CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Table 8 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 800C-4
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES --				-- ANIMALS AFFECTED -- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
LYMPH N., MAND.	NUMBER EXAMINED:	49	47	47	46	47	47	48	47
-N- LYMPHOSARCMA		2	1	0	1	0	1	0	2
-M- FIBROUS HISTIOCYTOMA		1	0	0	0	0	0	0	1
-N- MALIGNANT MELANOMA		0	0	0	0	1	0	0	0
SPLEEN	NUMBER EXAMINED:	50	48	50	49	49	48	50	49
-N- LYMPHOSARCOMA		3	2	2	2	0	1	1	0
-M- HEMANGIOSARCOMA		0	0	0	1	0	0	0	0
-N- ADENOCARCINOMA		0	0	0	0	1	1	0	2
-N- OSTEOSARCOMA		1	0	0	0	0	0	0	0
THYMUS	NUMBER EXAMINED:	38	45	49	39	42	48	47	37
-N- LYMPHOSARCOMA		0	1	0	0	0	2	0	1
-M- FIBROUS HISTIOCYTOMA		3	1	1	0	0	0	0	0
-M- THYMOMA		0	0	0	0	1	0	0	0
-N- ADENOCARCINOMA		0	0	0	0	1	1	0	0
-M- ADENOCARCINOMA		0	1	0	0	0	0	0	0
LUNG	NUMBER EXAMINED:	49	50	50	50	50	50	50	50
-N- LIPOSARCOMA		0	0	0	1	0	0	0	0
-N- ADENOCARCINOMA		0	0	0	0	2	3	0	1
-N- FIBROUS HISTIOCYOMA		2	2	1	0	0	0	0	1
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	1
-B- PULMONARY ADENOMA		1	1	0	0	0	0	0	0
-M- BRONCHOGENIC CARCINOMA		1	0	0	0	0	0	0	0
-N- FIBROSARCOMA		0	0	0	0	1	0	0	0
ESOPHAGUS	NUMBER EXAMINED:	49	50	50	49	50	49	50	50
-N- FIBROSARCOMA		0	0	0	0	1	0	0	0
DIAPHRAGM	NUMBER EXAMINED:	49	50	50	49	49	49	50	50
-N- ADENOCARCINOMA		0	0	0	0	1	1	1	1
-N- FIBROUS HISTIOCYTOMA		1	1	1	0	0	0	0	0
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
-N- FIBROSARCOMA		0	0	0	0	0	0	1	0

CARCINOGENICITY STUD. OF RATS TREATED WITH VIGABATRIN

Table 8 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 800C-4
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS -- MALES --				AFFECTED -- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
HEART	NUMBER EXAMINED:	49	50	50	50	50	50	50	50
-N- LYMPHOSARCOMA		0	1	0	1	0	0	0	0
-N- LIPOSARCOMA		0	0	0	1	0	0	0	0
-N- FIBROUS HISTIOCYTOMA		2	0	0	0	0	0	0	1
-M- HEMANGIOSARCOMA		0	0	0	0	0	0	0	1
-N- ADENOCARCINOMA		0	1	0	0	0	0	0	0
AORTA	NUMBER EXAMINED:	49	50	50	50	50	50	50	50
-N- FIBROUS HISTIOCYTOMA		1	1	0	0	0	0	0	0
-N- LYMPHOSARCOMA		0	1	0	0	0	1	0	0
TONGUE	NUMBER EXAMINED:	48	49	49	50	50	49	50	49
-N- FIBROSARCOMA		0	0	0	0	1	0	0	0
-B- FIBROMA		0	0	1	0	0	0	0	0
LIVER	NUMBER EXAMINED:	50	50	50	49	50	50	50	50
-N- LYMPHOSARCOMA		3	2	2	2	0	1	1	1
-B- BASOPHILIC FOCI		0	0	0	1	0	0	0	0
-N- LIPOSARCOMA		0	0	0	1	0	0	0	0
-B- HEPATOCELLULAR ADENOMA		0	1	0	1	2	0	0	2
-M- HEPATOCELLULAR CARCINOMA		0	0	1	1	1	0	0	0
-N- ADENOCARCINOMA		0	0	0	0	1	2	0	1
-N- FIBROUS HISTIOCYTOMA		2	1	0	0	0	0	0	1
-B- CLEAR CELL FOCI		1	0	0	1	2	1	2	1
-B- NODULAR HYPERPLASIA		2	0	0	0	1	0	0	0
-N- FIBROSARCOMA		0	1	0	0	0	0	0	0
-B- EOSINOPHILIC FOCI		2	7	1	1	4	2	6	1
STOMACH	NUMBER EXAMINED:	50	49	50	50	50	50	50	50
-N- FIBROUS HISTIOCYTOMA		1	0	0	0	0	0	0	1
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
-N- ADENOCARCINOMA		0	0	0	0	0	0	1	0
-B- LEIOMYOMA		0	0	1	0	0	0	0	0
PANCREAS	NUMBER EXAMINED:	44	44	48	46	47	44	47	47
-N- LIPOSARCOMA		0	0	0	1	0	0	0	0
-N- ADENOCARCINOMA		0	0	0	0	1	1	1	2
-B- ISLET CELL ADENOMA		1	1	0	0	0	0	0	0
-M- ACINAR ADENOCARCINOMA		0	0	0	0	1	1	0	0
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
-N- FIBROSARCOMA		0	1	0	0	0	0	0	0
-N- FIBROUS HISTIOCYTOMA		1	0	0	0	0	0	0	0

CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Table 8 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 800C-4
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS -- MALES --				AFFECTED -- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
JEJUNUM	NUMBER EXAMINED:	36	40	46	40	43	38	40	47
-N- ADENOCARCINOMA		0	0	0	0	2	0	0	1
-M- ADENOCARCINOMA		0	0	0	0	1	0	0	0
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
COLON	NUMBER EXAMINED:	44	43	48	47	45	45	46	48
-N- ADENOCARCINOMA		0	0	0	0	0	0	1	1
LYMPH N., MESEN.	NUMBER EXAMINED:	48	47	50	45	48	46	49	47
-N- LYMPHOSARCOMA		2	2	0	1	0	1	0	0
-N- LIPOSARCOMA		0	0	0	1	0	0	0	0
-B- HEMANGIOMA		0	0	0	0	0	0	0	1
-N- ADENOCARCINOMA		0	0	0	0	2	1	0	0
-N- FIBROSARCOMA		0	1	0	0	0	0	0	0
KIDNEY	NUMBER EXAMINED:	50	50	50	50	50	50	50	50
-N- LYMPHOSARCOMA		1	1	1	1	0	1	0	1
-B- LIPOMA		0	0	1	1	0	1	0	0
-N- FIBROUS HISTIOCYTOMA		0	0	0	0	0	0	1	1
-N- ADENOCARCINOMA		0	0	0	0	1	2	0	0
URINARY BLADDER	NUMBER EXAMINED:	50	50	49	50	48	50	50	47
-N- ADENOCARCINOMA		0	0	0	0	0	0	0	1
MAMMARY GLAND	NUMBER EXAMINED:	49	50	50	49	50	49	48	48
-N- LYMPHOSARCOMA		0	0	0	1	0	0	0	0
-M- ADENOCARCINOMA		0	0	0	0	7	1	4	2
-B- ADENOMA		0	0	0	0	2	1	1	0
-B- FIBROADENOMA		0	0	0	0	3	6	1	0
-M- FIBROSARCOMA		0	1	0	0	0	0	0	0
-B- LIPOMA		1	0	0	0	0	0	2	0
MUSCLE (l.dorsi)	NUMBER EXAMINED:	50	50	50	50	50	50	50	50
-N- FIBROUS HISTIOCYTOMA		0	1	0	0	0	0	0	1
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0

