CL 73 : 87 : 885

A REPORT PREPARED

FOR

RECKITT & COLMAN

----oOo----

EPILIM

TERATOGENICITY STUDIES IN THE RABBIT, RAT [redacted]

Reckitt & Colman Products Ltd.,
Dansom Lane,
Hull.

[redacted]

October, 1973

INTRODUCTION

SODIUM DIPROPYLACETATE, also known as EPILIM is an anticonvulsant intended for use in the treatment of epilepsy.

This report describes studies carried out in the rabbit, rat and mouse to investigate the teratogenic and/or embryotoxic potential of the material* PHENYTOIN, a well established anticonvulsant, has been used as a positive control. The first part of the study was performed using Dutch Belted rabbits.

MATERIALS & METHOD

Test Material - The test material was a white crystalline powder SODIUM DIPROPYL ACETATE also known as EPILIM. All of the test material was from batch PD1 lot 167. The EPILIM was hygroscopic and the material was kept in airtight containers.

PHENYTOIN was used as a comparative control. The sample was prepared by grinding tablets of PHENYTOIN SODIUM B.P. [redacted]

Animals - One hundred and twenty Dutch Belted rabbits were used in the study. They were obtained from Goodchild Bros., Crawley, Sussex.

Housing - The rabbits were housed in individual grid-floor cages all in the same area under natural lighting and with the ambient temperature thermostatically maintained at 20 ± 1°C. At the end of the dosing period the animals in the littering groups were transferred to individual solid-floor cages in the same area and provided with nesting material.

Diet - With the exception of one control group all the rabbits were allowed free access to water and to a commercial pellet diet R.G.P. (Dixons, Ware, Herts.)

The control group was allowed free access to water but during the dosing period was pair fed to match the food consumption of one of the top dose level groups.

* These studies were undertaken subsequent to studies performed in Japan using the rat and mouse which indicated that under the experimental conditions employed EPILIM was teratogenic.

Mating - The rabbits were fertilised by artificial insemination using a pooled semen sample with a sperm count of 27 - 98 million per ml. Ovulation was induced by the intravenous administration of 25 I.U. of CHORIONIC GONADOTROPHIN [redacted]. Mating was completed over a 6 day period and to avoid a temporal bias the rabbits from each day's fertilizations were allocated equally to all of the dosing groups. The day of fertilization was taken as Day 0 of gestation.

Choice of Dosage - Data supplied by the clients indicated that a dose level of 400 mg/kg would be tolerated by the rabbits and this was selected as the top dose. The clinical dose of EPILIM is expected to be about 25 mg/kg and this dose therefore represents a multiple of 16 times the clinical dose. Additional levels of 315 mg/kg, 252 mg/kg, 200 mg/kg and 100 mg/kg were used. A dose level of 150 mg/kg of PHENYTOIN was chosen. The clinical dose is approximately 5 mg/kg and this therefore represents a multiple of about 30 times the clinical dose.

Treatment - The EPILIM solutions and the PHENYTOIN suspension were prepared freshly each day and formulated to give a constant dose volume of 10 ml/kg. The rabbits were divided into treatment groups of 12 and dosed by gavage with the respective materials daily between Days 6 and 18 of gestation.

Intermediate Examination - The general condition of the animals was noted daily and the body weights recorded every 3 days throughout gestation.

The food consumption of the 400 mg/kg dose level rabbits was recorded daily during the dosing period and the control group 7 animals were pair-fed this quantity of food on the corresponding day of gestation.

One hour after the final dosing a 2 ml sample of blood was withdrawn by cardiac puncture. The plasma was seperated, frozen and forwarded to the clients for blood level determinations.

Terminal Examination - With the exception of the animals in the littering groups the rabbits were killed by cervical dislocation on Day 28 of gestation. The ovaries and uteri were removed, examined and the following data recorded :

1. Numbers of corpora lutea.

2. Numbers and positions of foetuses, resorptions and resorption sites.

3. Foetal weights.

4. Crown/rump lengths.

N.B. In these reports the term "resorption" refers to an implantation site showing both placental and embryonic or foetal tissue and "resorption sites" to implantation sites showing placental or decidual tissue only.

The foetuses were killed by an intracardiac injection of NEMBUTAL (Pentobarbitone Sodium - 60 mg/kg - Abbott). They were then skinned, examined for external and internal deformities, sexed, eviscerated and the carcasses checked for skeletal defects by X-ray examination using KODAK INDUSTREX - M film and a Machlett OEG50A tube. Free-hand razor sections were then made through the head region and examined for ocular and ventricular defects.

Littering Groups - Duplicate top dose level and control groups were set up and allowed to litter normally and to rear their young to weaning. The following data were observed :

1. Duration of gestation.

2. Numbers of pups born and weaned.

3. Pup weights at Day 7 and at weaning.

The pups were weaned at 6 weeks of age and after checking for defects in sight, hearing and balance were autopsied and sexed.

RESULTS

General Observations - The general condition of the animals remained good throughout the study and no adverse behavioural effects were noted. A number of fatalities were recorded but in each case death was due to massive haemorrhage into the pericardium following cardiac puncture. There were no dose-related trends in the group pregnancy rates and the values varied between 60.0% and 100.0%.

Body Weights - (See Table 4 and Fig. 1) - N.B. In this study the group mean body weights have been compiled from data from all the surviving animals since previous experience has shown that weight changes are not influenced by the pregnancy status of the animals.

All the test groups showed a weight decrease during the dosing period. The decreases were dose-related as was the duration of the decrease. At the 100 mg/kg and 200 mg/kg levels the decreases amounted to only 20 g per rabbit and were reversed after Day 9. In the 252 mg/kg group the weight decrease was extended to Day 12 and a similar result was obtained in the 315 mg/kg group but in this case the weight levelled off and did not increase until Day 15. The greatest weight loss in the EPILIM-treated animals was recorded at the 400 mg/kg dose level when the overall decrease of 80 g per rabbit extended to Day 15 of gestation.

The weight curves for the normal and pair-fed control groups were similar in shape and showed an uninterrupted weight gain throughout gestation.

The most severe weight changes were observed in the PHENYTOIN group where an overall weight loss of some 230 g per animal was recorded during the dosing period. The body weights increased only slightly after Day 18 whereas in all the other groups the rates of weight gain during the later stages of gestation were similar.

Food Consumption - (See Table 5 and Fig. 2) - Administration of 400 mg/kg EPILIM evoked a clear anorectic response in the rabbits with the mean food intake falling to about 50% that of the control animals. The consumption had a wide day-to-day variation in both groups but the value for the test group rarely approached that of the controls. During the second half of the dosing period the consumption in the test group tended to decrease rapidly but this trend was reversed after Day 18 although the control group value was never attained.

Implantations - (See Tables 2 and 7) - The mean implantation rate per mother ranged between 4.67 in the 252 mg/kg group and 6.80 in the 200 mg/kg group.

A measure of pre-implantation loss was made by relating the numbers of implantations with the numbers of corpora lutea according to the formula :

$$\% \text{ pre-implantation loss} = \frac{(\text{No. corpora lutea} - \text{No. implantations})}{\text{No. corpora lutea}} \times 100$$

The pre-implantation loss for the control group was 8.7% and 15.6% for the pair-fed control group. The values for the EPILIM treated groups ranged from 7.1% in the 100 mg/kg group to 28.2% in the 252 mg/kg group. The pre-implantation loss in the top dose level group was 10.3% and for the PHENYTOIN group 15.3%.

Uterine Deaths - (See Tables 2 & 7) - A total of 142 embryos died in utero. Deaths occurring late in gestation showed little inter-group variation and ranged between 0.00 in the 315 mg/kg group to 0.50 in the 400 mg/kg, 252 mg/kg and pair-fed control groups. The majority of the deaths occurred early in gestation and were thus represented as resorption sites. The mean distribution per mother in the EPILIM-treated groups showed a dose-related increase from 0.45 in the 100 mg/kg group to 4.70 in the 400 mg/kg group. The control group value was 1.91 per mother and the pair-fed control value 0.08. One point nine zero sites per mother were recorded in the PHENYTOIN group. The increase in resorption sites in the 400 mg/kg EPILIM group was statistically signification in a χ^2 test in comparison with the control group value which was itself rather higher than normal.

Foetuses - (See Tables 2, 6 & 7) - N.B. In this report mean values have been subscripted as Mean 1 or Mean 2. Mean 1 values include data from all animals having corpora lutea in the ovaries whereas Mean 2 values are limited to data from those animals which produced live young. A total of 312 foetuses were examined all being alive when removed from the uterus. None of the 400 mg/kg level rabbits produced any live young although 10 of the animals had been pregnant. The mean control and pair-fed control group values of foetuses per mother were 4.64 and 4.83 respectively and none of the other group values, except for the top dose level, differed significantly from these. The figures ranged from 3.25 in the 315 mg/kg group to 6.10 in the 200 mg/kg group. The PHENYTOIN group value was 3.80 foetuses per mother.

The mean foetal weights of the group 2 and 4 litters were significantly reduced at 24.8 g and 26.9 g respectively in

Student's "t" tests in comparison with the control group value of 28.5 g. The group 3 value (252 mg/kg) was raised, however, at 32.3 g as was the value for the pair-fed control group at 30.6 g. A slight but statistically non-significant decrease was noted in the PHENYTOIN group with a mean value of 27.7 g.

Only the 315 mg/kg group value of mean crown/rump lengths was significantly reduced with a value of 82.3 mm in comparison with the control group value of 87.5 mm. The values for the other groups ranged from 86.4 mm in the 200 mg/kg group to 89.4 mm in the 252 mg/kg group.

There was little variation in the sex ratios of the foetuses except for the PHENYTOIN group. The value of 1 : 2.17 in this group was rather higher than for the other groups where the values ranged from 1 : 0.86 in the 315 mg/kg group to 1 : 1.35 in the 200 mg/kg group.

Abnormalities have been classified as either major or minor defects with the latter restricted to the presence of supernumary ribs. Minor defects are discounted unless present in greatly increased numbers. Increases in minor defects, 35.00% and 26.23% respectively, were recorded in the 252 mg/kg and 200 mg/kg groups compared with the control group value of 15.69%. The values for the other groups ranged from 11.54% in the 315 mg/kg group to 20.69% in the pair-fed control group. Major defects were observed in all of the test groups. A strong teratogenic response was provoked in the 315 mg/kg group with a level of 30.77% deformities (8 foetuses from a total of 26). These defects consisted of 3 sibs with unilateral kidney agenesis, 3 foetuses with vertebral fusions in the lumbar region of the spine, one of which had an extra transverse process between lumbar vertebrae 3 and 4, one foetus with a pair of ribs fused together and one foetus with the intermediate lung lobe absent and the sternebrae fused obliquely. The abnormalities observed in the other EPILIM groups were of unrelated types frequently seen in the control animals. One foetus from the 252 mg/kg group had a double gall bladder giving a group total of 5.00%. One 200 mg/kg group foetus had a forelimb muscle flexure giving a group total of 1.64% and a single foetus in the 100 mg/kg group, representing 1.72% of the group total, had a double gall bladder. Three defects were observed in the normal control group giving a level of 5.88%. These consisted of single foetuses showing a double gall bladder, absence of the intermediate lung lobe and a hypoplastic spleen respectively. Two abnormal foetuses in the pair-fed control group had respectively, a muscle flexure and a double gall bladder giving a group total of 3.45%.

A clear teratogenic response was observed in the PHENYTOIN-treated animals where 23.68% of abnormalities was produced consisting of 9 foetuses showing various combinations of cleft palate, ablepharia and muscle flexures.

Littering Groups - (See Tables 3 & 8) - The results from this section were of academic interest since none of the EPILIM-treated rabbits produced any live pups. Autopsy revealed that 10 of the animals had been pregnant and that their litters were totally resorbing.

The control animals gave a normal series of results through weaning with a 90% pregnancy producing a mean of 5.67 pups per mother after a 31.7 day gestation. At weaning 4.00 pups per mother had survived. A measure of pre-weaning loss was made by relating the numbers of pups born with the numbers weaned according to the formula :

$$\% \text{ Pre-weaning loss} = \frac{(\text{No. pups born} - \text{No. pups weaned}) \times 100}{\text{No. pups born}}$$

The loss in the control group was 29.5% and the weight gain during weaning 535%. The sex ratios of the surviving pups was 1 : 1.40 and no abnormalities were observed.

DISCUSSION

It is a common observation that teratogenic effects are seen only at dose levels which are close to those producing complete uterine death. This was observed in this study and a clear cut off point seem to exist between the 315 mg/kg and 252 mg/kg dose levels, the latter producing completely normal results except for an increased pre-implantation loss. The unusually high incidence of uterine deaths in the control group masks the fact that the level of uterine deaths was also increased at the 315 mg/kg level.

EPILIM treatment was clearly anorectic but the data from the pair-fed control group demonstrated that this anorexia could not be responsible for either the high incidence of uterine deaths or the teratogenic activity.

The responses induced by the PHENYTOIN treatment were typical of those of a teratogenic compound with an increases in uterine deaths, a decrease in the weight of the surviving foetuses and an increase in the incidence of abnormalities. There was a

tendency towards a selective uterine toxicity against the male foetuses but since the sex ratio can vary quite widely we do not attribute any significance to this observation. The syndrome of abnormalities produced by PHENYTOIN was different from those induced by EPILIM with the palate, eyes and limbs being the target organs.

CONCLUSION

The teratogenic range for EPILIM was not wider than 252 - 400 mg/kg/day which values correspond approximately to 10 - 16 times the human therapeutic dose (1.5 g per day).

