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Safety and Health Research

Interspecies Comparison of Embryo-fetal Data among control groups of Sprague-Dawley rats, New Zealand White rabbits and Gottingen minipigs

France-Helene Paradis1, Robert Tavcar[1], Francine Beaudry[1], Anne Marie Downey[1], Raluca Kubaszky[1], Sisse Ellemann-Laursen[2], Andy Makin[2], Katherine Hill[3], Rhonda Gilmore[3], Pramila Singh[4], Judit Hargitai[5], Francois Spézia[4], Roy Forster[4], Simon Authier[1]

[1]Citoxlab North America, Laval, Canada. [2]Citoxlab Denmark, Ejby, Denmark. [3]Citoxlab USA, LLC, Stilwell, USA. [4]Citoxlab France, Evreux, France. [5]Citoxlab Hungary, Veszprem, Hungary.

INTRODUCTION

Embryo-fetal developmental (EFD) toxicity studies are required by regulatory agencies to assess the potential teratogenicity of chemicals and pharmaceuticals; rat and rabbit are typically the rodent and non-rodent species of choice for these studies. Recently, the minipig has also emerged as an important alternative model when the rat and rabbit are inappropriate, as it presents numerous advantages over other large animals. Defining the fundamental similarities and differences between the rat, rabbit and minipig is necessary to understand the advantages and limitations of each species and aid in selecting the appropriate model system. Evaluation of laboratory-generated control data is a useful and practical method to obtain knowledge of the incidence of spontaneous congenital abnormalities. Here, we assessed the incidence of external, visceral and skeletal alterations, as well as pregnancy outcome in rat, rabbit and minipig control data from EFD toxicity studies conducted at our laboratory facilities over the last two decades.

MATERIALS AND METHODS

Control data was compiled and evaluated from 44 rat (Sprague-Dawley), 21 rabbit (New Zealand White) and 4 minipig (Göttingen) EFD toxicity studies conducted in our facilities located in France[1], USA[2] and Denmark[3] between 1993 and 2016. Details of these studies are presented in Table 1. Pregnant dams received vehicle or reference item (RI) throughout the period of major organogenesis. Caesarian-sections were performed on pregnant dams at the end of gestation, prior to parturition. Ovaries and uterine contents were examined to assess pregnancy outcome and fetuses were examined for external, internal and skeletal malformations and variations.

Table 1 - *Summary of Study Design and Method*

Dosing Period	Rat	GD 6-17
	Rabbit	GD 7-19
	Minipig	GD 11-35
Exposure Routes	DA, OG, IM, IV, SC	
Vehicle/RI	Various commercial diluent [4]	
Caesarian Section	Rat	GD 21
	Rabbit	GD 29
	Minipig	GD 110-112
Evaluations	- Pregnancy outcome - External and internal malformations and variations [5] - Head examination by Wilson's Technique for malformation and variations - Alazarin red S stained skeletal examination for malformations and variations	
Fetuses Evaluated	Rat	5948 fetuses from 871 litters
	Rabbit	4352 fetuses from 322 litters
	Minipig	378 fetuses from 70 litters

GD: Gestational Day; DA: Dietary admixture;
OG: Oral gavage; IM: Intramuscular;
IV: Intravenous; SC: Subcutaneous

[1]Citoxlab France (Rat and Rabbit),
[2]Citoxlab USA (Rat),
[3]Citoxlab Denmark (Mini-pig),
[4]Vehicles/Reference items: water, saline, corn oil, corn oil/maisine/cremophor EL, Tween80, glycerol, phosphate/glycerol, gelatin/mannitol, Tris-buffered saline, phosphate buffered saline, NaPO3 buffer, methylcellulose, carboxymethylcellulose, carboxymethylcellulose/ Tween80, hydroxymethylcellulose hydropopylcellulose/sodium-lauryl-sulfate, hydroxypropylcyclodextrin, alhydrogel, SAFE A04C, PEG400, tetralose hydrate,
[5]Many external, skeletal and visceral malformations and variations were observed at low incidence in at least one of the three species investigated. However, in this study we reported selected findings which were observed in two species or more.