Table 8 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 800C-4
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX: / DOSAGE GROUP: / NO. IN GROUP:	-- ANIMALS -- MALES -- 1 50	2 50	3 50	4 50	AFFECTED -- FEMALES -- 1 50	2 50	3 50	4 50
SKIN/SUBCUTIS	NUMBER EXAMINED:	50	50	50	50	49	50	50	50
-M- SQUAMOUS CELL CARCINOMA		0	0	1	1	0	1	0	0
-B- FIBROMA		3	0	1	1	1	0	1	0
-M- FIBROUS HISTIOCYTOMA		2	3	1	0	0	0	0	1
-B- LIPOMA		1	1	1	1	1	0	0	0
-M- LIPOSARCOMA		1	0	0	0	0	0	0	0
-B- PAPILLOMA		1	1	0	0	0	0	0	0
-M- MYXOSARCOMA		2	0	1	0	0	0	0	0
-M- FIBROSARCOMA		1	1	2	0	2	0	1	0
-M- NEUROFIBROSARCOMA		0	0	1	0	0	0	0	0
-N- LYMPHOSARCOMA		0	1	0	0	0	0	0	0
TESTIS	NUMBER EXAMINED:	49	49	48	50				
-B- INTERSTITIAL CELL TUMOR		0	0	0	1				
-B- SERTOLI CELL TUMOR		0	0	0	1				
-N- LYMPHOSARCOMA		0	1	0	0				
EPIDIDYMIS	NUMBER EXAMINED:	49	49	48	50				
-N- LYMPHOSARCOMA		1	0	0	1				
-N- FIBROUS HISTIOCYTOMA		1	0	0	0				
PROSTATE	NUMBER EXAMINED:	49	48	49	48				
-N- LYMPHOSARCOMA		1	0	0	0				
SEMINAL VESICLES	NUMBER EXAMINED:	50	47	49	46				
-N- LYMPHOSARCOMA		0	0	0	1				
-N- FIBROUS HISTIOCYTOMA		1	0	0	0				
OVARY	NUMBER EXAMINED:					50	50	49	48
-N- LYMPHOSARCOMA						0	1	0	1
-N- ADENOCARCINOMA						1	1	0	0
-M- FIBROSARCOMA						0	0	1	0
-M- GRANULOSA CELL TUMOR						0	1	1	0
UTERUS	NUMBER EXAMINED:					50	50	50	49
-M- ADENOCARCINOMA						5	4	3	4
-B- STROMAL POLYP						2	1	1	5
-N- ADENOCARCINOMA						1	0	0	0
-M- STROMAL CELL SARCOMA						1	1	1	0
-B- ENDOMETRIAL GLAND POLYP						1	0	0	0

CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Table 8 (continued)

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS -- MALES --				AFFECTED -- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
VAGINA	NUMBER EXAMINED:					47	49	50	47
-M- LEIOMYOSARCOMA						0	1	0	0
STBRAE (MARROW)	NUMBER EXAMINED:	50	50	50	49	50	50	50	50
-N- LYMPHOSARCOMA		0	2	2	1	0	1	0	1
NOSE/TURBINATE	NUMBER EXAMINED:	0	1	1	4	0	0	1	2
-B- PAPILLOMA OF THE NOSE		0	0	0	1	0	0	0	0
-N- LYMPHOSARCOMA		0	1	0	0	0	0	0	0
SPINAL CORD	NUMBER EXAMINED:	0	2	0	4	0	1	0	0
ABDOMINAL CAVITY	NUMBER EXAMINED:	7	5	8	4	4	3	8	1
-M- MYXOSARCOMA		1	0	0	1	0	0	0	0
-M- LIPOSARCOMA		0	0	0	2	0	0	0	0
-N- ADENOCARCINOMA		0	0	0	0	1	1	1	1
-N- MYXOSARCOMA		1	0	0	0	0	0	0	0
-N- FIBROUS HISTIOCYTOMA		1	1	0	0	0	0	0	0
-M- FIBROSARCOMA		1	1	0	0	1	0	0	0
-M- UNDIFFERENTIATED ADENOCARCINOMA		0	0	0	0	1	0	0	0
-N- FIBROSARCOMA		0	0	0	0	0	0	1	0
-M- RETICULUM CELL SARCOMA		0	0	1	0	0	0	0	0
-N- SARCOMA		0	0	0	0	0	1	0	0
LYM./HEMOPOIETIC	NUMBER EXAMINED:	3	2	2	2	0	2	1	2
-M- LYMPHOCYTIC LEUKEMIA/LYMPHOSARCOMA		3	2	2	2	0	2	1	2
EAR	NUMBER EXAMINED:	0	0	1	1	1	1	0	0
-M- MALIGNANT MELANOMA		0	0	0	1	1	0	0	0
-B- SQUAMOUS PAPILLOMA		0	0	1	0	0	0	0	0
COAGULATE GLAND	NUMBER EXAMINED:	0	0	0	1	0	0	0	0
TAIL	NUMBER EXAMINED:	0	7	4	2	0	1	0	0
THORACIC CAVITY	NUMBER EXAMINED:	0	0	0	1	0	0	0	1
-N- LIPOSARCOMA		0	0	0	1	0	0	0	0
-N- ADENOCARCINOMA		0	0	0	0	0	0	0	1

CARCINOGENICITY STUD. OF RATS TREATED WITH VIGABATRIN

Table 8 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER B00C-4
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS -- MALES --				AFFECTED -- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
PARATHYROID	NUMBER EXAMINED:	1	2	1	1	0	0	0	0
PAW/FOOT	NUMBER EXAMINED:	0	6	1	0	1	0	0	0
-M- OSTEOGENIC OSTEOSARCOMA		0	0	0	0	1	0	0	0
RECTUM	NUMBER EXAMINED:	0	0	0	0	0	1	0	0
-M- SQUAMOUS CELL CARCINOMA		0	0	0	0	0	1	0	0

CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Table 8 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY
CINCINNATI, OHIO 45215

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 800C-4

PRINTED: 25-AUG-86
PAGE: 1

ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

SUMMARY REPORT	ANIMAL S E X: DOSAGE GROUP: NO. IN GROUP:	-- ANIMALS AFFECTED -- -- MALES -- 1 50	2 50	3 50	4 50	-- FEMALES -- 1 50	2 50	3 50	4 50
TOTAL PRIMARY NEOPLASMS		57	50	36	36	77	50	46	40
ANIMALS WITH ONE OR MORE		35	33	29	26	42	34	31	30
PERCENT WITH ONE OR MORE		70	66	58	52	84	68	62	60
TOTAL BENIGN NEOPLASMS		39	40	23	26	53	36	34	28
ANIMALS WITH ONE OR MORE		27	28	21	19	38	27	28	23
PERCENT WITH ONE OR MORE		54	56	42	38	76	54	56	46
TOTAL MALIGNANT NEOPLASMS		18	10	13	10	24	14	12	12
ANIMALS WITH ONE OR MORE		15	9	11	9	19	12	11	11
PERCENT WITH ONE OR MORE		30	18	22	18	38	24	22	22
METASTATIC NEOPLASMS									
ANIMALS WITH ONE OR MORE		9	7	3	3	4	6	4	5
PERCENT WITH ONE OR MORE		18	14	6	6	8	12	8	10

2 WEEK ORAL RANGEFINDING STUDY IN BEAGLE DOGS

A) Dosage

1 M + 1 F at 0, 100, 300, 600, or 1000 mg/kg/day, by gavage (aqueous solution)

B) Results

1. Observed signs

   a. Sporadic emesis at 300 mg/kg and above (no more than on 3 days per dog).

   b. Dark urine on 1-3 days in the M at 600 and 1000 mg/kg.

2. Mortality

   Death of HD F and HD M on days 11 and 13, resp. (See below for pathology results).

3. Bodyweight

   Decreased at 300 mg/kg and above.

4. Food consumption

   Decreased at 300 mg/kg and above.

5. Laboratory tests

   (Performed pre-and post-study, except for the HD F which died day 11).

   a. Hematology

      1. Several substantial abnormalities seen in the HD M: Elevated Hct, Hb, WBC, % band N, % PMN, % monocytes. Decrease in % lymphocytes although absolute number of lymphocytes was also elevated. The report concludes that these changes were consistent with a gastritis and myocarditis which were considered incidental lesions in this dog.

      2. No consistent effects in other dogs on above parameters plus RBC, % reticulocytes, Heinz bodies, platelets.

   b. Blood chemistry

      1. Changes seen in the HD M: slight decrease in total protein and albumin, elevated glucose (although this dog, in contrast to the

2 WEEK ORAL RANGEFINDING STUDY IN BEAGLE DOGS

A) Dosage

1 M + 1 F at 0, 100, 300, 600, or 1000 mg/kg/day, by gavage (aqueous solution)

B) Results

1. Observed signs

   a. Sporadic emesis at 300 mg/kg and above (no more than on 3 days per dog).

   b. Dark urine on 1-3 days in the M at 600 and 1000 mg/kg.