TABLE 1

RABBIT: GROUP CODES & DOSAGES USED

Group	Treatment	Duration of Dosage	No. of Pregnancies
1	400 mg/kg Epilim (gavage)	Days 6-18 inc.	10
2	315 mg/kg Epilim (gavage)	"	8
3	252 mg/kg Epilim (gavage)	"	6
4	200 mg/kg Epilim (gavage)	"	10
5	100 mg/kg Epilim (gavage)	"	11
6	10 ml/kg Water (gavage)	"	11
7	10 ml/kg Water (Pair-Fed) (gavage)	"	12
8	150 mg/kg Phenytoin (gavage)	"	10
9	400 mg/kg Epilim (gavage)	"	10
10	10 ml/kg Water (gavage)	"	9

TABLE 2

RABBIT: GROUP MEAN DATA (CAESARIAN DELIVERED GROUPS)

Parameter	MEAN	Group 1 400 mg/kg Epilim	Group 2 315 mg/kg Epilim	Group 3 252 mg/kg Epilim	Group 4 200 mg/kg Epilim
No. Pregnancies		10/11	8/10	6/10	10/12
% Pregnancies	1	90.9	80.0	60.0	83.3
No. Foetuses		-	26	20	61
Mean No. per Mother	1	0.00	3.25	3.33	6.10
No. Resorptions		5	-	3	2
Mean No. per Mother	1	0.50	0.00	0.50	0.20
No. Resorption Sites		47	16	5	5
Mean No. per Mother	1	4.70	2.00	0.83	0.50
Mean No. Uterine Deaths per Mother	1	5.20	2.00	1.33	0.70
Mean No. Implantations per Mother	1	5.20	5.25	4.67	6.80
Mean No. Corpora Lutea per Mother	1	5.80	6.13	6.50	7.70
% Pre-Implantation Loss	1	10.3	14.4	28.2	11.7
Litter Wt. (g)	2	-	129	129	164
Mean Foetal Wt. (g)	2	-	24.8	32.3	26.9
Mean Crown/Rump Length (mm)	2	-	82.3	89.4	86.7
No. Males		-	14	10	26
No. Females		-	12	10	35
Ratio Males:Females		-	1:0.86	1:1.00	1:1.35
No. Minor Defects		-	3	7	16
% Minor Defects		-	11.54	35.00	26.23
No. Major Defects		-	8	1	1
% Major Defects		-	30.77	5.00	1.64

TABLE 2 (Cont'd)

Parameter	MEA	Group 5 100 mg/kg Epilim	Group 6 10 ml/kg Water	Group 7 10 ml/kg Water (Pair-Fed)	Group 8 150 mg/kg Phenytoin	Remarks
No Pregnancies		11/12	11/12	12/12	10/11	
% Pregnancies	1	91.7	91.7	100.0	90.9	Little Variation
No. Foetuses		58	51	58	38	312 in all
Mean No. per Mother	1	5.27	4.64	4.83	3.80	Significant decrease at top dose level.
No. Resorptions		2	1	6	4	23 in all.
Mean No. per Mother	1	0.18	0.09	0.50	0.40	Little Variation
No. Resorption Sites		5	21	1	19	119 in all
Mean No. per Mother	1	0.45	1.91	0.08	1.90	Significant increase at top dose level
Mean No. Uterine Deaths per Mother	1	0.63	2.00	0.58	2.30	Significant increase at top dose level
Mean No. Implantations per Mother	1	5.90	6.64	5.41	6.10	
Mean No. Corpora Lutea per Mother	1	6.36	7.27	6.42	7.20	
% Pre-Implantation Loss	1	7.1	8.7	15.6	15.3	Increased at 252mg/kg level
Litter Wt. (g)	2	151	145	148	150	
Mean Foetal Wt. (g)	2	28.7	28.5	30.6	27.7	Decreased : groups 2, 4 and 8
Mean Crown/Rump Length (mm)	2	87.8	87.5	88.8	87.9	Decreased : groups 2 and 4
No. Males		28	24	26	12	140 in all
No. Females		30	27	32	26	172 in all
Ratio Males:Females		1:1.07	1:1.13	1:1.23	1:2.17	Slight increase in group 8
No. Minor Defects		11	8	12	6	
% Minor Defects		18.97	15.69	20.69	15.79	Increased in groups 3 and 4
No. Major Defects		1	3	2	9	
% Major Defects		1.72	5.88	3.45	23.68	Teratogenic at 315 mg/kg

TABLE 3

RABBIT: GROUP MEAN DATA (LITTERING GROUPS)

Parameter	Group 9 400 mg/kg Epilim	Group 10 10 ml/kg Water
No. Pregnancies	10/12	9/10
% Pregnancies	83.3	90.0
Mean Duration Pregnancy (Days)	-	31.7
No. Pups Born	-	51
Mean No. per Mother	-	5.67
No. Pups Weaned	-	36
Mean No. per Mother	-	4.00
% Pre-Weaning Loss	-	29.5
Mean Pup Wt. Day 7 (g)	-	104
Mean Pup Wt. Weaning (g)	-	660
% Wt. Increase	-	535
No. Males	-	15
No. Females	-	21
Ratio Males:Females	-	1:1.40
No. Deformities	-	-
% Deformities	-	0.00

TABLE 4

RABBIT: GROUP MEAN BODY WEIGHTS IN kg

Day	Groups 1&9 400 mg/kg Epilim	Group 2 315 mg/kg Epilim	Group 3 252 mg/kg Epilim	Group 4 200 mg/kg Epilim	Group 5 100 mg/kg Epilim	Groups 6&10 10 ml/kg Water	Group 7 10 ml/kg Water (Pair Fed)	Group 8 150 mg/kg Phenytoin
0	2.33	2.26	2.28	2.56	2.38	2.35	2.54	2.14
3	2.37	2.28	2.28	2.56	2.37	2.37	2.54	2.16
6	2.41	2.29	2.30	2.56	2.39	2.37	2.56	2.17
9	2.37	2.27	2.29	2.54	2.37	2.38	2.57	2.03
12	2.36	2.25	2.27	2.55	2.38	2.39	2.57	1.99
15	2.33	2.25	2.30	2.56	2.39	2.41	2.58	1.94
18	2.34	2.26	2.31	2.56	2.41	2.42	2.59	1.96
21	2.35	2.27	2.33	2.58	2.42	2.44	2.59	1.97
24	2.38	2.29	2.38	2.60	2.44	2.45	2.61	1.97
28	2.40	2.30	2.41	2.63	2.45	2.47	2.64	1.98

Rabbit Group Mean Body Weights in kg

Fig. 1

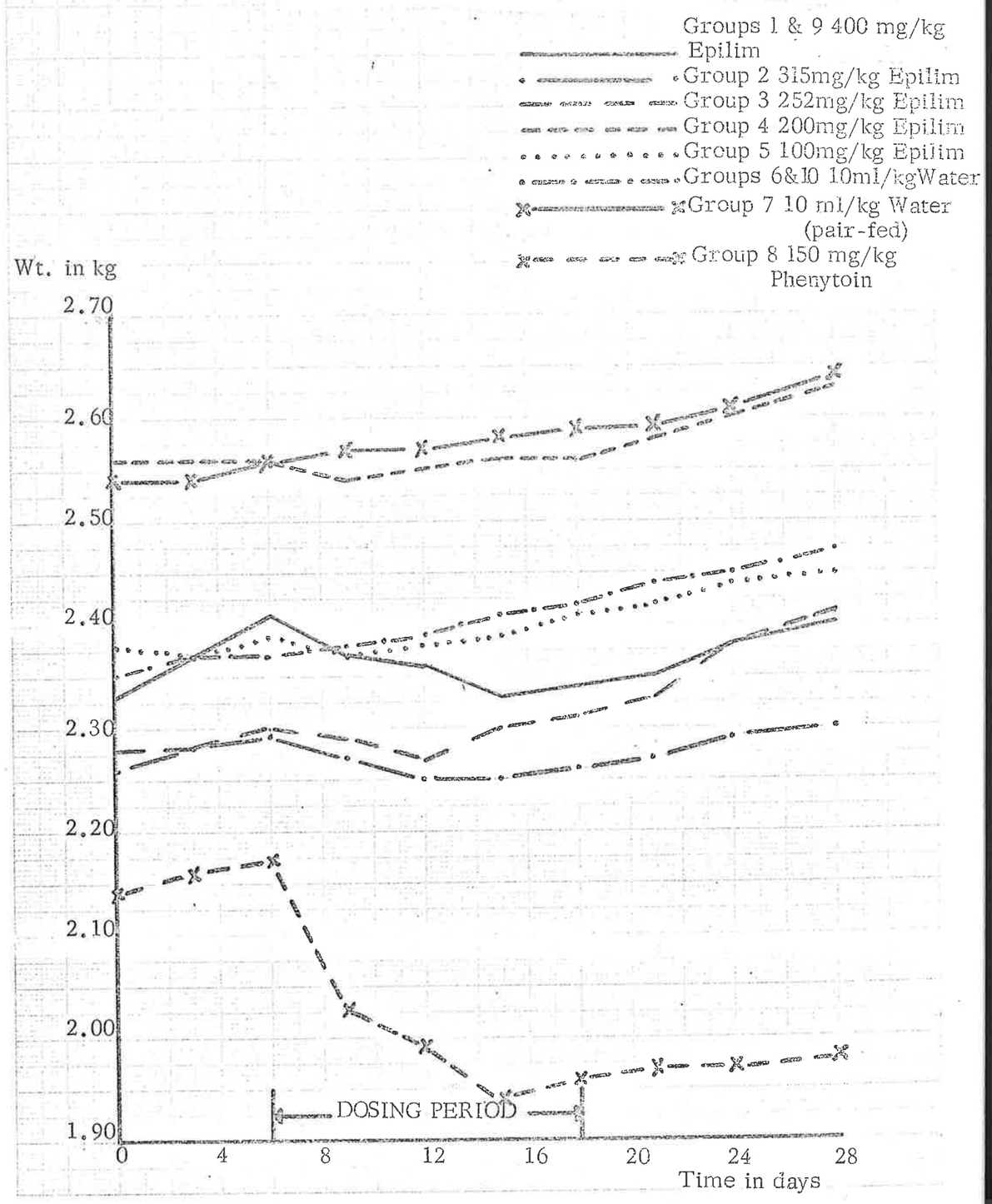

TABLE 5

RABBIT: GROUP MEAN DAILY FOOD CONSUMPTION (g)

Day	Group 1 400 mg/kg Epilim	Group 6 10 ml/kg Water
6- 7	31	71
7- 8	47	95
8- 9	64	99
9-10	38	108
10-11	48	104
11-12	67	81
12-13	74	83
13-14	63	89
14-15	53	96
15-16	37	88
16-17	33	86
17-18	33	84
18-19	48	78
19-20	64	83
20-21	71	105
21-22	66	95

Fig. 2

Rabbit Group Mean Food Consumption in g

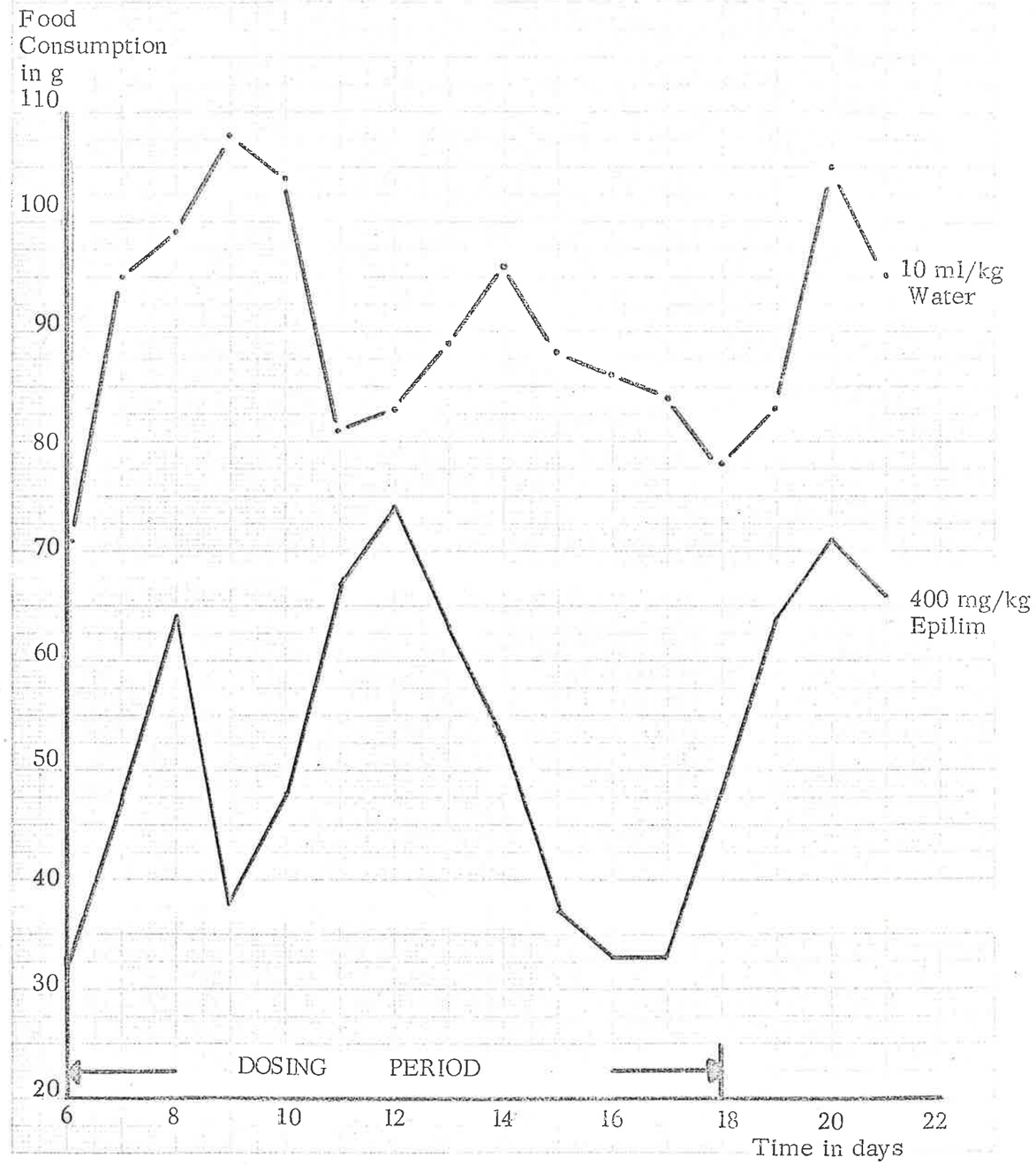

TABLE 6

RABBIT: NATURE AND INCIDENCE OF ABNORMALITIES

Abnormality	Group 1 400 mg/kg Epilim	Group 2 315 mg/kg Epilim	Group 3 252 mg/kg Epilim	Group 4 200 mg/kg Epilim	Group 5 100 mg/kg Epilim	Group 6 10 ml/kg Water	Group 7 10 ml/kg Water (Pair Fed)	Group 8 150 mg/kg Phenytoin
Total No. Foetuses	-	26	20	61	58	51	58	38
No. Foetuses showing Defects	-	11	8	17	12	11	14	15
No. Foetuses showing minor defects only	-	3	7	16	11	8	12	6
No. foetuses showing major defects	-	8	1	1	1	3	2	9
Minor Defects								
Percentage	-	11.54	35.00	26.23	18.97	15.69	20.69	15.79
13 pair Ribs	-	1	6	12	5	4	8	5
13 ribs one side	-	4	1	5	6	4	3	2
Major Defects								
Percentage	-	30.77	5.00	1.64	1.72	5.88	3.45	23.68
Muscle Flexure	-	-	-	1	-	-	1	5
Double Gall Bladder	-	-	1	-	1	1	1	-
Hypoplastic Spleen	-	-	-	-	-	1	-	-
Intermediate Lung Lobe Absent	-	1	-	-	-	1	-	-
Cleft Palate	-	-	-	-	-	-	-	4
Ablepharia	-	-	-	-	-	-	-	2
Oblique fusion of sternebrae	-	1	-	-	-	-	-	-

