.7	4.4	7.1	5.5	1.0	3.6	7.5	6.1	0.6	4.8	7.4
CI (mEq/L)	102.3	1.8	99	106	102.9	2.7	98	107.9	101.1	2.3	96	104	103.5	2.3	100	107	101.4	6.5	88.4	114.5	99.4	8.1	83.1	115.6	99.6	4.4	90.8	108.5	101.9	2.1	97.6	106.2
ALP (U/L)	96.4	22.6	63.1	135.8	85.3	19.5	55.1	121.6	68.3	18.9	39	106.2	66.9	16.5	32.9	98.2	99.2	37.3	24.6	173.9	65.3	18.4	28.5	102.0	90.6	60.7	0.0	212.1	56.8	15.9	25.0	88.6
ALT (U/L)	39.4	6.4	28.9	55.2	41.7	8.9	25.1	59	37.8	7.8	25.6	50.4	42.8	5.6	34.9	53.2	40.1	10.9	18.4	61.9	41.9	10.3	21.4	62.5	45.8	15.8	14.2	77.5	45.2	9.8	25.6	64.8
AST (U/L)	32.1	7.7	21	47	30.9	8.7	19	51.8	27.7	7.5	18	43.2	28.1	5.7	17.6	38	54.4	57.6	0.0	169.6	29.9	13.8	2.4	57.4	45.0	42.7	0.0	130.5	35.7	32.0	0.0	99.7
TBIL (mg/dL)	0.15	0.06	0.1	0.29	0.13	0.05	0.1	0.2	0.13	0.05	0.1	0.2	0.1	0	0.1	0.1	0.2	0.1	0.0	0.3	0.1	0.0	0.0	0.2	0.2	0.1	0.1	0.3	0.2	0.1	0.0	0.3
DBIL (mg/dL)	0.03	0.02	0	0.05	0.03	0.01	0.01	0.04	0.03	0.01	0.02	0.05	0.03	0.04	0	0.1	0.1	0.1	0.0	0.3	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
CK (U/L)	370	193.7	113	718	390.8	213.6	165	828.8	454.7	274.8	175.3	1246.1	283.8	116	156	541.3	486.0	550.1	0.0	1586.2	505.1	733.7	0.0	1972.6	499.3	617.7	0.0	1734.7	612.3	727.8	0.0	2067.9
GGT (U/L)	44.3	9	27.2	58.9	46.1	11.5	26.4	70.8	44.5	9.5	29	63.1	48.3	10.5	29.9	66.3	192.9	347.8	0.0	888.5	40.1	13.5	13.1	67.2	131.1	178.8	0.0	488.6	49.4	17.4	14.6	84.2
TP (g/dL)	6.2	0.6	5.1	7.1	6.1	0.4	5.4	6.8	6.6	0.4	5.9	7.3	6.2	0.2	5.8	6.7	6.6	0.5	5.5	7.7	6.2	0.8	4.6	7.9	7.0	0.6	5.7	8.2	6.8	0.5	5.8	7.7
ALB (g/dL)	4.1	0.4	3.1	4.8	3.9	0.3	3.5	4.4	4.2	0.3	3.8	4.7	4	0.2	3.5	4.3	4.0	0.5	3.0	5.1	3.7	0.9	1.9	5.4	5.1	8.4	0.0	22.0	3.7	0.8	2.1	5.3
GLOB (g/dL)	2.2	0.4	1.5	3	2.2	0.3	1.7	2.8	2.6	0.4	1.8	3.2	2.3	0.3	1.8	2.8	2.6	0.6	1.4	3.7	2.6	0.5	1.7	3.5	3.1	0.7	1.6	4.5	14.0	61.4	0.0	136.8
A\G ratio	1.8	0.4	1.2	2.7	1.8	0.4	1.1	2.4	1.7	0.4	1	2.4	1.8	0.3	1.2	2.2	1.7	0.5	0.7	2.6	1.5	0.5	0.5	2.5	1.4	0.4	0.5	2.3	1.3	0.4	0.4	2.2
AMY (U/L)	1044.3	250.5	678.1	1534.5	930.3	228.1	591	1462.3	1015.3	266.8	586.8	1511.7	972.9	195.4	643.6	1349.3	1075.1	531.3	12.4	2137.8	917.1	293.7	329.8	1504.5	959.2	269.2	420.8	1497.6	972.8	242.7	487.3	1458.2
Ca (mg/dL)	11.3	0.6	10.5	12.5	11.4	0.5	10.3	12.3	11.1	0.5	10.4	12.3	10.8	0.5	10.1	11.7	11.2	0.8	9.6	12.9	10.5	1.7	7.2	13.9	11.2	0.6	10.0	12.4	11.1	0.6	9.8	12.4
PHOS (mg/dL)	8.1	0.8	6.3	9.3	8.1	0.6	7	9.3	8.1	0.9	6.8	9.7	7.5	0.5	6.5	8.3	7.8	0.9	6.0	9.7	7.1	1.0	5.1	9.0	7.4	0.9	5.7	9.2	7.3	0.4	6.4	8.1
CHOL (mg/dL)	94.9	24.8	51.1	129.7	105.5	29.8	62.3	160.9	103.2	19.8	67	138.2	92.4	12.9	68.5	113.2	94.9	24.7	45.4	144.3	100.2	24.8	50.6	149.9	82.3	14.4	53.5	111.1	127.6	28.2	71.2	184.0
TRIG (mg/dL)	43.8	15	23.7	79.4	42.5	15.8	22.1	81.8	34.1	11.7	18	59.2	33.2	9.3	18.9	54.1	43.3	17.9	7.5	79.1	43.3	17.8	7.7	78.9	37.5	12.8	11.9	63.1	46.7	20.1	6.5	86.9
LDH (U/L)	513.5	126.7	287.7	753	526.6	63.8	427	666	397.4	68.2	263	489.3	497.2	66.2	371.9	636.6	400.4	117.9	164.6	636.1	424.4	99.2	226.1	622.7	454.2	252.1	0.0	958.3	496.2	286.3	0.0	1068.8