2. Mortality

   Death of HD F and HD M on days 11 and 13, resp. (See below for pathology results).

3. Bodyweight

   Decreased at 300 mg/kg and above.

4. Food consumption

   Decreased at 300 mg/kg and above.

5. Laboratory tests

   (Performed pre-and post-study, except for the HD F which died day 11).

   a. Hematology

      1. Several substantial abnormalities seen in the HD M: Elevated Hct, Hb, WBC, % band N, % PMN, % monocytes. Decrease in % lymphocytes although absolute number of lymphocytes was also elevated. The report concludes that these changes were consistent with a gastritis and myocarditis which were considered incidental lesions in this dog.

      2. No consistent effects in other dogs on above parameters plus RBC, % reticulocytes, Heinz bodies, platelets.

   b. Blood chemistry

      1. Changes seen in the HD M: slight decrease in total protein and albumin, elevated glucose (although this dog, in contrast to the

others, was not fasted prior to bleeding), increased BUN (no renal pathology noted), increased total bilirubin, and decreased Cl.

2. Elevated AP in the M at 600 mg/kg (no specific organ pathology noted, aside from G.I. irritation which was also seen in other dogs at the higher doses).

3. Elevated BUN in the LD F (no renal pathology noted).

4. ALT decreased in the F at 300 and in both dogs at 600 mg/kg, possibly a result of anti-transaminase activity of the drug.

5. No effects on: A/G ratio, Ca, Pi, AST, CO_2.

c. Urinalysis

1. In HD M: presence of occult blood, and decreased SG.

2. No effects on pH, bilirubin, ketones, glucose, protein, sediment.

6. Organ weights

Necropsy was not performed in controls, and organ weights not given for HD, thus data are difficult to interpret. The absolute and relative weights of the following organs were increased in M at 600 mg/kg compared to the 2 lower dosage groups: thyroid, liver, kidney, and adrenals. Prostate weights were decreased at 300 and 600 mg/kg (dose-related) compared to 100 mg/kg. In F, absolute and relative weights of thyroid and gonads were lower at 600 mg/kg compared to the 2 lower dosage groups.

7. Gross pathology

(Not performed in controls).

Hemorrhages of heart and stomach seen in HD M only. Reddening of various G.I. tract structures seen at the 2 highest doses.

8. Microscopic pathology

(Not performed in controls).

The following were seen in the HD M only: heart - hemorrhage, myocardial necrosis, myocarditis; stomach - submucosal necrosis, suppurative gastritis; congestion of thymus, liver, jejunum, and mesenteric lymph node; atrophy of thymus (also seen in the M at 600 mg/kg). The report concludes that these were probably spontaneous lesions. No significant lesions were seen in the HD F which died.

3 MONTH ORAL TOXICITY IN BEAGLE DOGS

A. Dosage

3 M + 3 F at 0, 30, 100, or 300 mg/kg/day, in gelatin capsules. Diet supplementation in 2 HD on various days to attempt to overcome anorexia and weight loss.

B. Results

1. Observed signs

a. Loose stool and/or diarrhea in 0/3, 3/3, 1/3, and 2/3 M in controls, LD, MD, and HD, resp., on 1-3 days each.

b. Anorexia and emaciation in 1 M and 1 F at HD. (the F was sacrificed day 33).

c. Emesis in HD F on day 28 only.

2. Mortality

1 HD F was sacrificed on day 33 in poor condition (anorexic, emaciated).

3. Bodyweight

Slight weight loss in some HD dogs.

4. Food consumption

Decreased in some HD dogs.

5. ECG

Performed on all dogs pre-drug and on days 8 and 90, approximately 2 hr. post-dosing. No drug effects were noted. No quantitative data was presented.

6. Ophthalmoscopic exam

Performed pre-and post-treatment. No drug effects were noted, although 1 MD M had a bilateral streak of corneal dystrophy noted on day 93.

7. Hematology

Performed pre-treatment and on days 35 and 91 (except for the HD F which was sacrificed - performed day 33).

a. Large decreases (to about ½ pre-drug values) in RBC, Hct, and Hb were seen in the 2 HD dogs which became emaciated. In the M the

decrease was no greater on day 91 than on day 35. The other 2 HD M and one other HD F had moderate decreases in these parameters, mainly on day 91.

b. A large increase in WBC at termination (day 33) was seen in the HD F which was sacrificed. Four nucleated RBC/100 WBC were present. There was a large increase in % band N and a large decrease in absolute number of and % lymphocytes; the absolute number of PMN's was increased in proportion to the increase in total WBC. A substantial number of metamyelocytes were present. In the HD M which became emaciated, a large increase in WBC was noted, but only on day 35. This was associated with an increased % band N and a decreased % of lymphocytes. (Absolute number of lymphocytes was unaltered). Absolute number of PMN's were increased in proportion to the increase in total WBC. A substantial number of metamyelocytes was present on both days 35 and 91. One MD M had an increased N (both bands and PMN) and decreased L, with slight elevation of total WBC, on day 91.

c. No drug effects on platelets, PT, PTT

8. Blood chemistry

Performed pre-treatment and on days 35 and 91 (except for the HD F which was sacrificed-performed day 33).

a. An elevation of AP (~4x pre drug) at termination was seen in both HD dogs which became emaciated. (See below for pathology results). The HD M also had an elevated ALT (3.5x) at termination. One MD F had an elevated AP at termination; no specific organ pathology was noted.

b. Slight decreases in Ca (10-20% less than pre-drug values) were seen in most dogs at MD and HD at termination.

c. Decreases in ALT were seen in a few MD and HD dogs, possibly a result of the anti-transaminase activity of the drug.

d. No drug effects on: total protein, albumin, A/G ratio, Pi, glucose, BUN, total bilirubin, AST, CO_2, Cl, Na, K.

9. Urinalysis

Performed pre-and post-treatment. No drug effects were noted (pH, SG, occult blood, bilirubin, ketones, glucose, protein, sediment.)

10. Organ weights

a. Liver-rel. wt. increased in 2 HD M (about 2x controls) without change in absolute weight. In 1 of these, histopathology showed pale swollen hepatocytes and hematopoiesis.

b. Absolute and relative weights of uterus and ovaries greatly increased in 1 LD F; small increases seen in a few a MD and HD.

c. Spleen - increased abs. and rel. wt in 1 HD F (about 2x control). Post-mortem exam showed enlarged spleen ("not well bled out") with hematopoiesis.

d. Aside from those mentioned above, the organ weight changes were not associated with any specific histopathology.

11. Gross pathology

Performed on all dogs.

There were no clearly drug-related effects. Nodules on lungs were seen in 1 control M, 1 LD F, 0 at MD, and 3 HD M. Microscopic exam showed granulomas in 1 control and 2 HD which were considered as probably parasitic in origin in the control and 1 of the HD; the 3rd HD showed no microscopic abnormality in lung. See below for gross findings in the 2 HD which became emaciated.

12. Microscopic pathology

a. Although the initial report concluded no effects on brain, brain sections were subsequently re-evaluated based on findings of brain vacuolization in other ongoing studies. (Some sections were re-cut "to allow good examination of the columns of the fornix and optic tract [location where the lesion is most notable]". Also, in addition to H + E staining, "sections from the cerebrum were stained with luxol fast blue in order to further evaluate any effects on myelin". Results are shown in the attached table. Vacuolization was seen at MD and HD in the areas shown. (Note that, as indicated in the table, "not all of the listed structures were examined in every instance"; thus the appropriate denominations are not clear). It was stated that Luxol fast blue slides showed no "evidence of segmental demyelination as is seen in demyelinating diseases such as multiple sclerosis" (but again, it is not clear how many sections in how many animals were examined). It was stated that there were no drug effects in cerebellum, pons, or lumbar spinal cord. (Sciatic nerve and eyes were said to have been examined; presumably there were no drug effects).

b. Hematopoiesis in spleen was seen in 2 HD M and 1 HD F, and sternal marrow hyperplasia was seen in 1 HD M and 2 HD F. The text states that this was probably a compensatory response to the anemia seen in this group.

c. Findings in the HD F which became emaciated and sacrificed day 33 not seen in other dogs:

1. Gross:

Thoracic cavity - blood-containing fluid

Lung - ulcers and adhesions

Stomach - numerous ulcerations and erosions in fundus

Colon - a few tiny eroded areas

Heart - pale areas

2. Microscopic:

Thymus - atrophic (also seen in the HD M which became emaciated; considered by report to be a response to stress).