DISCUSSION AND CONCLUSIONS:

- Our results indicate that pregnancy rates across species were similar. However, pre-implantation loss was more frequent than post-implantation loss in the rat and rabbit, whereas the opposite was observed in the minipig.
- Fused sternebrae and fused or absent ribs were observed in all three species. Incomplete ossification of the frontal, parietal and hyoid bones of the skull were variations frequently observed in the rat and minipig compared to the rabbit whereas a higher incidence of incomplete ossification of the interparietal and supraoccipital bones was observed in the rat. In all species, a higher incidence in incomplete ossification of the centrum was observed in more rostral vertebrae (cranial and thoracic) than in the more caudal vertebrae (lumbar and caudal).
- In addition, the minipig presents a higher spontaneous incidence of heart malformations consistent with humans, as congenital heart defects are the most common type of birth defects in humans (approximately 1% of births); notably, all septum defects and malformation of the heart valves were more prevalent in the mini-pig. In contrast, the rabbit presented the most malformations of the major blood vessels.
- Species differences in spontaneous malformations highlight potential translational considerations during embryo-fetal development study interpretation.

Table 2 - *Pregnancy Outcome*

		Rat	Rabbit	Minipig
Maternal Outcomes	No. Inseminated	983	549	74
	No. Pregnant	908	525	70
	Pregnancy Rate (%)	92.4	95.6	94.7
	No. Alive at Term	875	486	70
	Pregnancy Survival (%)	96.4	92.6	100.0
Uterine Findings	Total Resorptions	0.6	0.5	0.7
	No. Implantations	13.9	10.0	6.2
	No. Corporo Lutea	15.7	11.5	6.7
	Pre-Implantation Loss	10.9	13.8	7.5
	Post-Implantation Loss	4.7	8.1	12.5
Fetal Outcomes	No. Live Fetuses per Litter	13.3	9.2	5.5
	No. Dead Fetuses per Litter	0.02	0.19	0.01
	Dead/Alive Fetuses per Litter (%)	0.17	2.09	0.09

Table 3 - *Selected External Alterations*

		Rat (n=11460)		Rabbit (n=4486)		Mini-Pig (n=378)	
		No.	%	No.	%	No.	%
Malformations	Anal Atresia	1	0.01	2	0.04	1	0.26
	Acaudate	3	0.03	1	0.01	-	-
	Cleft Palate	2	0.02	2	0.04	-	-
	Ombilical Hernia	1	0.01	3	0.7	-	-
	Gastroschisis	4	0.03	7	0.16	-	-
	Omphalocele	7	0.06	1	0.02	-	-
Variations	Pale Fetus (Skin Discoloration)	2	0.02	1	0.02	-	-
	Domed Head	1	0.01	2	0.04	3	0.79
	Open eye	2	0.02	-	-	6	1.60
	Malrotated Paw	2	0.02	19	0.42	-	-
	Malrotated Limb	2	0.02	2	0.04	-	-

Table 4 - *Selected Skeletal Alterations*

		Rat (n=5948)		Rabbit (n=4352)		Mini-Pig (n=209)	
		No.	%	No.	%	No.	%
Malformations	Fused Sternebrae	5	0.08	68	1.56	67	32.06
	Fused Rib	4	0.07	12	0.28	2	0.96
	Absent Rib	3	0.05	1	0.02	1	0.48
Variations	Frontal Incomplete Ossification	82	1.38	21	0.48	18	8.61
	Parietal Incomplete Ossification	207	3.48	2	0.05	13	6.22
	Interparietal Incomplete Ossification	597	10.04	57	1.31	-	-
	Supraoccipital Incomplete Ossification	195	3.28	-	-	-	-
	Hyoid Incomplete Ossification	467	7.85	79	1.82	19	9.09
	Cervical Vertebrae Incomplete Ossification of Centrum	338	5.68	56	1.29	7	3.35
	Thoracic Vertebrae Incomplete Ossification of Centrum	926	15.57	13	0.30	3	1.44
	Lumbar Vertebrae Incomplete Ossification of Centrum	10	0.17	-	-	2	0.96
	Caudal Vertebrae Incomplete Ossification of Centrum	28	0.47	24	0.55	-	-