TABLE 6 (Cont'd)

Abnormality	Group 1 400 mg/kg Epilim	Group 2 315 mg/kg Epilim	Group 3 252 mg/kg Epilim	Group 4 200 mg/kg Epilim	Group 5 100 mg/kg Epilim	Group 6 10 ml/kg Water	Group 7 10 ml/kg Water (Pair Fed)	Group 8 150 mg/kg Phenytoin
Unilateral kidney Agenesis	-	3	-	-	-	-	-	-
3rd & 4th lumbar vertebrae fused	-	1	-	-	-	-	-	-
5th & 6th lumbar vertebrae fused	-	1	-	-	-	-	-	-
1st & 2nd lumbar vertebrae fused	-	1	-	-	-	-	-	-
Extra transverse process between 3rd and 4th lumbar vertebrae	-	1	-	-	-	-	-	-
6th and 7th ribs fused	-	1	-	-	-	-	-	-

TABLE 7 a)

RABBIT: INDIVIDUAL ANIMAL DATA (CAESARIAN DELIVERED GROUPS)

Group 1 400 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
281		NOT PREGNANT				
282	6	6	0.0	0	0	0
283	6	5	16.7	0	0	0
284	5	4	20.0	0	0	0
325	4	4	0.0	0	0	0
326	7	6	14.3	0	0	0
307		DID NOT SURVIVE TEST		0	0	0
308	4	4	0.0	0	0	0
316	8	7	12.5	0	0	0
317	5	5	0.0	0	0	0
319	7	7	0.0	0	0	0
320	6	4	33.3	0	0	0
Total	58	52	-	0	0	0
Mean	5.80	5.20	10.3	0.00	0.00	0.00
± S.E.M.	0.42	0.39				

TABLE 7 a) (Cont'd)

Group 2 315 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
199	DID NOT SURVIVE TEST					
200	4	3	25.0	0	0	-
201	NOT PREGNANT					
202	8	6	25.0	5	1	1:0.20
203	8	6	25.0	3	2	1:0.67
204	NOT PREGNANT					
211	5	5	0.0	0	0	-
212	DID NOT SURVIVE TEST					
213	7	7	0.0	0	0	-
214	6	6	0.0	2	4	1:2.00
215	6	6	0.0	3	3	1:1.00
216	5	3	40.0	1	2	1:2.00
Total	49	42		14	12	
Mean	6.13	5.25	14.4			1:0.86
± S.E.M.	0.52	0.53				

TABLE 7 a) (Cont'd)

Group 3 252 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
205	NOT PREGNANT					
206	4	3	25.0	2	1	1:0.50
207	NOT PREGNANT					
208	4	4	0.0	0	0	-
209	7	5	28.6	2	3	1:1.50
210	DID NOT SURVIVE TEST					
217	NOT PREGNANT					
218	DID NOT SURVIVE TEST					
219	7	4	42.9	0	2	-
220	9	4	55.6	2	1	1:0.50
221	NOT PREGNANT					
222	8	8	0.0	4	3	1:0.75
Total	39	28		10	10	
Mean	6.50	4.67	28.2			1:1.00
± S.E.M.	0.85	0.71				

TABLE 7 a) (Cont'd)

Group 4 200 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
285	7	5	28.6	1	1	1:1.00
286	6	6	0.0	3	3	1:1.00
287	8	7	12.5	4	3	1:0.75
288		NOT PREGNANT				
290	9	8	11.1	5	3	1:0.60
293	3	3	0.0	2	1	1:0.50
351	8	7	12.5	3	3	1:1.00
352	9	5	44.4	2	3	1:1.50
353		NOT PREGNANT				
354	11	11	0.0	2	7	1:3.50
355	8	8	0.0	2	5	1:2.50
356	8	8	0.0	2	6	1:3.00
Total	77	68		26	35	
Mean	7.70	6.80	11.7			1:1.35
$\pm$ S.E.M.	0.67	0.70				

TABLE 7 a) (Cont'd)

Group 5 100 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
333	6	5	16.7	2	2	1:1.00
334	5	4	20.0	1	3	1:3.00
335	7	6	14.3	2	4	1:2.00
336		NOT PREGNANT				
337	3	3	0.0	2	1	1:0.50
338	6	6	0.0	2	3	1:1.50
381	7	7	0.0	3	4	1:1.33
382	9	8	11.1	2	1	1:0.50
383	9	9	0.0	5	4	1:0.80
384	7	7	0.0	4	3	1:0.75
385	4	4	0.0	2	2	1:1.00
386	7	6	14.3	3	3	1:1.00
Total	70	65		28	30	
Mean	6.36	5.91	7.1			1:1.07
$\pm$ S.E.M.	0.56	0.55				

TABLE 7 a) (Cont'd)

Group 6 10 ml/kg Water

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
294	3	3	0.0	2	1	1:0.50
295	7	7	0.0	3	4	1:1.33
296	11	10	9.1	4	4	1:1.00
297	10	8	20.0	3	4	1:1.33
298	6	6	0.0	3	3	1:1.00
299	10	10	0.0	1	0	-
369	7	4	42.9	2	2	1:1.00
370	9	8	11.1	4	4	1:1.00
371	3	3	0.0	0	3	-
372	6	6	0.0	2	2	1:1.00
373	8	8	0.0	0	0	-
374		NOT PREGNANT				
Total	80	73		24	27	
Mean	7.27	6.64	8.7			1:1.13
± S.E.M.	0.81	0.75				

TABLE 7 a) (Cont'd)

Group 7 10 ml/kg Water (Pair Fed)

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
327	6	6	0.0	2	4	1:2.00
328	4	4	0.0	1	3	1:3.00
329	6	5	16.7	2	3	1:1.50
330	7	6	14.3	3	3	1:1.00
331	5	5	0.0	2	3	1:1.50
332	3	3	0.0	0	3	-
345	7	3	57.1	1	2	1:2.00
346	11	10	9.1	4	3	1:0.75
347	11	9	18.2	0	5	-
348	5	4	20.0	4	0	-
349	5	4	20.0	4	0	-
350	7	6	14.3	3	3	1:1.00
Total	77	65		26	32	
Mean	6.42	5.42	15.6			1:1.23
± S.E.M.	0.71	0.87				

TABLE 7 a) (Cont'd)

Group 8 150 mg/kg Phenytoin

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
357		DID NOT SURVIVE TEST				
358	8	7	12.5	0	0	-
359	6	5	16.7	2	2	1:1.00
360	4	2	50.0	0	2	-
361	10	9	10.0	3	4	1:1.33
362		NOT PREGNANT				
375	5	5	0.0	0	0	-
376	11	10	9.1	1	8	1:8.00
377	9	8	11.1	3	4	1:1.33
378	5	5	0.0	1	4	1:4.00
379	8	6	25.0	0	0	-
380	6	4	33.3	2	2	1:1.00
Total	72	61		12	26	
Mean	7.20	6.10	15.3			1:2.17
± S.E.M.	0.74	0.77				

TABLE 7 b)

Group 1 400 mg/kg Epilim

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
281		NOT PREGNANT					
282	0	0	6	0	-	-	-
283	0	0	5	0	-	-	-
284	0	0	4	0	-	-	-
325	0	0	4	0	-	-	-
326	0	0	6	0	-	-	-
307		DID NOT SURVIVE TEST					
308	0	0	4	0	-	-	-
316	0	2	5	0	-	-	-
317	0	0	5	0	-	-	-
319	0	3	4	0	-	-	-
320	0	0	4	0	-	-	-
Total	0	5	47	0			
Mean $\frac{1}{2}$	0.00	0.50	4.70	0.00	-	-	-
$\pm$ S.E.M	0.00	0.34	0.26				

TABLE 7b) (Cont'd)

Group 2 315 mg/kg Epilim

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
199	DID NOT SURVIVE TEST						
200	0	0	3	-	-	-	-
201	NOT PREGNANT						
202	6	0	0	1 f+j)	159	26.5	86.7
203	5	0	1	3 3k)	110	22.0	78.2
204	NOT PREGNANT						
211	0	0	5	-	-	-	-
212	DID NOT SURVIVE TEST						
213	0	0	7	-	-	-	-
214	6	0	0	2 a)b)	113	18.8	74.2
215	6	0	0	3 b+l)m) b+n+p)	167	27.8	85.0
216	3	0	0	2 b) o+p)	95	31.7	90.7
Total	26	0	16	11			
Mean 1 2	3.25	0.00	2.00	1.38	129	24.8	82.3
± S.E.M.	1.01	0.00	0.96	0.46	14.30		

TABLE 7 b) (Cont'd)

Group 3 252 mg/kg Epilim

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
205	NOT PREGNANT						
206	3	-	-	-	101	33.7	92.3
207	NOT PREGNANT						
208	0	0	4	-	-	-	-
209	5	0	0	1 a)	129	25.8	85.0
210	DID NOT SURVIVE TEST						
217	NOT PREGNANT						
218	DID NOT SURVIVE TEST						
219	2	1	1	1 a)	53	26.5	84.5
220	3	1	0	2 b)d)	103	34.3	90.7
221	NOT PREGNANT						
222	7	1	0	4 4a)	259	37.0	92.1
Total	20	3	5	8			
Mean $\frac{1}{2}$	3.33	0.50	0.83	1.33	129	32.3	89.4
$\pm$ S.E.M.	0.99	0.22	0.65	0.61	34.7		

TABLE 7 b) (Cont'd)

Group 4 200 mg/kg Epilim

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
285	2	0	3	1 b+c)	44	22.0	79.0
286	6	0	0	-	171	28.5	91.0
287	7	0	0	2 a)b)	207	29.6	89.8
288		NOT PREGNANT					
290	8	0	0	-	211	26.4	86.5
293	3	0	0	3 2a)b)	81	27.0	90.0
351	6	0	1	-	165	27.5	88.3
352	5	0	0	3 2a)b)	147	29.4	89.8
353		NOT PREGNANT					
354	9	2	0	1 a)	189	21.0	77.2
355	7	0	1	5 5a)	205	29.3	91.1
356	8	0	0	2 a)b)	220	27.5	85.3
Total	61	2	5	17			
Mean $\frac{1}{2}$	6.10	0.20	0.50	1.70	164	26.9	86.7
$\pm$ S.E.M.	0.21	0.20	0.31	0.52	18.6		

TABLE 7 b) (Cont'd)

Group 5 100 mg/kg Epilim

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
333	4	1	0	-	122	30.5	91.0
334	4	0	0	-	128	32.0	91.5
335	6	0	0	2 a)b)	171	28.5	88.0
336		NOT PREGNANT					
337	3	0	0	1 d)	100	33.3	92.3
338	5	1	0	-	148	29.6	90.0
381	7	0	0	-	186	26.6	89.1
382	3	0	5	1 b)	106	35.3	94.7
383	9	0	0	5 3a)2b)	211	23.4	85.7
384	7	0	0	1 b)	206	29.4	77.1
385	4	0	0	1 a)	120	30.0	90.5
386	6	0	0	1 b)	164	27.3	87.7
Total	58	2	5	12			
Mean $\frac{1}{2}$	5.27	0.18	0.45	1.09	151	28.7	87.8
$\pm$ S.E.M.	0.57	0.12	0.45	0.44	11.8		

TABLE 7 b) (Cont'd)

Group 6 10 ml/kg Water

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
294	3	0	0	-	108	36.0	96.7
295	7	0	0	2 2a)	198	28.3	88.4
296	8	0	2	3 2b)e)	167	20.9	78.8
297	7	1	0	2 d)f)	177	25.3	81.7
298	6	0	0	1 b)	165	27.5	88.5
299	1	0	9	-	27	27.0	85.0
369	4	0	0	-	133	33.3	90.5
370	8	0	0	2 2a)	245	30.6	91.3
371	3	0	0	1 b)	91	30.3	90.3
372	4	0	2	-	140	35.0	93.3
373	0	0	8	-	-	-	-
374		NOT PREGNANT					
Total	51	1	21	11			
Mean $\frac{1}{2}$	4.64	0.09	1.91	1.00	145	28.5	87.5
± S.E.M.	0.83	0.09	1.01	0.33	19.8		