Lung - Abscesses and pleuritis

Diaphragm - Pleuritis

Liver - brownish pigment in Kuppfer cells (also seen in another HD F).

Heart - Areas of myocardial degeneration

Stomach - focal ulcerations

d. Findings in the HD M which became emaciated not seen in other dogs:

1. Gross - nothing specific

2. Microscopic

Thymus - atrophic (also seen in the HD F, above).

Liver - pale swollen hepatocytes and hematopoiesis

Pancreas - occasional necrobiotic cell.

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3 MONTH DOG

HISTOPATHOLOGIC REEVALUATION OF BRAIN TISSUE

Table 2

Summary of Vacuolar Brain Changes*

Animal No.	Sex	Optic Tract	Columns of Fornix	Fimbria of Fornix	Colliculus	Hypothalamus
				Control		
77-151	M	-	-	+	-	-
77-152	M	+	-	+	-	-
78-105	M	-	-	-	-	-
78-89	F	-	-	+	-	-
78-110	F	-	-	-	-	-
78-111	F	+	-	+	-	-
				30 mg/kg/day		
78-101	M	-	-	-	-	-
78-103	M	-	-	-	-	-
78-104	M	-	-	-	-	-
78-86	F	-	-	-	-	-
78-88	F	-	-	-	-	-
78-106	F	-	-	-	-	-
				100 mg/kg/day		
78-75	M	-	++	-	+	+
78-97	M	-	++	-	+	-
78-98	M	-	-	-	-	-
78-87	F	+++	+++	-	-	-
78-107	F	+	++++	-	-	-
78-112	F	++	++	-	+	-
				300 mg/kg/day		
78-99	M	++++	+++++	+++	-	++
78-100	M	++++	++++	-	+++	++
78-102	M	++++	++++	-	++ [a]	-
78-90	F	-	++++	-	+	++
78-108	F	++++	+++++	-	++	+++
78-113	F	+++	+++++	-	+	++

[a] Also slight vacuolation of hippocampus

* See ~~previous~~ following page for Histopathologic Grading Classification. Not all of the listed structures were examined in every instance

3 MONTH DOG

Histopathologic Grading Classification (See previous page)

0	None or very rare isolated vacuoles
+	Occasional vacuoles (sometimes seen in controls)
++	Slight vacuolation (slightly more than ever seen in controls)
+++	Mild vacuolation
++++	Moderate vacuolation
+++++	Severe diffuse vacuolation

ONE YEAR P.O. TOXICITY IN BEAGLE DOGS:

A) Dosage

4/sex at 0, 50, or 100 mg/kg/day, and 5/sex at 200 mg/kg/day, in capsules.

Sacrifice times were as follows (see table): 1/sex/group at 6 months, 1/sex at HD after 7 months' treatment + 4 month recovery period, 1-2/sex/group at 12 months, and 1-2/sex/group after 12 months' treatment + 6 month recovery period.

Lot #: 71,754-40 (days 1-133)
71,754-46 (days 134-367)

B) Results

1) Observed signs

No clear drug effects. Loose stools were seen on rare occasions among treated dogs; too infrequent to be considered to be drug-related by sponsor. (See table).

2) Mortality
One control F and 1 MD F died of accidental strangulation.

3) Bodyweight

No drug effect

4) Food Consumption

No drug-effect. (Subjective evaluation).

5. Ophthalmoscopic and physical exam.

Said to have been done pre-study, after 6 and 12 months, and after 6 month recovery period following 12 months' treatment. It was stated that there were no drug effects, although the only results presented were for ophthalmoscopic exam at 11 months.

6. EKG

(Performed pre-study, and approximately 2 and 24 hr. post-dosing at 3, 6, and 12 months.)

No drug effects. (No quantitative data presented).

7) Hematology

(Performed pre-study and at 3, 6, 9, and 12 months).

No drug effects.

Parameters measured: RBC, Hb, Hct, WBC, differential, PT, PTT, platelets.

8) Blood chemistry

(Performed pre-study and at 3, 6, 9, and 12 months)

No clear drug effects aside from a decreased ALT in MD and HD M and all F groups, mainly at 3 months. (Attributed to drug-induced transaminase inhibition, although it is not clear why it was not more widely manifest at the later time points).

Other parameters measured: AST, AP, total bilirubin, arginase, total protein, albumin, A/G ratio, glucose, BUN, Ca, Pi, CO_2, Cl, Na, K.

9) Urinalysis

(Done pre-study and at 6 and 12 months).

No drug effects.

10) Organ weights

Drug effects difficult to determine due to small N sacrificed at each time point. Large decreases in absolute and relative uterus weights were seen at 6 and 12 months at all doses; however this was not D-R, was not seen after the 6 month recovery period, and was not associated with drug-induced histopathological changes.

11) Macroscopic organ exam

No clear drug effects; however such effects would be difficult to determine in view of the small N sacrificed at each time point.

12) Microscopic organ exam

(In addition to routine exam, after 12 months' treatment + 6 months' recovery "a standard transverse cerebral section through the optic tract and another section through the cerebellum were stained with Luxol fast blue for myelin and similar cerebellar sections were stained with Sevier Munger silver stain for nerve endings and neurofibrils and phosphotungstic acid hematoxylin for neuroglial fibers.").

As above, some drug effects may be difficult to determine due to the small N at each sacrifice. The only effect considered to be drug-related was vacuolation in several brain areas as indicated in the attached table. It was seen only at MD and HD at 6 months and at all doses at 12 months. The severity was dose-related. There were no clear drug effects after the recovery periods; the slightly more widespread occurrence of vacuoles seen after the 4 month recovery period is equivocal. In addition to the vacuolation, a mild gliosis was seen in the optic tract of 1 HD at 12 months.

There were no drug effects in peripheral nerve, spinal cord, or eye.

ONE YEAR TOXICITY STUDY OF MDL 71,754 ADMINISTERED ORALLY TO DOGS

Table 1

Disposition of Animals

Dosage mg/kg/day	6 months treatment Interim sacrifice #1		7 months treatment approx. 4 months recovery Interim sacrifice #2		12 months treatment Interim sacrifice #3		12 months treatment 6 months recovery Final sacrifice[c]	
	M	F	M	F	M	F	M	F
Control[a]	1 (#1)	1 (#18)	-	-	2 (#2, 3)	1 (#20)	1 (#4)	1 (#21)
50	1 (#5)	1 (#22)	-	-	2 (#6, 7)	2 (#23,24)	1 (#8)	1 (#25)
100 [b]	1 (#9)	1 (#26)	-	-	1 (#10)	1 (#27)	2 (#11,12)	1 (#28)
200	1 (#13)	1 (#30)	1 (#14)	1 (#31)	1 (#15)	1 (#32)	2 (#16,17)	2 (#33,34)

[a] Dog #19 died on day 40 from accidental cause
[b] Dog #29 died on day 179 from accidental cause
[c] Still being observed

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ONE YEAR TOXICITY STUDY OF MDL 71,754 ADMINISTERED ORALLY TO DOGS

Table 2

Summary of Clinical Observations

	Dose Groups (mg/kg/day)							
	Control		50		100		200	
	4M	4F	4M	4F	4M	4F	5M	5F
Aggressive/hyperirritable	-	-	-	-	-	-	1/253	-
Skin - abrasion	-	-	-	1/197	1/38	-	-	-
- swelling (between toes)	-	-	-	1/6	-	-	-	-
Emesis	-	-	-	-	-	-	1/1	-
Alopecia	-	1/18	-	-	-	-	-	-
Loose stool	-	-	-	1/1	3/5	1/2	3/10	-
Capsule found on cage floor	-	-	-	1/1	-	1/1	1/1	-
Obese	-	-	1	-	-	-	-	
Nothing significant	4	2	3	2	1	2	1	5

NOTE: The data is expressed as a ratio ("X/Y") in which "X" is the number affected and "Y" is the total number of times it was observed