Table 3. Ophthalmology Findings

Observation	n	Left	Right
Discharge	148	80 (54%)	79 (53%)
Discharge 2	148	22 (15%)	24 (16%)
Congestion	148	6 (4%)	6 (4%)
Corneal Opacity	148	1 (1%)	0

Table 4. ECG Parameters

			Male		Female Female							
Parameter	Mean	SD	R	Range	Mean	SD	Range					
	Weatt	30	Min	Max	Weali	30	Min	Max				
HR (bpm)	94.0	20.9	60.9	153.9	100.9	16.9	72.1	157.3				
RR (msec)	672.8	144.8	389.9	998.0	614.6	95.1	381.7	833.9				
PR (msec)	99.6	12.0	87.1	133.2	97.8	14.4	79.3	155.1				
QRS (msec)	57.6	8.6	38.8	72.0	56.3	11.4	31.7	72.7				
QT (msec	268.6	33.5	212.7	344.4	255.3	18.4	194.7	295.1				
QTc [Fridericia Correction] (msec)	308.0	31.8	254.2	379.3	301.6	23.9	242.7	349.3				

 Table 5. Pathology Findings

Gross Pathology (n=25):	No gross findings were identified at necropsy								
Microscopic Findings (n=25):	Incidence:	Comparison:							
Mononuclear cell infiltrates	High incidence in larynx (25/25) and kidney (21/25), often accompanied by renal tubular degeneration. Low incidence (≤4/25) noted in lung (peribronchialoar), brain (perivascular), liver, salivary glands, skin, and adrenal glands.	Common per INHAND: Nonproliferative and Proliferative Lesions of the Minipig. (Skydsgaard, 2021)							
Mineralization in the pituitary gland	High incidence (17/25) of minimal multifocal	Common per INHAND. (Skydsgaard, 2021)							
Testes background changes Sexual maturity also assessed: 21 mature (bilateral), 1 mature/immature (unilateral), 1 peripubertal.	Low incidence (3/23; 13%) of hypospermatogenesis or degeneration/atrophy consistent with unilateral atrophy/degeneration of testis (prostate and contralateral testis mature/peripubertal).	Common per INHAND. (Skydsgaard, 2021) (Up to 50% incidence in Göttingen Minipigs.)							
Retained cartilage cores	High incidence (18/24) of was observed in the sternum/rib but not long bones.	Not mentioned in INHAND							