Table 5 - *Selected Visceral Alterations, excluding heart and major vessel alterations*

		Rat (n=5507)		Rabbit (n=4352)		Mini-Pig (n=378)	
		No.	%	No.	%	No.	%
Malformations	Absent Gallbladder	-	-	2	0.05	1	0.26
	Fused Kidneys	-	-	1	0.02	3	0.79
Variations	Dilated Cerebral Ventricle	7	0.13	10	0.23	-	-
	Abdominal Edema	2	0.04	12	0.28	1	0.26
	Small Gallbladder	-	-	9	0.21	2	0.53
	Dilated Renal Pelvis	62	1.13	23	0.53	-	-

Table 6 - *Visceral Alterations of the Heart*

		Rat (n=5507)		Rabbit (n=4352)		Mini-Pig (n=378)	
		No.	%	No.	%	No.	%
Malformations	Misshapen Heart	-	-	-	-	1	0.26
	Thin Cardiac Wall	-	-	2	0.05	-	-
	Cardiomegaly	-	-	3	0.07	-	-
	Reduced Heart Size	8	0.15	-	-	-	-
	Cor Trinocular	-	-	1	0.02	-	-
	Ventricular Septum Defect	-	-	1	0.02	7	1.85
	Thick Ventricular Septum	-	-	1	0.02	-	-
	Atrium Septum Defect	-	-	-	-	8	2.12
	Atrioventricular Septum Defect	-	-	-	-	1	0.26
	Absent Atrioventricular Valve	-	-	-	-	1	0.26
	Absent Aortic Valve	-	-	-	-	1	0.26
	Absent Chordae Tendinae	-	-	-	-	1	0.26
	Absent Papillary Muscle	-	-	-	-	1	0.26
Variations	Hydropericardium	-	-	4	0.09	-	-
	Enlarged Ventricular Chamber	-	-	6	0.14	-	-
	Enlarged Ventricular Wall	-	-	2	0.05	-	-
	Enlarged Atrial Chamber	2	0.04	4	0.09	-	-

Table 7 - *Visceral Alterations of the Major Blood Vessels*

		Rat (n=5507)		Rabbit (n=4352)		Mini-Pig (n=378)	
		No.	%	No.	%	No.	%
Malformations	Dilated Aortic Arch	-	-	13	0.3,	-	-
	Narrowed Aortic Arch	-	-	2	0.05	-	-
	Absent Aortic Arch	-	-	3	0.07	-	-
	Narrowed Descending Aorta	-	-	1	0.02	-	-
	Dilated Pulmonary Trunk	-	-	10	0.23	1	0.26
	Narrowed Pulmonary Trunk	-	-	3	0.07	-	-
	Dilated Pulmonary Artery	-	-	1	0.02	-	-
	Malpositioned Subclavian Artery	-	-	2	0.05	1	0.26
	Retroesophageal Subclavian Artery	-	-	2	0.05	-	-
	Absent Subclavian Artery	-	-	1	0.02	-	-
	Supernumerary Artery	-	-	5	0.11	-	-
	Transposition of the Great Vessels	1	0.02	-	-	-	-
Variations	Short Innominate Artery	35	0.64	453	10.41	24	6.35
	Long Innominate Artery	-	-	-	-	2	0.53
	Absent Innominate Artery	36	0.65	296	6.80	39	10.32
	Absent Brachioencephalic Trunk	-	-	192	4.41	-	-
	Dilated Brachioencephalic Trunk	-	-	2	0.05	-	-
	Dilated Ductus Arteriosus	-	-	-	-	1	0.26
	Subclavian Artery Branching Variation	-	-	-	-	1	0.26
	Malpositioned Subclavian Artery Origin	-	-	-	-	3	0.79
	Dilated Subclavian Artery	-	-	1	0.02	-	-
	Malpositioned Carotid Artery	-	-	1	0.02	-	-
	Dilated Carotid Artery	-	-	1	0.02	-	-
	Left Side Umbilical Artery	15	0.27	-	-	-	-