TABLE 2

ORAL TOXICITY STUDY OF VIGABATRIN IN DOGS:
~~INCIDENCE AND SEVERITY~~ OF BRAIN MICROVACUOLATION
DATA FROM INDIVIDUAL ANIMALS

A

ANATOMIC LOCATION	6-MONTH TREATMENT								7-MO. TREATMENT-4-MO. RECOVERY	
DOSE GROUP (MG/KG/DAY)	CONTROL M	CONTROL F	50 M	50 F	100 M	100 F	200 M	200 F	200 M	200 F
HIPPOCAMPUS	-	-	-	-	2	-	-	2	-	-
THALAMUS	-	-	-	-	3	3	2	4	-	-
COLUMN OF FORNIX	-	-	-	-	3	3	3	4	1	1
OPTIC TRACT	-	-	-	-	1	3	-	3	-	1
INFERIOR COLLICULUS	-	-	-	-	-	1	-	2	-	-
FIMBRIA OF THE FORNIX	-	-	-	-	-	-	-	-	1	-
ANTERIOR COMMISSURE	-	-	-	-	-	-	-	-	1	-

B

ANATOMIC LOCATION	ONE-YEAR TREATMENT										ONE-YEAR TREATMENT-SIX-MONTH RECOVERY									
DOSE GROUP (MG/KG/DAY)	CONTROL M	CONTROL M	CONTROL F	50 M	50 M	50 F	50 F	100 M	100 F	200 M	200 F	CONTROL M	CONTROL F	50 M	50 F	100 M	100 M	100 F	200 M	200 M	200 F	200 F
HIPPOCAMPUS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
THALAMUS	-	-	-	-	-	-	-	2	2	3	2	-	-	-	-	-	-	-	-	-	-	-
COLUMN OF FORNIX	1	-	1	1	2	1	-	2	2	3	3	-	-	-	-	-	-	-	-	-	-	-
OPTIC TRACT	-	-	-	-	2	1	-	-	2	4	-	-	-	-	-	-	-	-	-	-	-	-
INFERIOR COLLICULUS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FIMBRIA OF THE FORNIX	-	-	-	-	2	-	-	-	2	3	3	1	-	-	-	-	-	-	-	-	-	-
ANTERIOR COMMISSURE	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-

GRADES FOR DEFINING THE AMOUNT OF CHANGE ARE AS FOLLOWS:
- = NO CHANGE
1 = MINIMUM OR VERY SLIGHT DEGREE OR AMOUNT (SOMETIMES SEEN IN CONTROLS)
2 = SLIGHT DEGREE OR SMALL AMOUNT PRESENT
3 = MODERATE, MEDIAN, OR MIDDLE SEVERITY OR AMOUNT
4 = MARKED SEVERITY OR DEGREE OF CHANGE, LARGE AMOUNT PRESENT

In the brains of two dogs given 200 mg/kg/day of vigabatrin for seven months, followed by four months off drug, and in those animals treated for one year, followed by six months off drug, microvacuolation was similar in severity to that of controls (Tables 2A and 2B). No residual

6 YEAR P.O. TOXICITY IN MONKEYS:

A) Dosage

5/sex at 0, 50, 100, and 300 mg/kg/day, by gavage. The following interim sacrifices were performed:

1) 3 months: 1/sex/group

2) 6 months: 1/sex/group

3) 16 months: 1 M + 2 F in control group, and the remaining 3 M + 3 F at HD

4) 6 years: 3 M + 3 F in control group (1 M + 2 F were added at 19 months to replace the controls sacrificed at 16 months), and the remaining 3 M + 3 F at LD and MD.

(A rangefinding study was also performed, using 2/sex at 0, 500, 750, and 1000 mg/kg given for 4 weeks; an additional 2/sex received 300 mg/kg b.i.d. for 1 week. Results of this study will be presented where appropriate).

Strain: cynomolgus monkeys (Macaca fascicularis).

Lot #s: D-2890, DX-2916, D-2957, D-2978, and D-2990

B) Results

1) Observed signs

Occasional, transient instances of loose stools or diarrhea at HD. (Intermittent diarrhea was also seen in 1 LD and 1 MD, but this was considered to be due to an infectious process and not drug-related). (In the rangefinding study, loose stool/diarrhea was seen at all doses, i.e., 500 mg/kg/day and above; this began within the first 3 days of treatment and lasted throughout the study).

2) Mortality

None

3) Bodyweight gain

No drug effects in main study; in rangefinding study slight weight loss was seen in some monkeys at 750 and 1000 mg/kg which was attributed to drug by the sponsor; however the data provided showed similar weight loss in some controls.

4) Food consumption

6 YEAR P.O. TOXICITY IN MONKEYS:

A) Dosage

5/sex at 0, 50, 100, and 300 mg/kg/day, by gavage. The following interim sacrifices were performed:

1) 3 months: 1/sex/group

2) 6 months: 1/sex/group

3) 16 months: 1 M + 2 F in control group, and the remaining 3 M + 3 F at HD

4) 6 years: 3 M + 3 F in control group (1 M + 2 F were added at 19 months to replace the controls sacrificed at 16 months), and the remaining 3 M + 3 F at LD and MD.

(A rangefinding study was also performed, using 2/sex at 0, 500, 750, and 1000 mg/kg given for 4 weeks; an additional 2/sex received 300 mg/kg b.i.d. for 1 week. Results of this study will be presented where appropriate).

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2) Mortality

None

3) Bodyweight gain

No drug effects in main study; in rangefinding study slight weight loss was seen in some monkeys at 750 and 1000 mg/kg which was attributed to drug by the sponsor; however the data provided showed similar weight loss in some controls.

4) Food consumption

No drug effect in main study; in rangefinding study it was stated that food consumption was decreased at 750 and 1000 mg/kg based on "subjective evaluation".

5) Ophthalmoscopic exam

(Done pre-study and periodically thereafter).

No drug effects, although in most cases specific results were not given.

6) Lab tests

(Done pre-study and periodically thereafter).

a) Hematology

No clear drug effects. Values for Hb, Hct, and RBC were equivocally very slightly decreased at some time points, primarily at HD. (This equivocal trend was also seen in the rangefinding study).

Other parameters measured: WBC, differential, platelets and, in rangefinding study only, PT and PTT.

b) Blood chemistry

No clear drug effects, except for a decrease in ALT, mainly at MD and HD, considered to be due to the pharmacologic action of the drug (i.e., inhibition of transaminase). A few LD and MD had sporadic elevations in triglycerides after long term treatment (i.e., after all HD had been terminated).

Other parameters measured: BUN, glucose, AST, AP, total bilirubin, total protein, albumin, globulin, cholesterol, Na, K, Cl, Ca, PO_4.

c) Urinalysis

No drug effects.

7. Organ weights

No clear drug effects, although this was difficult to determine in view of the small N sacrificed at each time point.

8) Gross pathology

No clear drug effects; however note small N at each sacrifice time.

9) Microscopic pathology

As above, drug effects are difficult to determine in view of the small N at each sacrifice time. The only finding considered to be possibly drug-related was vacuolization in brain. Such vacuolization was assessed by several consultants in addition to assessment by the sponsor; individual conclusions ranged from no drug effect through possible drug effect through slight drug effect, with the latter two only pertaining to HD monkeys sacrificed at 16 months. Where a possible or slight drug effect was concluded the vacuolization was generally in the optic tract; one consultant also concluded possible effects in corpus callosum, septum, and cerebellum. One monkey also had an "unusual amount" vacuoles in optic nerve, although this was not considered drug related by the sponsor. (In the 4 week rangefinding study, it was stated that vacuolization seen in the corpus callosum in one monkey each at 500 and 750 mg/kg was possibly drug related since a similar lesion was not seen in controls; however this conclusion cannot be made confidently in view of the vacuolization seen in corpus callosum of some controls in the main study).

Some consultants considered at least some of the vacuolization to be artifactual. Where a drug effect was concluded it was assumed that the vacuolization was similar to the intramyelenic edema found in other species although apparently EM exams were not done in monkeys. At any rate, if an effect is present in monkeys, it appears to be less severe and less widespread than in the other affected species.

No drug effects were apparent in sciatic nerve, spinal cord, or eye.