TABLE 7 b) (Cont'd)

Group 7 10 ml/kg Water (Pair Fed)

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
327	6	0	0	2 a)b)	170	28.3	87.7
328	4	0	0	-	118	29.5	88.0
329	5	0	0	3 2a)c)	140	28.0	88.2
330	6	0	0	1 a)	181	30.2	89.7
331	5	0	0	-	142	28.4	88.6
332	3	0	0	-	94	31.3	90.7
345	3	0	0	1 b)	96	32.0	91.0
346	7	2	1	2 a)b)	206	29.4	88.4
347	5	4	0	2 b)d)	164	32.8	88.4
348	4	0	0	-	140	35.0	90.3
349	4	0	0	-	114	28.5	86.3
350	6	0	0	3 3a)	209	34.8	89.7
Total	58	6	1	14			
Mean $\frac{1}{2}$	4.83	0.50	0.08	1.17	148	30.6	88.8
$\pm$ S.E.M.	0.37	0.36	0.08	0.34	11.3		

TABLE 7 b) (Cont'd)

Group 8 150 mg/kg Phenytoin

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
357		DID NOT SURVIVE TEST					
358	0	0	7	-	-	-	-
359	4	1	0	2 a)g)	114	28.5	87.5
360	2	0	0	-	54	27.0	88.5
361	7	2	0	5 a)b)h) a+g)g+h)	164	23.4	84.1
362		NOT PREGNANT					
375	0	0	5	-	-	-	-
376	9	1	0	5 b)3c)c+g)	221	24.6	84.6
377	7	0	1	2 2a)	210	30.0	91.4
378	5	0	0	-	152	30.4	89.8
379	0	0	6	-	-	-	-
380	4	0	0	1 c)	138	34.5	94.0
Total	38	4	19	15			
Mean $\frac{1}{2}$	3.80	0.40	1.90	1.50	150	27.7	87.9
$\pm$ S.E.M.	1.03	0.22	0.91	0.64	21.6		

KEY TO ABNORMALITIES

a)	13 pair ribs	Minor defect
b)	13 ribs one side	Minor defect
c)	Muscle flexure	Major defect
d)	Double Gall bladder	Major defect
e)	Hypoplastic Spleen	Major defect
f)	Intermediate Lung Lobe Absent	Major defect
g)	Cleft Palate	Major defect
h)	Ablepharia	Major defect
j)	Oblique fusion of sternebrae	Major defect
k)	Unilateral kidney agenesis	Major defect
l)	3rd and 4th lumbar vertebrae fused	Major defect
m)	6th and 7th ribs fused	Major defect
n)	5th and 6th lumbar vertebrae fused	Major defect
o)	Extra transverse process between 3rd and 4th lumbar vertebrae	Major defect
p)	1st and 2nd lumbar vertebrae fused	Major defect

TABLE 8 a)

RABBIT: INDIVIDUAL ANIMAL DATA (LITTERING GROUPS)

Group 9 400 mg/kg Epilim

No.	No. Pups Born	No. Pups Weaned	% Pre-Weaning Loss	No. Males	No. Females	Ratio Males: Females	No. Deformities
309		NOT PREGNANT					
310		NO PUPS BORN					
311		" "	"				
312		" "	"				
313		" "	"				
314		" "	"				
339		" "	"				
340		NOT PREGNANT					
341		NO PUPS BORN					
342		" "	"				
343		" "	"				
344		" "	"				

TABLE 8 a) (Cont'd)

Group 10 10 ml/kg Water

No.	No. Pups Born	No. Pups Weaned	% Pre-Weaning Loss	No. Males	No. Females	Ratio Males: Females	No. Deformities
300	7	5	28.6	2	3	1:1.50	-
301	9	6	33.3	3	3	1:1.00	-
302	6	5	16.7	1	4	1:4.00	-
303	NOT PREGNANT						
304	4	4	0.0	4	-	-	-
306	5	0	100.0	-	-	-	-
363	DID NOT SURVIVE TEST						
364	7	6	14.3	2	4	1:2.00	-
365	3	3	0.0	1	2	1:2.00	-
366	NOT PREGNANT						
367	4	3	25.0	-	3	-	-
368	6	4	33.3	2	2	1:1.00	-
Total	51	36		15	21		-
Mean	5.67	4.00	29.5			1:1.40	0.00
± S.E.M.	0.62	0.62					

TABLE 8 b)

Group 9 400 mg/kg Epilim

No.	Mean Pup Wt. Day 7 (g)	Mean Pup Wt. Weaning (g)	% Wt. Increase	Duration of Pregnancy (Days)
309	NOT PREGNANT			
310	NO PUPS BORN			
311	" " "			
312	" " "			
313	" " "			
314	" " "			
339	" " "			
340	NOT PREGNANT			
341	NO PUPS BORN			
342	" " "			
343	" " "			
344	" " "			

TABLE 8 b) (Cont'd)

Group 10 10 ml/kg Water

No.	Mean Pup Wt Day 7 (g)	Mean Pup Wt. Weaning (g)	% Wt. Increase	Duration of Pregnancy (Days)
300	97	634	554	31
301	106	711	571	30
302	128	786	514	33
303	NOT PREGNANT			
304	94	599	537	31
306	-	-	-	32
363	DID NOT SURVIVE TEST			
364	111	641	477	32
365	118	712	503	33
366	NOT PREGNANT			
367	96	603	528	31
368	85	597	602	32
Mean	104	660	535	31.7
± S.E.M.	4.5	24.33		

APPENDIX 1

CUMULATIVE CONTROL DATA ON DUTCH BELTED RABBITS

CORRECT TO JULY 1st. 1971

Parameter	Control Animals Only	Animals From Inactive Drugs	Total
Pregnancy Rate			
Percentage	75.6	71.7	72.7
Animals Involved	301/398	820/1143	1121/1541
Range	57.1 - 100.0	60.0 - 100.0	57.1 - 100.0
Corpora Lutea			
Mean Nos. per Mother	8.36	9.02	8.84
Total Nos. Involved	2516	7396	9912
Range	6.36 - 10.00	5.91 - 9.78	5.91 - 10.00
Individual Animal Range	1 - 19	1 - 16	1 - 19
Implantations			
Mean No. per Mother	6.94	7.02	7.00
Total Nos. Involved	2089	5757	7846
Range	3.00 - 9.00	4.62 - 8.70	3.00 - 9.00
Individual Animal Range	1 - 12	1 - 12	1 - 12
Pre - Implantation Loss			
% Loss	17.00	22.2	20.8
Range	11.3 - 38.1	9.1 - 34.0	9.1 - 38.1
Foetuses			
Mean No. per Mother	6.18	6.00	6.05
Total Nos. Involved	1860	4918	6778
Range	3.00 - 9.00	2.63 - 7.85	2.63 - 9.00
Individual Animal Range	0 - 13	0 - 12	0 - 13
Resorptions			
Mean No. per Mother	0.24	0.34	0.31
Total Nos. Involved	73	278	351
Range	0.00 - 1.00	0.00 - 1.10	0.00 - 1.10
Individual Animal Range	0 - 8	0 - 8	0 - 8

Parameter	Control Animals Only	Animals From Inactive Drugs	Total
Resorption Sites			
Mean No. per Mother	0.52	0.68	0.64
Total Nos. Involved	156	561	717
Range	0.00 - 2.00	0.00 - 2.86	0.00 - 2.86
Individual Animal Range	0 - 10	0 - 9	0 - 10
Uterine Deaths			
Mean No. per Mother	0.76	1.02	0.95
Total Nos. Involved	229	839	1068
Range	0.10 - 2.12	0.40 - 3.29	0.10 - 3.29
Individual Animal Range	0 - 10	0 - 9	0 - 10
Foetal Weights			
Mean Weight (g)	29.92	30.12	30.04
Total Nos. Involved	1860	3918	6778
Range	15.9 - 41.0	14.3 - 40.0	14.3 - 41.0
Individual Animal Range	12 - 47	11 - 46	11 - 47
Mean Crown/Rump Length			
Mean Length (mm)	87.4	87.6	87.6
Total Nos. Involved	1860	4918	6778
Range	71.9 - 96.0	71.4 - 97.3	71.4 - 97.3
Individual Animal Range	68 - 105	69 - 106	68 - 106
Sex Ratio			
Total No. Males	452	716	1168
Total No. Females	553	931	1484
Ratio Males:Females	1:1.22	1:1.30	1:1.27
Range	1:0.76 - 1:2.75	1:0.89 - 1:2.75	1:0.76 - 1:2.75
Major Defects			
Mean Percentage	2.42	3.58	3.26
Total Nos. Involved	45	176	221
Range	0.00 - 9.43	0.00 - 8.69	0.00 - 9.43
Minor Defects			
Mean Percentage	12.31	11.16	11.48
Total Nos. Involved	229	549	778
Range	0.00 - 36.38	0.00 - 34.70	0.00 - 36.38

Abnormality	Control Animals Only		Animals From Inactive Drugs		Total	
	No.	%	No.	%	No.	%
Major Defects						
Acephaly	3	0.16	1	-	4	0.06
Anencephaly	1	-	1	-	2	0.03
Exencephaly	1	-	7	0.14	8	0.12
Hydrocephaly	-	-	4	0.08	4	0.06
Anophthalmia	-	-	2	0.04	2	0.03
Ablepharia	-	-	3	0.06	3	0.04
Cleft Palate	-	-	10	0.20	10	0.15
External Nares Absent	-	-	2	0.04	2	0.03
Muscle Flexure	16	0.86	52	1.06	68	1.00
Oligodactyly	4	0.22	12	0.24	16	0.24
Adactylion Foot	1	-	-	-	1	-
One Rib Absent	4	0.22	15	0.31	19	0.28
6 Pair Ribs Only	-	-	1	-	1	-
Rib Fusion	1	-	2	0.04	3	0.04
Hemicentric Vertebrae	1	-	4	0.08	5	0.07
Lumbar Vertebrae Absent	-	-	1	-	1	-
Caudal Vertebrae Absent	-	-	1	-	1	-
4 Thoracic Vertebrae Only	-	-	1	-	1	-
Unossified Sternum	9	0.48	5	0.10	14	0.21
Oblique Fusion of Sternebrae	-	-	4	0.08	4	0.06
Split Sternum	3	0.16	-	-	3	0.04
Umbilical Hernia	4	0.22	14	0.28	18	0.27
One Kidney Absent	-	-	4	0.08	4	0.06
Fused Kidneys	-	-	1	-	1	-
Hydronephrosis	2	0.11	4	0.08	6	0.08
Gonads Absent	-	-	1	-	1	-
Imperforate Anus	1	-	3	0.06	4	0.06
Bladder Absent	-	-	1	-	1	-
External Heart	3	0.16	-	-	3	0.04
Double Gall Bladder	5	0.27	37	0.75	42	0.62
Gall Bladder Absent	-	-	3	0.06	3	0.04
Intermediate Lung Lobe Absent	6	0.32	4	0.08	10	0.15
Minor Defects						
13 Pair Ribs	127	6.83	228	4.74	355	5.24
13 Ribs One Side	102	5.45	321	6.66	423	6.24

CUMULATIVE DATA ON LITTERING DUTCH BELTED RABBITS

CORRECT TO JULY 1st 1971

Parameter	Control Animals Only	Animals From Inactive Drugs	Total
Pregnancy Rate			
% Pregnancies	61.1%	62.8%	61.8%
No. of Rabbits Involved	116/190	86/137	202/327
Range	53.3 - 69.2	53.3 - 66.7	53.3 - 69.2
Duration of Pregnancy			
Mean Duration (Days)	31.3	31.2	31.2
Range	30.7 - 31.9	30.2 - 31.8	30.2 - 31.9
Individual Animal Range	28 - 34	28 - 33	28 - 34
Birth Rate			
Mean No. per Mother	5.74	5.80	5.77
Total Numbers Involved	666	499	1165
Range	5.13 - 7.00	4.50 - 6.75	4.50 - 7.00
Individual Animal Range	1 - 10	3 - 9	1 - 10
Weaning Rate			
Mean No. per Mother	4.03	2.83	3.51
Total Numbers Involved	467	243	710
Range	3.11 - 5.22	2.67 - 4.80	2.67 - 5.22
Individual Animal Range	0 - 9	0 - 8	0 - 9
Pre - Weaning Loss			
% Loss	29.8	51.2	39.2
Range	19.5 - 40.4	22.6 - 70.0	19.5 - 70.0
Individual Animal Range	0.0 - 100.0	0.0 - 100.0	0.0 - 100.0

Parameters	Control Animals Only	Animals From Inactive Drugs	Total
Pup Weights			
Mean Pup Wt. Day 7 (g)	119	105	114
Range	76 - 142	70 - 127	70 - 127
Mean Pup Wt. Weaning (g)	766	793	777
Range	443 - 844	368 - 921	368 - 921
% Wt. Increase	544	655	582
Range	490 - 747	374 - 915	374 - 915
Sex Ratios			
Mean Ratio	1:1.14	1:1.15	1:1.15
Total No. Males	218	113	331
Total No. Females	249	130	379
Range	1:0.59-1:1.92	1:0.85-1:1.50	1:1.15
Deformities			
Mean Percentage	0.86%	0.82%	0.85%
Total Numbers Involved	4	2	6
Range	0.00 - 3.57%	0.00 - 3.21%	0.00 - 3.57%

APPENDIX 2

Effect of Administration of Thalidomide on the outcome of Pregnancy in the Dutch Belted Rabbit

The following is a summary of the results from a typical teratogenicity study performed on Dutch Rabbits in the Laboratories using Thalidomide, the known human teratogen.