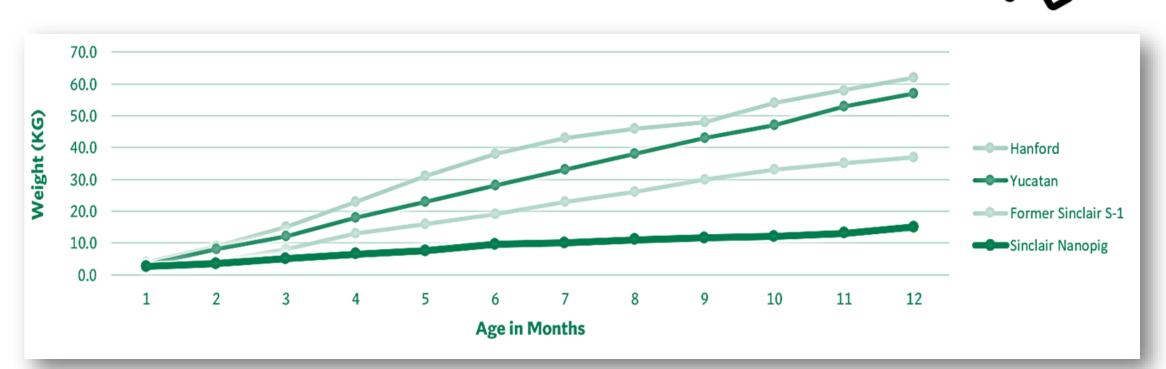


Figure 1:

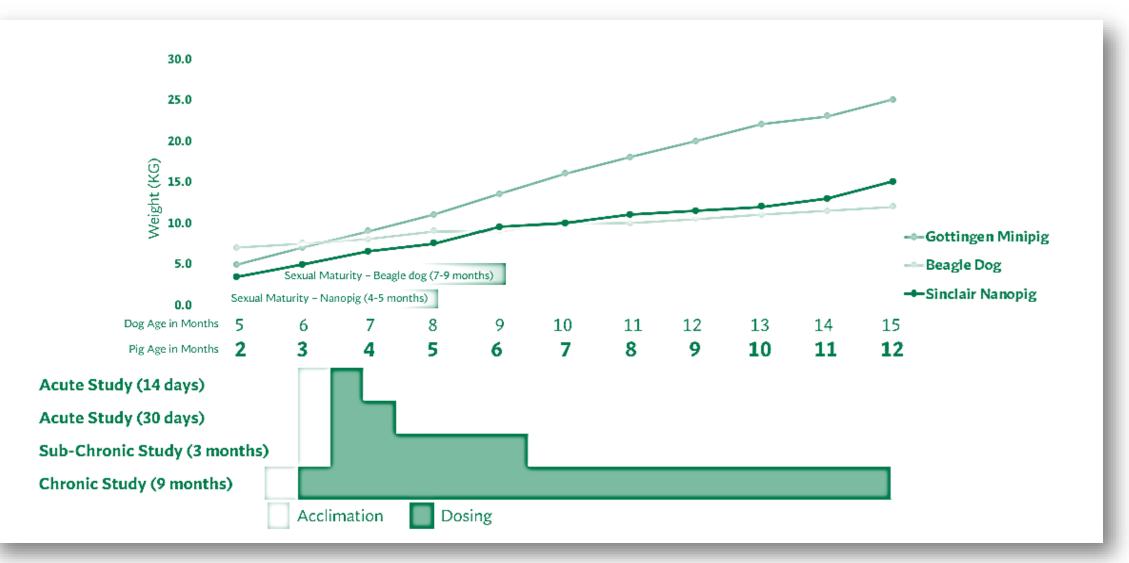


Figure 2:

RESULTS

Body weight data demonstrated that when the Sinclair Nanopig® is maintained on a low-fat, high-fiber diet, body weight measurements are close to those of a beagle dog. Hematology and serum chemistry data from each age group were analyzed to obtain the mean, standard deviation, and 95% reference range. All data (clinical and anatomic pathology) were then compared to that of the original Sinclair S-1. The majority of the hematology, serum chemistry, and ECG data of the Sinclair Nanopig® overlaps with that of the original Sinclair S-1 lineage. No differences in ophthalmological findings were observed between age groups or between males and females, consistent with findings from other lineages. The comparison of the pathology data demonstrates findings comparable to those of Göttingen minipigs®.

CONCLUSION

This data demonstrates consistency in the background data within the Sinclair S-1 lineage after downsizing the animal to the Nanopig. Comparison with data from other miniature swine lineages demonstrates that the Sinclair Nanopig[®] is a viable option when considering a miniature swine model for safety assessment studies and is comparable with other commonly used lineages. The weight of the Sinclair Nanopig[®] is closer to a beagle dog, especially when adjusted for age to match when sexual maturity begins, and the age at which dosing for IND-enabling studies most often occurs.

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