10) CSF pressure

Measured in 1 control M, 1 control F, 2 HD M, and 3 HD F which were sacrificed after 16 months. (Measurements made after animals anesthetized and prior to sacrifice). As shown in the attached table, there was no apparent drug effect, although note the relatively large inter-animal variation.

11) PK data

a) Plasma levels

Levels of parent drug obtained from monkeys approx. 22 hr. after dosing on days 7, 30, and 91 are shown in the attached table. Levels increased with increasing dose (but less than proportionately between MD and HD) and appeared to reach SS by day 7; however this is based on only 1/sex/dose/time point. Levels obtained at 6 and 22 hr. after 6 years' treatment at LD and MD are shown in the attached table. The 6 hr. values were similar between doses; the 22 hr. values were dose-proportional. Levels obtained at 6 hr. after 16 month's treatment at HD are shown in a separate table. (Plasma levels were also measured in the 4 week rangefinding study, at 2 and 24 hr. after the last dose; results, shown in the attached table, indicate no clear differences in levels across doses with the possible exception of lower levels at 500

mg/kg at 24 hr.).

b) CSF levels

Levels of parent drug were sampled approx. 24 hr. post-dosing on day 91, and approximately 6 hr. post dosing after 16 months and 6 years. Results are shown in attached tables. Mean values ranged from about 1.5 n moles/g (24 hr. after 50-100 mg/kg) to about 15 n moles/ml or more (6 hr. after 300 mg/kg). At the 6 year time point, levels were greater in F than in M, but note the small N.

c) Urinary levels

Measured at HD after 31 weeks and at LD and MD after 6 years. The % of the daily dose excreted as unchanged drug ranged from 5 to 37% across individual animals (mean approx. 10-20%). (Results in attached tables).

12) Brain GABA, GABA-T, and GAD, and CSF GABA

Measured 6 hr. after final dose. Results shown in attached tables. (Separate table for HD. Also, CSF GABA levels for LD and MD groups are in table with CSF drug levels, above). Brain GABA-T was decreased (30-37%); CSF GABA levels were increased (approx. 20-30%) but this was considered to be of questionable biological significance due to the small effect, lack of dose relationship, and lack of drug effect on brain GABA levels. The other measured parameters showed no drug effects.

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78

CSF PRESSURE

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Table 1

Cerebral Spinal Fluid Pressure Obtained at Necropsy
from Monkeys Administered Vigabatrin (MDL 71,754)
for 16 Months

Monkey-Number/Sex	Dose (mg/kg/day)	CSF Pressures (mm Hg)*[1,2]
180-F	0	No pressure obtained, respiratory failure
206-F	0	3.07 ± 0.02
321-M	0	10.18 ± 0.03
182-F	300	3.76 ± 0.01
210-F	300	3.13 ± 0.04
212-F	300	5.27 ± 0.09
307-M	300	6.46 ± 0.12
315-M	300	No pressure obtained, clot formation in cannula
323-M	300	9.51 ± 0.04

* Mean and standard error of ten readings at six second intervals

[1] Supplemental CSF pressure measurements performed on colony cynomolgus monkeys on 8/16/84 and 9/6/84 were as follows:

Animal No.	Pressure (mm Hg)	
334-F	2, 1	
336-F	2.6, 10	Mean = 3.6
372-F	2.5, 3.2	

[2] Additional values in rhesus monkeys have been found to range from 3.7 to 14 mm Hg (cited as 50 to 190 mm H_2O) measured by manometer at the cisterna magna.

(a) Hayreh, S.S. Pathogenesis of edema of the optic disc, Docum. Ophthal., (Den Haag), 1968, 24, 289-411

(b) McIntyre, J.W.R., Miller, J.O.R. and Weir, B.K.A. Anesthesia for cerebral angiography. Cardiovascular and cerebrospinal fluid pressure observations in monkeys under pentobarbital-halothane anesthesia. Canad. Anaesth. Soc. J., 1960, 16, 300-315.

TABLE 36

MDL 71,754: REVIEW OF THE CHRONIC TOXICITY STUDY IN CYNOMOLGUS MONKEYS THROUGH 18 MONTHS INCORPORATING CONSULTANTS OPINIONS AFTER HISTOLOGIC EXAMINATION OF BRAIN

Plasma Levels (µg/ml) of MDL 71,754 Obtained Approximately 22 Hours Following 0, 7, 30 and 91 Doses from Those Monkeys Necropsied at the end of the 3 Month Treatment Period

Dose Level (mg/kg/day)	Animal No./Sex	Number of Doses Administered			
		0	7	30	91
0	287-M				
	226-F				
50	317-M				
	126-F				
100	291-M				
	236-F				
300	289-M				
	204-F				

b(4)

*Average of two determinations/sample.

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6 YEAR DATA - plasma levels

TABLE 31

MDL 71,754: SIX-YEAR ORAL TOXICITY STUDY IN CYNOMOLGUS MONKEYS GIVEN 50 OR 100 MG/KG/DAY
PLASMA MDL 71,754 Concentrations in Cynomolgus Monkeys 6 and 22 Hours After 0-100 mg/kg/day Oral Doses.
Samples Were Taken After a 6-Year Treatment Period

Dose (mg/kg/day)	Sex	Animal Number	Plasma MDL 71,754 Concentrations (µg/mL) 6 Hours[a]	22 Hours[b]
0	F	218	NF[c]	NF
	F	362	NF	NF
	F	388	NF	NF
	M	325	NF	NF
	M	327	NF	NF
	M	569	NF	NF
50	F	196		
	F	198		
	F	228		
	M	293		
	M	297		
	M	329		
		MEAN	4.33	0.58
		SD	2.06	0.18
100	F	184		
	F	208		
	F	240		
	M	301		
	M	303		
	M	311		
		MEAN	5.57	1.21
		SD	3.16	0.33

[redacted]

b(4)

[a]Obtained 6 hours after the last dose and immediately after collection of a CSF sample from the anesthetized monkey.
[b]Obtained 22 hours after the dose given one week prior to the end of the study.
[c]Not found, less than lower limit of quantitation (<0.2 (µg/mL).

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81

300 mg/kg
16 month plasma levels.

TABLE 2

CONCENTRATION OF VIGABATRIN IN PLASMA AND URINE SIX HOURS AFTER THE LAST DOSE IN MONKEYS RECEIVING VIGABATRIN 300 MG/KG/DAY P.O. FOR 16 MONTHS

Monkey	Daily Dose Vigabatrin mg/kg p.o.	Vigabatrin Plasma nmol/ml	Vigabatrin Urine µmol/ml	Vigabatrin Urine µmol mg Creatinine
180	↑			
206	0			
321	↓			
182	↑			
210				
212	300			
307				
315				
323	↓			
Mean ± S.E.M.		67.5 ± 12.3	4.57 ± 2.21	5.03 ± 1.34

b(4)

*Using the method of Jones (4), the urine of monkey 321 contained a small peak equivalent to 0.10 µmol vigabatrin/mg creatinine. Using an alternative method (5) it was found that none of the control urines contained vigabatrin.

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82

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ORAL RANGE-FINDER STUDIES OF MDL 71,754 ADMINISTERED TO MONKEYS

Table 5

Concentration of GVG in Plasma of Monkeys 2 and 24 hr. After Final Dose[a]

Monkey No.	Sex	GVG dose mg/kg/day	Concentration of GVG (ug/ml) 2 hr. post dose	24 hr. post dose
1	M	0		
2	M			
9	F			
10	F			
3	M	500		
4	M			
11	F			
12	F			
5	M	750		
6	M			
13	F			
14	F			
7	M	1000		
8	M			
15	F			
16	F			

b(4)

[a] Composite of Tables 1 and 2 from Drug Metabolism Report D-84-04 entitled "Analysis of MDL 71,754 in Plasma Samples from Toxicology Study #300C-117"

[b] The mean of these two values is significantly less than the means for any of the other dose groups (male or female) $P < 0.05$

-- = No sample

~~206~~

TABLE 37

MDL 71,754: REVIEW OF THE CHRONIC TOXICITY STUDY IN CYNOMOLGUS MONKEYS THROUGH 18 MONTHS INCORPORATING CONSULTANTS OPINIONS AFTER HISTOLOGIC EXAMINATION OF BRAIN

Cerebrospinal Fluid Levels (ng/g) of MDL 71,754, obtained Approximately 24 hours following 91 Doses of MDL 71,754 from Monkeys Necropsied at the end of the 3 Month Treatment Period