Dosage Used

150 mg/kg administered in a dose volume of 2 ml/kg by gavage daily from Days 6 - 18 of gestation.

Parameter	Control Group	Thalidomide Group
No. Mated	20	19
No. Surviving	18	15
No. Pregnant	13	9
Percentage Pregnancies	65%	47%
No. Implantations	110	64
No. Implantations per Mother	8.46	7.11
No. Foetuses	103	42
No. Foetuses per Mother	7.92	4.67
No. Resorptions	1	6
No. Resorptions per Mother	0.08	0.67
No. Resorption Sites	6	16
No. Resorption Sites per Mother	0.48	1.78
No. Uterine Deaths per Mother	0.56	2.45
Mean Foetal Weight	31.30g	29.44g
No. Deformities	2	35
Percentage Deformities	1.94%	83.3%

Nature and Incidence of Deformities

Limb Flexure	-	30
Intermediate Lung Lobe Absent	-	9
Unilateral Kidney Agenesis	-	5
Short Tail	-	4
Cleft Palate	-	3
Oligodactyly	-	3
Hydronephrosis	2	-
Umbilical Hernia	-	1

INTRODUCTION

The second part of the study was performed using Sprague Dawley derived rats.

MATERIALS & METHODS

Test Material - The sample of EPILIM was from the same batch as that used for the rabbit study.

Animals - Ninety six Sprague Dawley derived rats of the CD strain were used. They were obtained from the [redacted]

Housing - The rats were housed in groups of 4 in grid-floor cages all in the same room under artificial lighting with a 12-hour photoperiod and with the ambient temperature thermostatically maintained at 22°C ± 1°C. At the end of the dosing period the animals in the littering groups were transferred to individual solid floor cages and supplied with nesting material.

Diet - Throughout the study all except one group of rats were allowed free access to water and to a commercial pellet diet No. 41B (Dixon & Sons, Ware, Herts.). The food consumption was recorded for the top dose level group and a control group of rats were pair-fed to match this consumption. These animals were allowed free access to water throughout the study.

Mating - Mating with proven males was carried out at the [redacted] The day on which sperm was observed in a vaginal smear was taken as Day 0 of gestation. The rats were admitted to the [redacted] by Day 3 of gestation.

Choice of Dosage - Data provided by the clients indicated that EPILIM was teratogenic in rats in the dose range 400 - 600 mg/kg. Dose levels of 600 mg/kg, 300 mg/kg and 150 mg/kg were therefore chosen for the study.

PHENYTOIN was used as a comparative control at a dose level of 100 mg/kg.

The clinical dose of EPILIM is expected to be about

25 mg/kg and that of PHENYTOIN is approximately 5 mg/kg. The dose levels of EPILIM used in this study therefore represent multiples of 24, 12 and 6 times the clinical dose, and the PHENYTOIN dosage is approximately 20 times the clinical dose.

Treatment - The rats were divided into treatment groups of 12 animals per group. The EPILIM solutions and PHENYTOIN suspension were prepared freshly each day and formulated to give a constant dose volume of 10 ml/kg. The control animals received distilled water only. The rats were dosed by gavage daily between Day 6 and Day 15 of gestation.

Intermediate Examination - The general condition of the animals was noted daily and the body weights were recorded every 3 days throughout gestation.

The food consumption per cage was recorded daily for the 600 mg/kg group 1 animals and the control group 5 animals were fed these quantities of food on the corresponding day in gestation. The food consumption per cage was also recorded for the control group 4.

One hour after the final administration of the test materials selected animals were bled by cardiac puncture under ether anaesthesia. Two millilitres of blood were withdrawn into lithuim heparin tubes and the separated frozen plasma was sent to the clients for blood level determinations.

Terminal Examination - With the exception of the animals in the littering groups the rats were killed by cervical dislocation on Day 21 of gestation.

The ovaries and uteri were removed, examined and the following data recorded :

1. Numbers of corpora lutea.
2. Numbers and positions of foetuses, resorptions and resorption sites.
3. Foetal weights.
4. Crown/rump lengths.

The foetuses were then killed by an intracardiac injection of NEMBUTAL, examined under x 2 magnification for external and internal defects, sexed, eviscerated and the carcasses checked for anomalies by X-ray examination. Free-hand razor sections were then made through the head region and examined for ocular and ventricular defects.

Littering Groups - Duplicate top dose level and control groups were set up and allowed to litter normally and to rear their young to weaning. The following data were recorded :

1. Duration of gestation.

2. Numbers of pups born and weaned.

3. Pup weights at day 7 and at weaning.

The pups were weaned at 21 days of age. After checks on balance, hearing and sight the pups were autopsied and sexed.

RESULTS

General Observations - The rats appeared to tolerate the treatment well and no adverse behavioural effects were noted. One top dose level rat was culled after contracting inner ear disease and one control animal died after undergoing cardiac puncture. Autopsy revealed massive haemorrhage into the pericardium. The pregnancy rates were not affected by treatment and ranged from 83.3% to 100.0%.

Body Weights - (See Table 4 and Fig. 1) - N.B. In this study the group mean values have been compiled from data from the surviving pregnant animals in both the Caesarian delivered and littering groups.

The weight curves for the test groups all showed a retardation in rate of weight gain, and in the case of the 600 mg/kg dose level an actual decrease, during the dosing period and even after Day 15 the rates of weight gain were always less than those of the control groups. The weight curves for the normal and pair-fed control groups were essentially similar in shape except for a slight lag in the pair-fed group between Days 15 and 18 of gestation. A similar lag was observed in the PHENYTOIN treated group. It was not possible to detect a dose relationship in the weight changes occurring in the treatment groups during the dosing period but such a relationship become apparent during the last stages of gestation.

Food Consumption - (See Table 5 and Fig. 2) - The mean group food consumption for the 600 mg/kg group showed a marked decrease during the early stages of treatment falling to almost 50% of the control group value. A gradual increase in the food consumption was noted during the dosing period and by Day 15 the decrease was only about 35%. The food consumption was not recorded after Day 15 of gestation but casual observation suggested that it increased only slightly even after the dosing was discontinued.

Implantations - (See Tables 2 and 7) - The mean implantation rate per mother showed a dose-related decrease in the treatment groups ranging from 9.30 in the 600 mg/kg group to 11.90 in the 150 mg/kg group compared with 12.22 in the control group and 11.00 in the pair-fed control group.

A measure of pre-implantation loss was made by relating the numbers of corpora lutea with the numbers of implantations according to the formula :

$$\% \text{ pre-implantation loss} = \frac{(\text{No. corpora lutea} - \text{No. implantations})}{\text{No. corpora lutea}} \times 100$$

There was very little inter-group variation in the pre-implantation losses with values ranging from 7.2% in the 300 mg/kg group to 12.7% in the pair-fed control group. No dose-related trends could be detected and all of the values were well within the normal range obtained from previous data in these laboratories (see Appendix 2).

Uterine Deaths - (See Tables 2 & 7) - A total of 133 embryos died in utero. Non dose-related increases in the incidence of resorptions per mother were observed in the treatment groups with the values ranging from 1.70 in the low dose level group to 0.80 in the mid-dose level group compared with 0.00 in the control group, 0.30 in the pair-fed control group and 0.09 in the PHENYTOIN treated group. Much greater increases in early uterine deaths were recorded with mean values of resorption sites per mother ranging from 6.30 in the top dose level group to 0.50 in the mid-dose level group compared with 0.22 in the control group.

The overall incidence of uterine deaths per mother showed significant, though not dose related, increases in all of

the test groups with values of 7.50 for the 600 mg/kg group, 1.30 for the 300 mg/kg group and 3.60 for the 150 mg/kg group compared with the control group value of 0.22 and the pair-fed control group value of 0.40. The PHENYTOIN treated group value was 0.27 uterine deaths per mother.

Foetuses - (See Tables 2, 6 & 7) - A total of 545 foetuses were examined all being alive when removed from the uterus. All of the test groups showed decreases with mean litter size values ranging from 1.80 in the 600 mg/kg group to 10.30 in the 300 mg/kg group compared with 12.00 in the control group, 10.60 in the pair-fed control group and 11.55 in the PHENYTOIN group. Only two of the eleven surviving top dose level rats produced any foetuses although ten of the animals were pregnant.

A non-dose-related decrease in the mean foetal weights was observed at the mid- and top dose levels. The values of 3.85 g and 3.92 g were significantly reduced in Student's 't' tests at $P = 0.05$ in comparison with the control group value of 4.55 g. The low dose level value was 4.60 g, the pair-fed control value 4.93 g and the PHENYTOIN group value 5.05 g.

Similar significant reductions were recorded in the mean crown/rump lengths with value of 38.2 mm and 37.9 mm respectively compared with the control group value of 40.9 mm. There was little difference between this value and those for the 150 mg/kg, pair-fed control and PHENYTOIN groups with values of 40.8 mm, 41.9 mm and 41.8 mm respectively.

There was little intergroup variation in the sex ratios of the surviving foetuses with the values ranging from 1 : 0.63 in the 150 mg/kg group to 1 : 1.20 in the control group.

Abnormalities have been classified as in the rabbit study except that bipartite thoracic centra have been ignored since they are normally present in high numbers in this strain of rat.

Minor defects were increased in all of the test groups but the increase showed an inverse correlation with dosage the

values ranging from 11.1% in the top dose level group to 19.28% in the low dose level group compared with the control group value of 5.56%. A slight increase was also recorded in the PHENYTOIN group with a value of 7.87%.

Major defects were recorded in all except the control group of Caesarian births.

A marked teratogenic action was noted in the EPILIM treated groups. The effect was dose-related and the incidence of abnormalities ranged from 66.67% in the 600 mg/kg group to 26.51% in the 150 mg/kg group. Two distinct syndromes were observed. The majority of the abnormalities were observed in the lumbar region of the spine with the vertebral centra either absent, hemicentric or bipartite. In addition some of the lumbar vertebrae were fused together. In the other syndrome the kidneys appeared to be the target organ with hydronephrosis being the most common effect but with ectopic, and hypoplastic renal tissue and unilateral renal agenesis also being recorded. (A full description of the abnormalities is given in Appendix 1).

The foetuses from the PHENYTOIN treated animals did not display any of the lumbar abnormalities but hydronephrosis was observed as well as cleft palates and exencephalocoeles. The overall level of abnormalities for this group was 5.51%. Only 2 of the pair-fed control group foetuses showed major defects - both unossified sternums giving a group total of 1.89% deformities.

Littering Groups - (See Tables 3 & 8) - Only one rat from the test group produced any pups although subsequent autopsy indicated that all of the rats had been pregnant. The duration of pregnancy for the test rat was 23 days compared with a group mean of 22.5 days for the control litters. Ten pups were born of which 9 survived to weaning giving a pre-weaning loss of 9.6% compared with the 4.0% in the control group. The mean number of pups born per mother in this group was 10.00 and at weaning 9.60 per mother were still alive.

The mean rate of weight gain during weaning in the control group was 220% a value which was considerably higher than the 147% recorded in the test litter.

There was little variation in the sex ratios of the surviving pups with 1 : 1.25 in the test litter and 1 : 0.96 in the control group.

No abnormalities were seen in either littering group.

DISCUSSION

The results from this study are broadly in agreement with previous data supplied by the clients and produced in Japan. The strain of rat used in the Consultox Laboratories appears to be somewhat more sensitive to EPILIM than does the Japanese, and marked teratogenic activity was observed even at the low dose level which represents a multiple of some 6 times the clinical dose. The deformities observed in the EPILIM treated foetuses were generally of a different type to those produced by PHENYTOIN although kidney anomalies were common to both treatments. The severe disorientation of the lumbar vertebrae has been previously recorded in these laboratories in only one foetus and the very high incidence observed in this study clearly points to drug action and repeats the results obtained in the Japanese study. The embryotoxicity and lethality associated with the EPILIM treatment were not observed in the PHENYTOIN treated group but this may reflect a difference in the dose-response curve for this material. It is interesting to note that PHENYTOIN was teratogenic at a dose level which did not produce any embryotoxicity or lethality whereas in the Japanese study EPILIM had no teratogenic activity at these dose levels. Although this is speculative it may imply that PHENYTOIN has a wider teratogenic range in this species.

Treatment with EPILIM severely reduced the food consumption in the pregnant rats but the data from the pair-fed control group clearly indicated that this anorexia was not responsible for the teratogenicity and embryotoxicity.

CONCLUSION

Administration of EPILIM by gavage at dose levels of 600 mg/kg, 300 mg/kg and 150 mg/kg to Sprague Dawley derived rats of the CD strain daily between Day 6 and Day 15 of gestation produces marked teratogenic and embryolethal activity at all dose levels, and embryotoxicity at all except the low dose level.