Dose Levels (mg/kg/day)	Animal No./ Sex	MDL 71,754 Levels
0	287-M 226-F	[redacted]
50	317-M 126-F	[redacted]
	Average:	200 (1.5 n mole/g)
100	291-M 236-F	[redacted]
	Average:	205 (1.6 n mole/g)
300	289-M 204-F	[redacted]
	Average:	1365 (10.5 n mole/g)

b(4)

*Not Detected (Detection Limit Approximately 150 ng/g)

16 MONTH SACRIFICE / 6 HR. POST-DOSE

TABLE 38

MDL 71,754: REVIEW OF THE CHRONIC TOXICITY STUDY IN CYNOMOLGUS MONKEYS THROUGH 18 MONTHS INCORPORATING CONSULTANTS OPINIONS AFTER HISTOLOGIC EXAMINATION OF BRAIN

Analysis of Vigabatrin in the Suboccipital CSF of Cynomolgus Monkeys By HPLC, GC/MS and Ion Exchange Methods

Dose mg/kg/day	Monkey Number	Sex	Concentration in the CSF (nmoles/ml) HPLC	ION EXCH.	GC/MS
	180-1+	F			
0	206-1	F			
	321-1+	M			
	182-4	F			
	210-4	F			
300	212-4	F			
	307-4	M			
	315-4+	M			
	323-4	M			
Mean ± S.E.M. (n)*			11.7 ± 1.2 (5)	13.2 ± 1.4 (5)	14.3 ± 1.1 (5)
Mean ± S.E.M. (n)**			20.3 ± 8.7 (6)	22.2 ± 9.1 (6)	24.8 ± 10.5 (6)

The mean ± S.E.M. value of vigabatrin in the CSF of the treated monkeys

*excluding monkey 323

**Including monkey 323

+CSF sample tinged with blood, yields noticea... protein precipitate upon acidification

b(4)

b(4)

CONFIDENTIAL

84

6 YEAR DATA / 6 HR. POST-DOSE

~~5 4952, 4.24~~

85

~~169~~

TABLE 33

MDL 71,754: SIX-YEAR ORAL TOXICITY STUDY IN CYNOMOLGUS MONKEYS GIVEN 50 OR 100 MG/KG/DAY

CSF MDL 71,754 and Total GABA Concentrations in Cynomolgus Monkeys 6 Hours after 0-100 mg/kg/day Oral Doses. Samples were Taken after the Final Dose of a 6-Year Treatment Period[a]

Dose (mg/kg/day)	Sex	Animal Number		Concentrations in CSF (µg/mL) MDL 71,754	Concentrations in CSF (µg/mL) Total GABA	Concentrations in CSF (nmoles/mL) MDL 71,754	Concentrations in CSF (nmoles/mL) Total GABA
0	F	218					
	F	362					
	F	388					
			Mean		0.987		9.57
			SD		0.278		2.70
	M	325					
	M	327					
	M	569					
			Mean		0.899		8.71
			SD		0.130		1.26
	OVERALL MEAN				0.943		9.14
	SD				0.200		1.94
50	F	196					
	F	198					
	F	228					
			Mean	0.783	1.403	6.06	13.61
			SD	0.299	0.291	2.32	2.83
	M	293					
	M	297					
	M	329					
			Mean	0.309	1.101	2.39	10.68
			SD	0.084	0.077	0.65	0.75
	OVERALL MEAN			0.546	1.252	4.23	12.14
	SD			0.325	0.252	2.52	2.45
100	F	184					
	F	208					
	F	240					
			Mean	1.473	1.313	11.41	12.73
			SD	0.579	0.065	4.48	0.63
	M	301					
	M	303					
	M	311					
			Mean	0.910	1.218	7.04	11.81
			SD	0.321	0.182	2.48	1.76
	OVERALL MEAN			1.192	1.265	9.23	12.27
	SD			0.520	0.133	4.03	1.29

b(4)

b(4)

b(4)

[a]CSF was obtained from monkeys anesthetized 6 hours after dose administration.
[b]Not found, less than lower limit of quantitation (<0.001 µg/01 mL sample).

TABLE 39

MDL 71,754: REVIEW OF THE CHRONIC TOXICITY STUDY IN CYNOMOLGUS MONKEYS THROUGH 18 MONTHS INCORPORATING CONSULTANTS OPINIONS AFTER HISTOLOGIC EXAMINATION OF BRAIN

Levels of MDL 71,754 in Urine Obtained for 3 Consecutive Days from Control and 300 mg/kg/day Monkeys after 31 Weeks of Treatment

Monkey	Daily Dose (mg)	Day 1 (12/5-6/83)			Day 2 (12/6-7/83)			Day 3 (12/7-8/83)		
		Conc. mg/ml	Total Amt.(mg)	% of Dose	Conc. mg/ml	Total Amt.(mg)	% of Dose	Conc. mg/ml	Total Amt.(mg)	% of Dose
307	1082									
315	919									
323	928									
182	821									
210	643									
212	621									

The assay value for each sample from the control monkey group was zero.

~~5-4931, v1.24~~

87

6 YEAR DATA

TABLE 32

MDL 71,754: SIX-YEAR ORAL TOXICITY STUDY IN CYNOMOLGUS MONKEYS GIVEN 50 OR 100 MG/KG/DAY
MDL 71,754 in Urine Collected for 24 Hours after Cynomolgus Monkeys were given 0-100 mg/kg/day Oral Doses.
Samples were Taken after a 6-Year Treatment Period[a]

Dose (mg/kg/day)	Sex	Animal Number	MDL 71,754 in Urine		
			Concentration (µg/mL)	Amount (mg)	Amount (% Dose)
0	F	218			
	F	362			
	F	388			
	M	325			
	M	327			
	M	569			
50	F	196			
	F	198			
	F	228			
	M	293			
	M	297			
	M	329			
		Mean			19.5
		SD			9.7
100	F				
	F				
	F				
	M				
	M				
	M				
		Mean			13.2
		SD			6.2

b(4)

b(4)

[a]Obtained one week prior to the end of the study.
[b]Not found, less than lower limit of quantitation (<10 µg/mL).

LEFT TEMPORAL CORTEX

6 YEAR TX

TABLE 34

MDL 71,754: SIX-YEAR ORAL TOXICITY STUDY IN CYNOMOLGUS MONKEYS GIVEN 50 OR 100 MG/KG/DAY
GABA-T and GAD Activities and Concentrations of GABA in the Brain of Monkeys Necropsied 6 Hours after Receiving the Final Daily Dose of MDL 71,754

FREE

Dose mg/kg	Sex	Monkey Number	GABA-T µmoles/hr/g brain	% Control	GAD µmoles/hr/g brain	% Control	GABA µmoles/g brain
0	F	83P0218					
0	F	83P0362					
0	F	83P0388					
0	M	83P0325					
0	M	83P0569					
0	M	83P0327					
Mean ± SEM			20.18 ± 0.68 N=6		8.08 ± 0.62 N=6		1.58 ± 0.11 N=6
50	F	83P0228					
50	F	83P0196					
50	F	83P0198					
50	M	83P0297					
50	M	83P0329					
50	M	83P0293					
Mean ± SEM			14.03 ± 1.17 N=6		8.40 ± 0.89 N=6		1.74 ± 0.21 N=6
100	F	83P0240					
100	F	83P0208					
100	F	83P0184					
100	M	83P0311					
100	M	83P0303					
100	M	83P0301					
Mean ± SEM			13.69 ± 1.22 N=6		6.38 ± 0.47 N=6		1.51 ± 0.14 N=6

b(4)

b(4)

b(4)

878

300 mg/kg
16 month data

~~B-86-02~~ ~~Page-8~~ 87 b

TABLE 1

ACTIVITIES OF GABA-T AND GAD IN THE BRAIN
AND CONCENTRATION OF GABA IN THE BRAIN AND CSF
SIX HOURS AFTER THE LAST DOSE IN MONKEYS RECEIVING VIGABATRIN 300 MG/KG/DAY, P.O.
FOR 16 MONTHS

Monkey	Sex	Body Wt.	Dose Vigabatrin mg/kg/day p.o.	Brain GABA-T Activity µmol/g/hr	Brain GAD Activity µmol/g/hr	Brain (FREE)* GABA+ Concentration µmol/g	CSF (TOTAL) GABA++ Concentration nmol/ml
180-1	F	2.81	↑				
206-1	F	2.54	0				
321-1	M	3.24	↓				
			Mean ± S.E.M.	36.2 ± 3.0	6.7 ± 0.6	1.44 ± 0.10	8.8 ± 1.6
182-4	F	3.10	↑				
210-4	F	2.43					
212-4	F	2.13	300				
307-4	M	4.34					
315-4	M	4.39	↓				
323-4	M	4.13					
			Mean ± S.E.M.	22.8 ± 0.6*	6.0 ± 0.6	1.46 ± 0.09	10.6 ± 0.4
			% Control ± S.E.M.	63.0 ± 0.6%	89 ± 8%		

b(4)

b(4)

+The method used measures free brain GABA.