TABLE 1

RAT: GROUP CODES & DOSAGES USED

Group	Treatment	Duration of Dosage	No. of Pregnancies
1	600 mg/kg Epilim (gavage)	Days 6-15 inc.	10
2	300 mg/kg Epilim (gavage)	"	10
3	150 mg/kg Epilim (gavage)	"	10
4	10 ml/kg Water (gavage)	"	9
5	10 ml/kg Water (Pair-fed) (gavage	"	10
6	100 mg/kg Phenytoin (gavage)	"	11
7	600 mg/kg Epilim (gavage)	"	12
8	10 ml/kg Water (gavage)	"	10

TABLE 2

RAT: GROUP MEAN DATA (CAESARIAN DELIVERED GROUPS)

Parameter	MEAN	Group 1 600 mg/kg Epilim	Group 2 300 mg/kg Epilim	Group 3 150 mg/kg Epilim	Group 4 10 ml/kg Water
No. Pregnancies		10/11	10/12	10/12	9/11
% Pregnancies	1	90.9	83.3	83.3	81.8
No. Foetuses		18	103	83	108
Mean No. per Mother	1	1.80	10.30	8.30	12.00
No. Resorptions		12	8	17	-
Mean No. per Mother	1	1.20	0.80	1.70	0.00
Resorption Sites		63	5	19	2
Mean No. per Mother	1	6.30	0.50	1.90	0.22
Mean No. Uterine Deaths per Mother	1	7.50	1.30	3.60	0.22
Mean No. Implantations per Mother	1	9.30	11.60	11.90	12.22
Mean No. Corpora Lutea per Mother	1	10.10	12.50	13.20	13.22
% Pre-Implantation Loss	1	7.9	7.2	9.8	7.6
Litter Wt. (g)	2	35.32	39.64	42.40	54.64
Mean Foetal Wt. (g)	2	3.92	3.85	4.60	4.55
Mean Crown/Rump Length (mm)	2	37.9	38.2	40.8	40.9
No. Males		10	47	51	49
No. Females		8	56	32	59
Ratio Males:Females		1:0.80	1:1.19	1:0.63	1:1.20
No. Minor Defects		2	13	16	6
% Minor Defects		11.1	12.62	19.28	5.56
No. Major Defects		12	30	22	-
% Major Defects		66.67	29.13	26.51	0.00

TABLE 2 (Cont'd)

Parameter	MEAN	Group 5 10 ml/kg Water	Group 6 100 mg/kg Phenytoin	Remarks
No. Pregnancies		10/12	11/12	
% Pregnancies	1	83.3	91.7	Little variation
No. Foetuses		106	127	545 in all
Mean No. per Mother	1	10.60	11.55	Significant Decrease in test groups
No. Resorptions		3	1	41 in all
Mean No. per Mother	1	0.30	0.09	
No. Resorption Sites		1	2	92 in all
Mean No. per Mother	1	0.10	0.18	
Mean No. Uterine Deaths per Mother	1	0.40	0.27	Significant increase in test groups
Mean No. Implantations per Mother	1	11.00	11.82	
Mean No. Corpora Lutea per Mother	1	12.60	12.91	
% Pre-Implantation Loss	1	12.7	8.4	Little variation
Litter Wt. (g)	2	52.29	58.34	
Mean Foetal Wt. (g)	2	4.93	5.05	Significant Decrease at Mid & Top Dose Levels
Mean Crown/Rump Length (mm)	2	41.9	41.8	Significant Decrease at Mid & Top Dose Levels
No. Males		58	60	275 in all
No. Females		48	67	270 in all
Ratio Males:Females		1:0.83	1:1.12	Little variation
No. Minor Defects		7	10	54 in all
% Minor Defects		6.60	7.87	Higher in Test Groups
No. Major Defects		2	7	73 in all
% Major Defects		1.89	5.51	Teratogenic

TABLE 3

RAT: GROUP MEAN DATA (LITTERING GROUPS)

Parameter	Group 7 600 mg/kg Epilim	Group 8 10 ml/kg Water	Remarks
No. Pregnancies	12/12	10/12	
% Pregnancies	100.0	83.3	Little Variation
Mean Duration Pregnancy (Days)	23.0	22.5	Little Variation
No. Pups Born	10	100	110 in all
Mean No. per Mother	0.83	10.00	Much lower in Test group
No. Pups Weaned	9	96	105 in all
Mean No. per Mother	0.75	9.60	Much lower in Test group
% Pre-Weaning Loss	9.64	4.00	Little Variation
Mean Pup Wt. Day 7 (g)	19	15	
Mean Pup Wt. Weaning (g)	47	48	
% Wt. Increase	147	220	Higher in Control group
No. Males	4	49	53 in all
No. Females	5	47	52 in all
Ratio Males:Females	1:1.25	1:0.96	Little Variation
No. Deformities	-	-	-
% Deformities	0.00	0.00	Non Teratogenic

TABLE 4

RAT: GROUP MEAN BODY WEIGHTS IN g.

Day	Groups 1 & 7 600 mg/kg Epilim	Group 2 300 mg/kg Epilim	Group 3 150 mg/kg Epilim	Group 4&8 10 ml/kg Epilim	Group 5 10 ml/kg Water (Pair-Fed)	Group 6 100 mg/kg Phenytoin
0	238	241	231	241	230	229
3	244	248	236	245	237	235
6	249	252	241	251	245	241
9	245	263	256	263	253	247
12	260	266	264	271	265	257
15	272	273	279	299	280	281
18	280	298	304	327	301	293
21	294	333	336	372	357	342

Rat Group Mean Body Weights in Grams

Fig. 1.

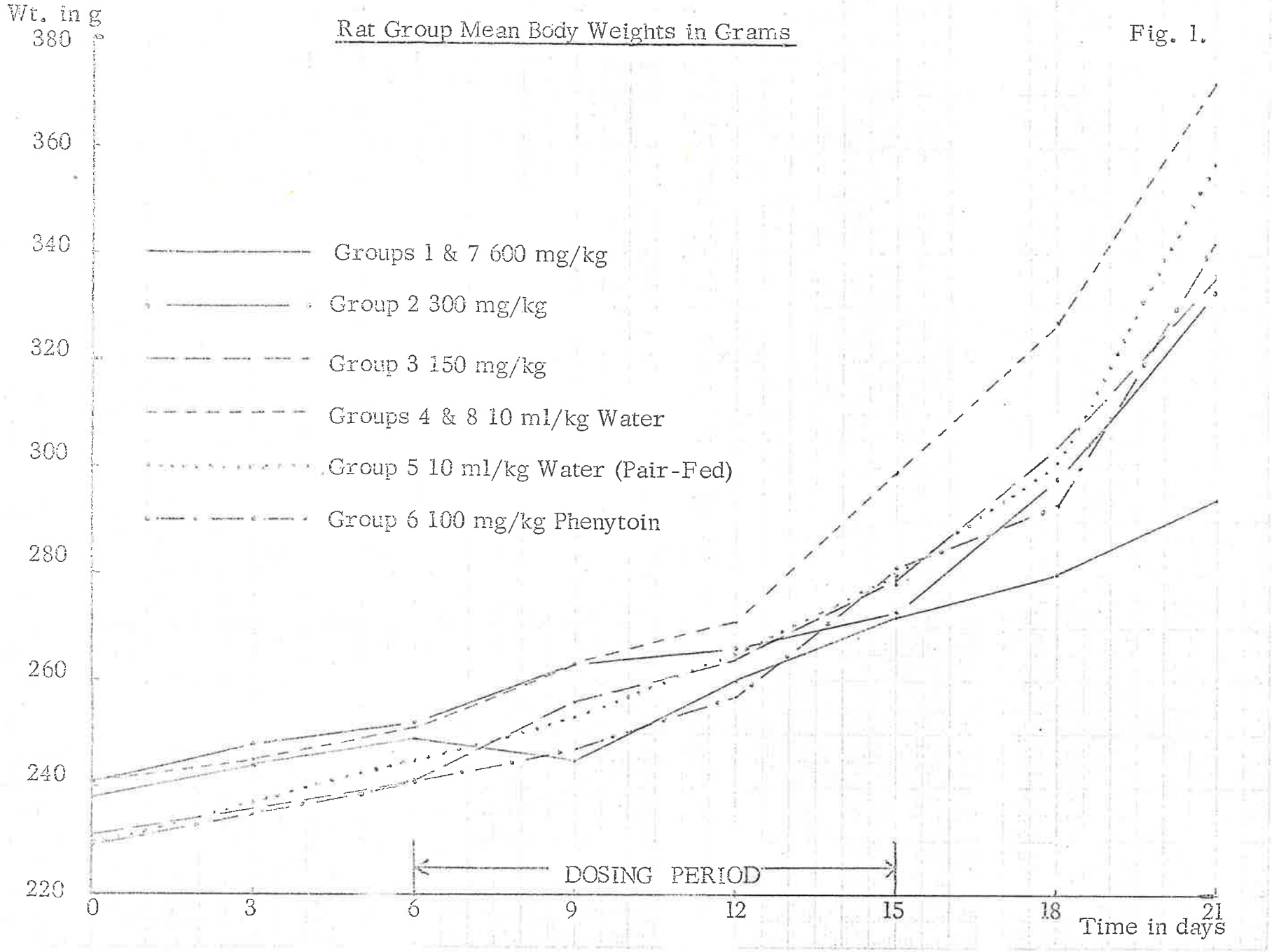

TABLE 5

RAT: FOOD CONSUMPTION (g)

600 mg/kg Epilim

Day	Cage 1	Mean	Cage 2	Mean	Cage 3	Mean	Group Mean
6-7	60	15	70	18	76	19	17
7-8	40	10	44	11	60	15	12
8-9	40	10	33	11	78	20	14
9-10	76	18	38	13	80	20	17
10-11	48	12	33	11	88	22	15
11-12	62	16	43	14	100	25	18
12-13	72	18	33	11	76	19	16
13-14	58	15	37	12	84	21	16
14-15	38	13	33	11	90	23	16
15-16	36	12	36	12	86	22	15

10 ml/kg Water (Group 4)

Day	Cage 1	Mean	Cage 2	Mean	Cage 3	Mean	Group Mean
6-7	90	23	100	25	90	23	24
7-8	100	25	100	25	90	23	24
8-9	100	25	100	25	100	25	25
9-10	100	25	96	24	100	25	25
10-11	100	25	96	24	96	24	24
11-12	90	23	96	24	96	24	24
12-13	96	24	96	24	100	25	24
13-14	100	25	96	24	92	23	24
14-15	100	25	100	25	90	23	24
15-16	96	24	93	23	92	23	23

Fig. 2

Rat Group Mean Food Consumption in Grams

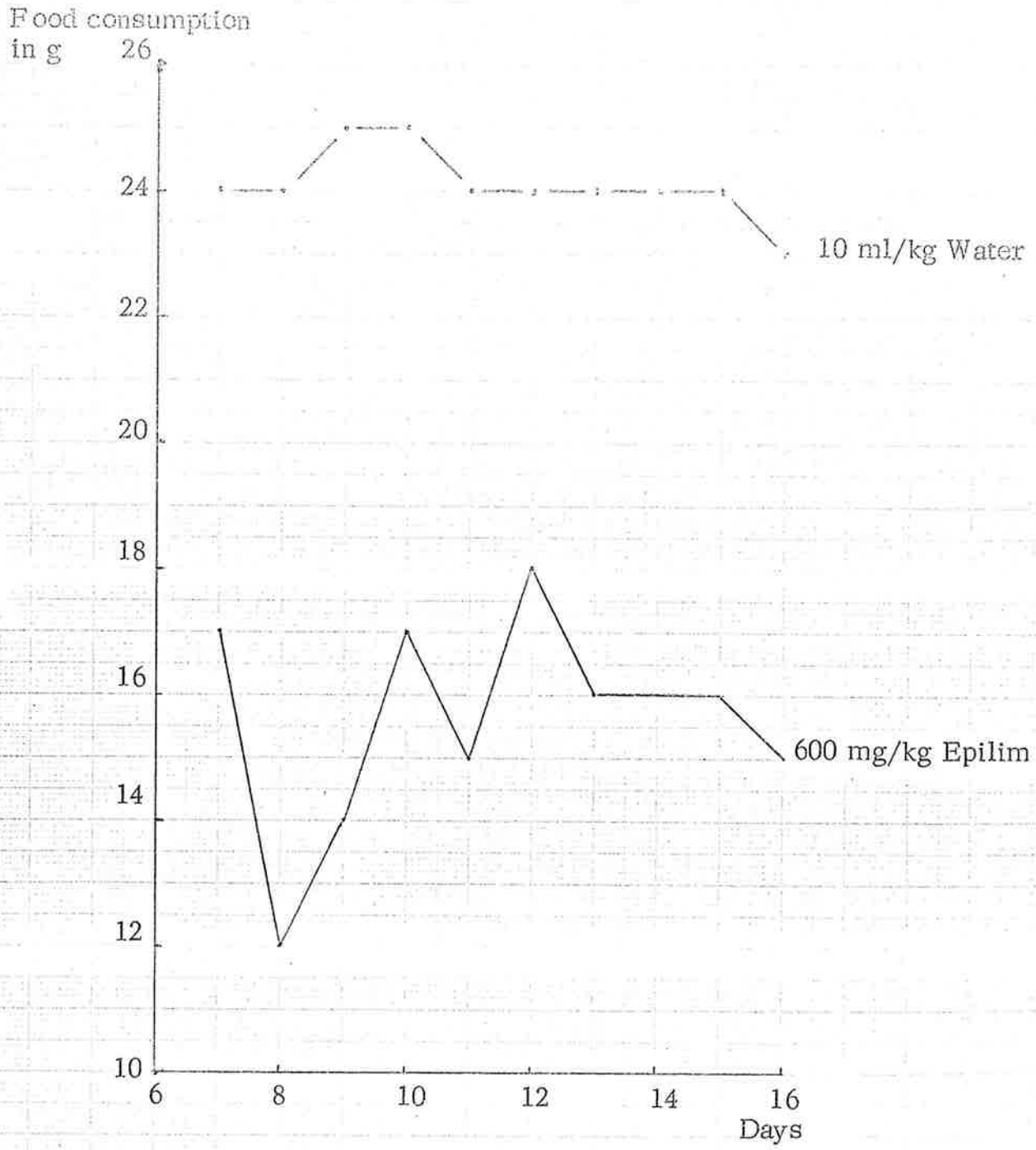

TABLE 6

RAT: NATURE & INCIDENCE OF ABNORMALITIES

Abnormality	Group 1 600 mg/kg Epilim	Group 2 300 mg/kg Epilim	Group 3 150 mg/kg Epilim	Group 4 10 ml/kg Water	Group 5 10 ml/kg Water (Pair Fed)	Group 6 100 mg/kg Phenytoin
Total No. of Foetuses	18	103	83	108	106	127
Foetuses showing Defects	14	43	38	6	9	17
Foetuses showing minor defects only	2	13	16	6	7	10
Foetuses showing major defects	12	30	22	-	2	7
Minor Defects						
Percentage	11.11	12.62	19.28	5.56	6.60	7.87
14 Pair Ribs	-	15	17	4	5	6
14 Ribs one side	6	6	2	2	2	4
Major Defects						
Percentage	66.67	29.13	26.51	0.00	1.89	5.51
Hydronephrosis	6	1	6	-	-	2
Kidney hypoplasia	-	-	1	-	-	-
Ectopic kidney	-	-	1	-	-	-
Unilateral kidney agenesis	-	1	-	-	-	-
Single lumbar vertebra hemicentric, acentric or bipartite	4	-	3	-	-	-
Several lumbar vertebrae hemicentric acentric or bipartite	2	5	9	-	-	-
All lumbar vertebrae hemicentric, acentric or bipartite	1	23	8	-	-	-
Several lumbar vertebrae fused	-	1	2	-	-	-
Ribs fused	-	-	4	-	-	-
Oligodactyly	-	-	2	-	-	-
Unilateral agenesis of radius	-	-	2	-	-	-

TABLE 6 (Cont'd)

Abnormality	Group 1 600 mg/kg Epilim	Group 2 300 mg/kg Epilim	Group 3 150 mg/kg Epilim	Group 4 10 ml/kg Water	Group 5 10 ml/kg Water (Pair Fed)	Group 6 100 mg/kg Phenytoin
Unossified sternum	1	-	-	-	2	-
Muscle flexure	-	1	-	-	-	1
Exencephalocoele	-	-	-	-	-	2
Cleft Palate	-	-	-	-	-	2

TABLE 7 a)

RAT: INDIVIDUAL ANIMAL DATA (CAESARIAN DELIVERED GROUPS)

Group 1 600 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
13	11	10	9.1	0	0	-
14		DID NOT SURVIVE TEST				
15	12	10	16.7	0	0	-
16	9	8	11.1	0	0	-
17	7	7	0.0	0	0	-
18	7	6	14.3	0	0	-
19		NOT PREGNANT				
20	8	8	0.0	0	0	-
21	9	9	0.0	0	0	-
22	14	13	7.1	7	4	1:0.57
23	11	11	0.0	0	0	-
24	13	11	15.4	3	4	1:1.33
Total	101	93		10	8	
Mean	10.10	9.30	7.9			1:0.80
± S.E.M.	0.78	0.67				

TABLE 7 a) (Cont'd)

Group 2 300 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
25	14	14	0.0	11	3	1:0.27
26		NOT PREGNANT				
27		NOT PREGNANT				
28	12	6	50.0	4	1	1:0.25
29	13	13	0.0	7	5	1:0.71
30	15	15	0.0	9	6	1:0.67
31	12	12	0.0	1	10	1:10.00
32	13	13	0.0	6	7	1:1.17
33	8	7	12.5	3	4	1:1.33
34	14	13	7.1	2	10	1:5.00
35	14	13	7.1	1	3	1:3.00
36	10	10	0.0	3	7	1:2.33
Total	125	116		47	56	
Mean	12.50	11.60	7.2			1:1.19
± S.E.M.	0.67	0.94				

TABLE 7 a) (Cont'd)

Group 3 150 mg/kg Epilim

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females.
37	13	13	0.0	0	0	-
38	12	11	8.3	10	1	1:0.10
39	7	5	28.6	1	0	-
40	10	10	0.0	8	2	1:0.25
41	15	14	6.7	4	10	1:2.50
42	19	14	26.3	6	8	1:1.33
43	13	12	7.7	8	4	1:0.50
44		NOT PREGNANT				
45		NOT PREGNANT				
46	16	13	18.8	2	0	-
47	14	14	0.0	9	5	1:0.56
48	13	13	0.0	3	2	1:0.67
Total	132	119		51	32	
Mean	13.20	11.90	9.8			1:0.63
± S.E.M.	1.03	0.87				

TABLE 7 a) (Cont'd)

Group 4 10 ml/kg Water

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males: Females
49	13	13	0.0	5	8	1:1.60
50	13	11	15.4	5	6	1:1.20
51	15	15	0.0	5	10	1:2.00
52	13	13	0.0	7	6	1:0.86
53	14	13	7.1	7	6	1:0.86
54		NOT PREGNANT				
55	13	10	23.1	3	7	1:2.33
56		DID NOT SURVIVE TEST				
57	9	8	11.1	5	2	1:0.40
58		NOT PREGNANT				
59	10	9	10.0	5	3	1:0.60
60	19	18	5.3	7	11	1:1.57
Total	119	110		49	59	
Mean	13.22	12.22	7.6			1:1.20
± S.E.M.	0.95	1.04				

TABLE 7 a) (Cont'd)

Group 5 10 ml/kg Water (Pair Fed)

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males Females
73		NOT PREGNANT				
74	14	8	42.9	7	1	1:0.14
75	12	10	16.7	6	4	1:0.67
76	11	11	0.0	5	3	1:0.60
77	13	12	7.7	7	5	1:0.71
78	14	12	14.3	4	8	1:2.00
79	12	11	8.3	7	3	1:0.43
80	11	10	9.1	6	4	1:0.67
81		NOT PREGNANT				
82	11	9	18.2	4	5	1:1.25
83	16	16	0.0	8	8	1:1.00
84	12	11	8.3	4	7	1:1.75
Total	126	110		58	48	
Mean	12.60	11.00	12.7			1:0.83
± S.E.M.	0.52	0.68				

TABLE 7 a) (Cont'd)

Group 6 100 mg/kg Phenytoin

No.	No. Corpora Lutea	No. Implantations	% Pre-Imp Loss	No. Males	No. Females	Ratio Males Females
85	9	9	0.0	5	4	1:0.80
86		NOT PREGNANT				
87	10	9	10.0	3	6	1:2.00
88	11	11	0.0	4	5	1:1.25
89	10	10	0.0	6	3	1:0.50
90	14	12	14.3	4	8	1:2.00
91	14	13	7.1	4	9	1:2.25
92	11	11	0.0	8	3	1:0.38
93	19	15	21.1	7	8	1:1.14
94	13	12	7.7	5	7	1:1.40
95	18	16	11.1	8	8	1:1.00
96	13	12	7.7	6	6	1:1.00
Total	142	130		60	67	
Mean	12.91	11.82	8.4			1:1.12
± S.E.M.	0.98	0.67				

TABLE 7 b)

Group 1 600 mg/kg Epilim

No.	No. Foetuses	No. Resorption	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
13	0	0	10	-	-	-	-
14		DID NOT SURVIVE TEST					
15	0	0	10	-	-	-	-
16	0	2	6	-	-	-	-
17	0	3	4	-	-	-	-
18	0	0	6	-	-	-	-
19		NOT PREGNANT					
20	0	0	8	-	-	-	-
21	0	2	7	-	-	-	-
22	11	0	2	8 b)2c)2d) b+d)b+c+g) b+c)	46.36	4.21	38.5
23	0	4	7	-	-	-	-
24	7	1	3	6 b)c)d)e) b+e)c+f)	24.28	3.49	37.0
Total	18	12	63	14			
Mean $\frac{1}{2}$	1.80	1.20	6.30	1.40	35.32	3.92	37.9
$\pm$ S.E.M.	1.24	0.47	0.86	0.94			

TABLE 7 b) (Cont'd)

Group 2 300 mg/kg Epilim

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
25	14	0	0	-	42.56	3.04	35.9
26		NOT PREGNANT					
27		NOT PREGNANT					
28	5	1	0	3 3f)	16.67	3.33	37.2
29	12	0	1	1 b)	46.02	3.84	38.0
30	15	0	0	2 a)h)	65.31	4.35	39.6
31	11	1	0	6 6a)	35.59	3.24	36.1
32	13	0	0	5 5a)	58.39	4.49	40.8
33	7	0	0	-	29.61	4.23	39.6
34	12	0	1	12 3e)5f) 2b+e) a+f)b+f+j)	48.98	4.08	37.8
35	4	6	3	4 4f)	13.25	3.31	36.3
36	10	0	0	10 5f)a+f) 2b+f)k) a+c+f)	40.00	4.00	38.9
Total	103	8	5	43			
Mean $\frac{1}{2}$	10.30	0.80	0.50	4.30	39.64	3.85	38.2
$\pm$ S.E.M	1.91	0.59	0.31	1.29	5.26		

TABLE 7 b)

Group 3 150 mg/kg Epilim

No.	No. Foetuses	No. Resorption	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
37	0	13	0	-	-	-	-
38	11	0	0	7 4a)2b) a+c)	54.15	4.92	42.0
39	1	4	0	-	3.85	3.85	38.0
40	10	0	0	-	51.13	5.11	42.6
41	14	0	0	1 a)	71.39	5.10	42.7
42	14	0	0	10 9a) a+e)	55.20	3.94	39.6
43	12	0	0	-	51.10	4.26	39.0
44		NOT PREGNANT					
45		NOT PREGNANT					
46	2	0	11	2 2c+f+1+m)	7.74	3.87	37.5
47	14	0	0	13 2d)4e)n)f) c+d)a+e) 3e+n)	63.51	4.54	40.9
48	5	0	8	5 c+f)f+j) f+o) c+f+j)f+p)	23.56	4.71	38.8
Total	83	17	19	38			
Mean $\frac{1}{2}$	8.30	1.70	1.90	3.80	42.40	4.60	40.8
$\pm$ S.E.M.	1.81	1.32	1.29	1.50	8.95		

TABLE 7 b)

Group 4 10 ml/kg Water

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
49	13	0	0	1 b)	46.66	3.59	36.5
50	11	0	0	-	38.60	3.51	37.7
51	15	0	0	-	48.39	3.23	37.9
52	13	0	0	-	66.96	5.15	42.8
53	13	0	0	-	71.05	5.47	42.3
54		NOT PREGNANT					
55	10	0	0	-	55.75	5.58	45.6
56		DID NOT SURVIVE TEST					
57	7	0	1	-	29.61	4.23	41.4
58		NOT PREGNANT					
59	8	0	1	3 3a)	36.96	4.62	44.8
60	18	0	0	2 a)b)	97.74	5.43	41.6
Total	108	0	2	6			
Mean $\frac{1}{2}$	12.00	0.00	0.22	0.67	54.64	4.55	40.9
$\pm$ S.E.M.	1.14	0.00	0.15	0.37	7.05		

TABLE 7 b) (Cont'd)

Group 5 10 ml/kg Water (Pair Fed)

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
73		NOT PREGNANT					
74	8	0	0	3 3a)	41.03	5.13	42.8
75	10	0	0	-	34.96	3.50	38.8
76	8	3	0	-	23.19	2.90	35.4
77	12	0	0	-	61.02	5.09	42.9
78	12	0	0	-	66.22	5.52	40.7
79	10	0	1	2 2a)	53.37	5.34	43.9
80	10	0	0	-	49.40	4.94	44.6
81		NOT PREGNANT					
82	9	0	0	1 g)	52.72	5.86	43.2
83	16	0	0	-	84.63	5.29	42.8
84	11	0	0	3 2b)g)	56.36	5.12	42.1
Total	106	3	1	9			
Mean 1 2	10.60	0.30	0.10	0.90	52.29	4.93	41.9
± S.E.M.	0.75	0.30	0.10	0.41	5.39		

TABLE 7 b) (Cont'd)

Group 6 100 mg/kg Phenytoin

No.	No. Foetuses	No. Resorptions	No. Resorption Sites	No. Deformities	Litter Wt. (g)	Mean Foetal Wt. (g)	Mean Crown/Rump Length (mm)
85	9	0	0	2 2a)	34.74	3.86	40.1
86		NOT PREGNANT					
87	9	0	0	2 b)h)	50.41	5.60	43.6
88	9	1	1	2 2a)	38.88	4.32	40.8
89	9	0	1	3 3a)	43.02	4.78	42.4
90	12	0	0	2 a)r)	55.46	4.62	41.8
91	13	0	0	1 c)	74.17	5.71	42.9
92	11	0	0	-	69.83	6.35	45.9
93	15	0	0	-	65.94	4.40	40.2
94	12	0	0	-	64.26	5.36	41.3
95	16	0	0	2 c)r)	81.13	5.07	40.4
96	12	0	0	3 3b)	63.88	5.32	41.5
Total	127	1	2	17			
Mean $\frac{1}{2}$	11.55	0.09	0.18	1.55	58.34	5.05	41.8
$\pm$ S.E.M.	0.74	0.09	0.12	0.34	4.54		

KEY TO ABNORMALITIES

a)	14 pair ribs	Minor defect
b)	14 ribs one side	Minor defect
c)	Hydronephrosis	Major defect
d)	Single lumbar vertebra acentric, hemicentric or bipartite.	Major defect
e)	Several lumbar vertebrae acentric, hemicentric or bipartite	Major defect
f)	All lumbar vertebrae acentric, hemicentric or bipartite	Major defect
g)	Unossified sternum	Major defect
h)	Muscle flexure	Major defect
j)	Several lumbar vertebrae fused	Major defect
k)	Unilateral kidney agenesis	Major defect
l)	Oligodactyly	Major defect
m)	One radius absent	Major defect
n)	Ribs fused	Major defect
o)	Kidney hypoplasia	Major defect
p)	Ectopic kidney	Major defect
q)	Cleft Palate	Major defect
r)	Exencephalocoele	Major defect

TABLE 8 a)

RAT: INDIVIDUAL ANIMAL DATA (LITTERING GROUPS)

Group 7 600 mg/kg Epilim

No.	No. Pups Born	No. Pups Weaned	% Pre-Weaning Loss	No. Males	No. Females	Ratio Males: Females	No. Deformities
1		NO PUPS BORN					
2		" " "					
3		" " "					
4		" " "					
5		" " "					
6		" " "					
7		" " "					
8		" " "					
9	10	9	10.0	4	5	1:1.25	-
10		NO PUPS BORN					
11		" " "					
12		" " "					
Total	10	9		4	5		-
Mean	0.83	0.75	9.64			1:1.25	0.00

TABLE 8 a) (Cont'd)

Group 8 10 ml/kg Water

No.	No. Pups born	No. Pups weaned	% Pre-Weaning Loss	No. Males	No. Females	Ratio Males:	No. Deformities
61	11	10	9.1	4	6	1:1.50	-
62	12	12	0.0	5	7	1:1.40	-
63	13	13	0.0	6	7	1:1.17	-
64	11	10	9.1	4	6	1:1.50	-
65	7	7	0.0	5	2	1:0.40	-
66	10	10	0.0	5	5	1:1.00	-
67		NOT PREGNANT					
68	12	11	8.3	9	2	1:0.22	-
69		NOT PREGNANT					
70	9	8	11.1	3	5	1:1.67	-
71	7	7	0.0	4	3	1:0.75	-
72	8	8	0.0	4	4	1:1.00	-
Total	100	96		49	47		-
Mean	10.00	9.60	4.00			1:0.96	0.00
± S.E.M.	0.68	0.65					

TABLE 8 [illegible]

Group 7 600 mg/kg Epilim

No.	Mean Wt. Day 7 (g)	Mean Wt. Weaning (g)	% Wt. Increase	Duration of Pregnancy (Days)
1		NO PUPS BORN		
2		" " "		
3		" " "		
4		" " "		
5		" " "		
6		" " "		
7		" " "		
8		" " "		
9	19	47	147	23
10		NO PUPS BORN		
11		" " "		
12		" " "		
Mean	19	47	147	23

TABLE 8 b) (Cont'd)

Group 8 10 ml/kg Water

No.	Mean Pup Wt. Day 7 (g)	Mean Pup Wt. Weaning (g)	% Wt. Increase	Duration of Pregnancy (Days)
61	14	50	257	22
62	13	45	246	22
63	14	40	186	22
64	15	47	213	23
65	20	64	220	23
66	15	47	213	23
67	NOT PREGNANT			
68	11	39	255	23
69	NOT PREGNANT			
70	14	44	214	23
71	16	51	219	22
72	17	54	218	22
Mean	15	48	220	22.5
± S.E.M.	0.77	2.30		0.18

APPENDIX I

SKELETAL ANOMALIES ASSOCIATED WITH EPILIM TREATMENT

600 mg/kg		
Rat No. 22	Foetus L2	1st lumbar vertebra bipartite
	" L3	14 ribs one side + 6th lumbar vertebra bipartite
	" L7	6th lumbar vertebra bipartite
	" R2	14 ribs + unossified sternum
Rat No. 24	Foetus L2	2nd lumbar vertebra hemicentric
	" L4	14 ribs + 1st & 2nd lumbar vertebrae bipartite
	" R1	All lumbar vertebrae hemicentric
	" R3	1st & 2nd lumbar vertebrae acentric
300 mg/kg		
Rat No. 28	Foetus L1	All lumbar vertebrae bipartite
	" L2	All lumbar vertebrae bipartite
	" L3	All lumbar vertebrae bipartite
Rat No. 34	Foetus L1	14 ribs + 3rd, 6th, & 7th lumbar vertebrae bipartite
	" L2	14 ribs + 3rd - 7th lumbar vertebrae acentric
	" L4	3rd - 7th lumbar vertebrae acentric
	" L5	All lumbar vertebrae acentric
	" L6	14 ribs + 2nd & 3rd lumbar vertebrae fused + all lumbar vertebrae acentric
	" L7	All lumbar vertebrae bipartite
	" R1	2nd, 3rd & 6th lumbar vertebrae bipartite
	" R2	14 pair ribs + all lumbar vertebrae acentric
	" R3	3rd, 7th & 8th lumbar vertebrae bipartite
	" R4	All lumbar vertebrae acentric
	" R5	All lumbar vertebrae bipartite
	" R6	All lumbar vertebrae bipartite

APPENDIX 1 (Cont'd)

Rat No. 35	Foetus	L1	All lumbar vertebrae hemicentric or bipartite
	"	L3	All lumbar vertebrae hemicentric or bipartite
	"	L5	All lumbar vertebrae hemicentric or bipartite
	"	L7	All lumbar vertebrae hemicentric or bipartite
Rat No. 36	Foetus	L1	14 pair ribs + all lumbar vertebrae hemicentric or bipartite
	"	L2	All lumbar vertebrae hemicentric or bipartite
	"	L3	All lumbar vertebrae hemicentric or bipartite
	"	L4	14 pair ribs + all lumbar vertebrae hemicentric or bipartite
	"	L5	14 ribs + all lumbar vertebrae hemicentric or bipartite
	"	L6	All lumbar vertebrae hemicentric or bipartite
	"	L7	All lumbar vertebrae hemicentric or bipartite
	"	R2	14 ribs + all lumbar vertebrae hemicentric or bipartite
	"	R3	14 pair ribs + all lumbar vertebrae hemicentric or bipartite
150 mg/kg			
Rat No 42	Foetus	L4	14 pair ribs + 2nd & 3rd lumbar vertebrae bipartite
Rat No. 46	Foetus	R1	Oligodactyly + right radius absent + all lumbar vertebrae hemicentric or bipartite
	"	R2	Oligodactyly + right radius absent + all lumbar vertebrae hemicentric or bipartite
Rat No. 47	Foetus	L1	10th & 11th ribs fused + 1st & 2nd lumbar vertebrae bipartite
	"	L2	1st - 3rd lumbar vertebrae bipartite
	"	L3	11th &12th ribs fused + 1st & 2nd lumbar vertebrae acentric
	"	L4	1st lumbar vertebrae acentric
	"	L5	12th & 13th ribs fused + 1st - 5th lumbar vertebrae acentric

APPENDIX 1 (Cont'd)

Rat No. 47	Foetus	L6	1st - 3rd lubar vertebrae bipartite
	"	R1	11th & 12th ribs fused
	"	R2	All lumbar vertebrae bipartite
	"	R3	1st & 5th lumbar vertebrae bipartite
	"	R4	3rd lumbar vertebrae bipartite
	"	R6	3rd lumbar vertebrae bipartite
	"	R7	14 pair ribs + 1st & 3rd lumbar vertebrae bipartite
	"	R8	1st, 4th & 5th lumbar vertebrae bipartite
Rat No. 48	Foetus	L1	All lumbar vertebrae bipartite
	"	L3	All lumbar vertebrae bipartite
	"	L5	All lumbar vertebrae fused and bipartite
	"	R1	All lumbar vertebrae fused and bipartite
	"	R2	All lumbar vertebrae hemicentric

APPENDIX

CUMULATIVE DATA ON SPRAGUE - DAWLEY RATS MAINTAINED IN CONSULTOX LABORATORIES ANIMAL HOUSE

CORRECT TO JULY 1st 1971

Parameter	Control Animals Only	Animals From Inactive	Total
Pregnancy Rate			
Percentage	85.7	82.4	83.4
Numbers Involved	306/357	660/801	966/1158
Range of Mean Values	66.7 - 100.0	60.0 - 100.0	60.0 - 100.0
Corpora Lutea			
Mean No. per Mother	13.21	14.40	14.02
Numbers Involved	4042	9504	13546
Range of Mean Values	10.36 - 15.23	9.91 - 15.42	9.91 - 15.42
Individual Animal Range	2 - 18	1 - 24	1 - 24
Implantations			
Mean No. per Mother	11.40	11.95	11.78
Numbers Involved	3488	7887	11375
Range of Mean Values	7.42 - 14.96	7.80 - 15.30	7.42 - 15.30
Individual Animal Range	1 - 14	1 - 20	1 - 20
Pre - Implantation Loss			
% Loss	13.7	17.0	16.0
Range	9.6 - 21.2	6.7 - 28.1	6.7 - 28.1
Foetuses			
Mean No. per Mother	10.90	11.37	11.22
Numbers Involved	3335	7504	10839
Range of Mean Values	8.50 - 13.25	7.55 - 14.40	7.55 - 14.40
Individual Animal Range	0 - 14	0 - 20	0 - 20
Resorptions			
Mean No. per Mother	0.25	0.35	0.32
Numbers Involved	76	231	307
Range of Mean Values	0.15 - 0.58	0.07 - 1.36	0.07 - 1.36
Individual Animal Range	0 - 5	0 - 7	0 - 7
Resorption Sites			
Mean No. per Mother	0.25	0.23	0.24
Numbers Involved	77	152	229
Range of Mean Values	0.08 - 0.42	0.07 - 0.45	0.07 - 0.45
Individual Animal Range	0 - 4	0 - 3	0 - 4

Parameter	Control Animals Only	Animals From Inactive Drugs	Total
Uterine Deaths			
Mean No. per Mother	0.50	0.58	0.56
Numbers Involved	153	383	536
Range of Mean Values	0.23 - 1.00	0.10 - 1.65	0.10 - 1.65
Individual Animal Range	0 - 6	0 - 8	0 - 8
Foetal Weights			
Mean Foetal Wt. (g)	4.60	4.96	4.86
Range of Mean Values	4.17 - 6.21	4.19 - 6.15	4.17 - 6.21
Individual Animal Range	3.42 - 6.88	3.56 - 7.12	3.42 - 7.12
Crown/Rump Lengths			
Mean Crown/Rump Length (mm)	44.1	42.3	42.8
Range of Mean Values	41.5 - 46.2	38.3 - 47.6	38.3 - 47.6
Individual Animal Range	34 - 49	33 - 52	33 - 52
Sex Ratios			
Number Males	1861	3956	5817
Number Females	1474	3548	5022
Range Males:Females	1:0.79	1:0.90	1:0.86
Range of Mean Values	1:0.52-1:1.34	1:0.60-1:1.37	1:0.52-1:1.37
Major Deformities			
Percentage	0.66	0.87	0.80
Numbers Involved	22	65	87
Range of Mean Values	0.00 - 1.27	0.00 - 1.54	0.00 - 1.54
Minor Deformities			
Percentage	4.44	4.96	4.80
Numbers Involved	148	372	520
Range of Mean Values	2.06 - 9.41	0.77 - 9.09	0.77 - 9.09

Deformity	Control Animals Only		Animals From Inactive Drugs		Total	
	No.	%	No.	%	No.	%
Major Defects						
Exencephaly	2	0.06	3	0.05	5	0.05
Agnathia	3	0.09	4	0.06	7	0.06
Micrognathia	2	0.06	2	0.03	4	0.04
Cleft Palate	1	-	4	0.06	4	0.04
Anophthalmia	1	-	1	-	2	0.02
Fused Ribs	1	-	2	0.03	3	0.03
Wavy Ribs	1	-	2	0.03	3	0.03
Rib Absent	1	-	2	0.03	3	0.03
Unossified Sternum	6	0.18	24	0.32	30	0.28
Oblique Fusion of Sternebrae	2	0.06	3	0.05	5	0.05
Caudal Vertebrae Absent	-	-	1	-	1	-
Lumbar Centra Absent	-	-	1	-	1	-
Sacral Centra Absent	-	-	1	-	1	-
Muscle Flexure	2	0.06	17	0.23	19	0.18
Oligodactyly	2	0.06	1	-	3	0.03
Hydronephrosis	3	0.09	1	-	4	0.04
Minor Defects						
14 Pair Ribs	90	2.70	202	2.69	306	2.82
14 Ribs One Side	58	1.74	170	2.27	214	1.97

LITTERING GROUPS

Parameter	Control Animals Only	Animals From Inactive Drugs	Total
Pregnancy Rate			
Percentage	86.67	86.67	86.67
Total Numbers Involved	156/180	156/180	312/360
Range	66.7 - 100.0	66.7 - 100.0	66.7 - 100.0
Duration of Gestation			
Mean Duration (Days)	21.9	21.7	21.8
Range	21.8 - 22.0	21.5 - 21.9	21.5 - 22.0
Individual Animal Range	20 - 23	20 - 23	20 - 23
Pups Born			
Mean Number per Mother	11.30	11.00	11.15
Total Numbers Involved	1763	1716	3479
Range	9.21 - 12.10	8.50 - 12.31	8.50 - 12.31
Individual Animal Range	6 - 14	7 - 15	6 - 15
Pups Weaned			
Mean Number per Mother	8.70	7.92	8.31
Total Numbers Involved	1357	1236	2593
Range	6.46 - 9.21	5.49 - 10.63	5.49 - 10.63
Individual Animal Range	0 - 12	0 - 13	0 - 13
Pre - Weaning Loss			
Percentage	23.0	28.0	25.5
Range	11.4 - 27.3	11.7 - 31.2	11.4 - 31.2
Weight Gain			
Mean Pup Wt. Day 7 (g)	11.3	11.6	11.5
Range	9.0 - 14.4	8.9 - 13.0	8.9 - 14.4
Mean Pup Wt. Weaning (g)	35.3	38.4	36.9
Range	26.1 - 49.2	27.9 - 43.3	26.1 - 49.2
Percentage Weight Increase	212	231	221
Range	170 - 282	189 - 288	170 - 288

Parameter	Control Animals Only	Animals From Inactive Drugs	Total
Sex Ratios			
Total Number Males	749	703	1452
Total Number Females	608	533	1141
Ratio Males:Females	1:0.81	1:0.76	1:0.79
Range	1:0.60-1:1.14	1:0.52-1:0.98	1:0.52-1:1.14

OVERALL DISCUSSION AND CONCLUSIONS

With interspecific differences administration of EPILIM to rabbits, rats [redacted] has evoked teratogenic, embryotoxic and embryolethal effects not less severe than those induced by PHENYTOIN. The nature of the teratogenic response was similar in the rat and the rabbit but was qualitatively different from that evoked by PHENYTOIN [redacted]

The teratogenic effects of PHENYTOIN in animals have previously been described [redacted] and its possible human teratogenic activities has been discussed [redacted], but it is generally accepted that the risk is small and that removal of this means of anti-convulsant therapy would constitute a severe risk in itself.

References

[redacted]