++The method used measures total CSF GABA.

*Significantly different from control (P<0.05).

* Another paper in the NDA also showed no drug effect on total brain GABA in these animals, although it is noted that the values for free brain GABA given in that paper [which also showed no drug effect on free GABA) were different from the above

Metabolism

Metabolism

GENOTOXICITY

A) Ames tests

(1) One lab performed two independent assays using salmonella strains TA 1535, 1537, 1538, 98, and 100; the highest concentration of GVG was 5000 ug/plate. (It was stated that this concentration was not bacteriotoxic). GVG was negative both with and without metabolic activation (Aroclor S9).

(2) A second lab performed an assay using the same strains as above. The highest concentration of GVG used was 500 ug per plate; it was implied that this concentration caused some toxicity but this was not explicitly stated and results of toxicity testing were not shown. GVG was negative both with and without metabolic activation (Aroclor S9).

(3) None of the above studies contained GLP statements or QA inspections.

B) Point mutation assay in Schizosaccharomyces Pombe and gene conversion test in Saccharomyces cerevisiae D_4

GVG was negative in both assays, with or without metabolic activation (Aroclor S9). The highest concentration of GVG used was 5000 ug/ml, which was said to be nontoxic (although results in the former assay, without activation, showed bacterial survival was decreased about 30% over the dose range studied).

These studies contained no GLP statements or QA inspections.

C) CHO/HGPRT forward mutation assay

Two assays were run, with and without metabolic activation, at GVG concentrations up to 5000 ug/ml, which were shown to be nontoxic. Results are shown in the attached tables. In one case (625 ug/ml, without activation, in one of 2 assays) the mutation frequency was statistically significantly above control (and above the historical control range); however since an increase was not seen at this concentration in the second assay and was not seen at the higher concentrations in any assay, it was not considered to be drug related.

D) In vitro chromosomal aberration assay in rat lymphocytes

GVG was negative with and without metabolic activation (Aroclor S9). Treatment time was "approximately" 4 hours. The highest concentration of GVG used was 5000 ug/ml, which did not alter the mitotic index.

E) Micronucleus test in mice.

Ten CD-1 mice per sex were given a single dose of 0, 170, 540, or 1700 mg/kg, by gavage, with sacrifice at 24 and 48 hr. post-dose. (5/sex/group at each time point). (The doses were based on "an estimated oral LD 50 of 2830 mg/kg"; no data on drug toxicity [observed signs, etc.] were given for the present study). GVG was negative in this assay.

Table 3B. Results of the Gene Mutation Assay in CHO Cells Treated with the Test Chemical in the Presence of S-9

Cell Line: CHO-K_1-BH_4; Passage: 15
Test Chemical: MDL 71,754 Pos. Control: 4.03 µg/ml 20-MCA; Neg.Control: Culture Medium
Assay No. 2

Treatment (µg/ml)	Toxicity Assay: No. Colonies in Individual Dishes	Toxicity Assay: RCS(%)[a]	Mutation Assay: TG^r Colonies in Individual Dishes[b]	Mutation Assay: Total	Cloning Efficiency (CE): No. of Colonies in Individual Dishes	Cloning Efficiency (CE): CE(%)[c]	TG^r Mutants per 10^6 Clonable Cells
Neg. Control	118,125,123	100.0	0, 1, 2, 2, 2	7	137,163,147,154,164	76.5	9.2
625	127,104,123	96.7	0, 1, 1, 0, 0	2	165,130,143,133,172	74.3	2.7
1250	121,118,109	95.1	3, 0, 1, 3, 0	7	119,155,149,152,127	70.2	10.0
2500	119,122,138	103.6	2, 4, 0, 0, 8	14	139,122,163,151,144	71.9	19.5
5000	116,119,135	101.1	1, 0, 0, 0, 1	2	135,141,168,133,154	73.1	2.7
Pos. Control	91, 83, 81	69.7	16,11, 6,11,14	58	120,115,114,106,112	56.7	102.3[d]

[a] Relative cell survival (%) = $\frac{\text{Mean number of colonies/dish in the treated} \times 100}{\text{Mean number of colonies/dish in the negative control}}$

[b] TG^r = 6-Thioguanine resistant

[c] CE(%) = $\frac{\text{Mean number of colonies/dish} \times 100}{\text{No. of cells seeded/dish}}$

[d] Significantly (alpha ≤ 0.01) different from the negative control.

Table 3A. Results of the Gene Mutation Assay in CHO Cells Treated with the Test Chemical in the Presence of S-9

Cell Line: CHO-K_1-BH_4; Passage: 15
Test Chemical: MDL 71,754 Pos. Control: 4.03 µg/ml 20-MCA; Neg.Control: Culture Medium
Assay No. 1

Treatment (µg/ml)	Toxicity Assay: No. Colonies in Individual Dishes	Toxicity Assay: RCS(%)[a]	Mutation Assay: TG^r Colonies in Individual Dishes[b]	Mutation Assay: Total	Cloning Efficiency (CE): No. of Colonies in Individual Dishes	Cloning Efficiency (CE): CE(%)[c]	TG^r Mutants per 10^6 Clonable Cells
Neg. Control	142,125,119	100.0	0, 0, 0, 1, 2	3	123,134,144,110,128	63.9	4.7
625	150,139,110	103.4	0, 0, 0, 0, 0	0	166,148,112,142,146	71.4	0
1250	139,135,128	104.1	0, 1, 2, 1, 3	7	132,148,154,150,144	72.8	9.6
2500	141,150,126	108.0	1, 2, 1, 0, 1	5	156,175,160,155,158	80.4	6.2
5000	129,153,116	103.1	0, 0, 0, 1, 0	1	172,132,142,154,134	73.4	1.4
Pos. Control	136,139,133	105.7	21,25,26,23,24	119	100, 83, 91, 96, 73	44.3	268.6[d]

[a]Relative cell survival (%) = $\frac{\text{Mean number of colonies/dish in the treated X 100}}{\text{Mean number of colonies/dish in the negative control}}$

[b]TG^r = 6-Thioguanine resistant

[c]CE(%) = $\frac{\text{Mean number of colonies/dish x 100}}{\text{No. of cells seeded/dish}}$

[d]Significantly (alpha ≤ 0.01) different from the negative control.

90

Table 2B. Results of the Gene Mutation Assay in CHO Cells Treated with the Test Chemical in the Absence of S-9

Cell Line: CHO-K_1-BH_4; Passage: 15
Test Chemical: MDL 71,754 Pos. Control: 621 µg/ml EMS; Neg. Control: Culture Medium
Assay No. 2

Treatment (µg/ml)	Toxicity Assay: No. Colonies in Individual Dishes	Toxicity Assay: RCS(%)[a]	Mutation Assay: TG^r Colonies in Individual Dishes[b]	Mutation Assay: Total	Cloning Efficiency (CE): No. of Colonies in Individual Dishes	Cloning Efficiency (CE): CE(%)[c]	TG^r Mutants per 10^6 Clonable Cells
Neg. Control	111,137,105	100.0	1, 2, 0, 0, 0	3	123,152,161,158,136	73.0	4.1
625	114,105,121	96.3	1, 2, 2, 0, 0	5	151,134,147,143,163	73.8	6.8
1250	136,122,138	112.2	1, 1, 1, 0, 0	3	139,133,123,144,160	69.9	4.3
2500	121,129, 98	98.6	1, 3, 0, 1, 0	5	132,145,141,123,145	68.6	7.3
5000	119,101,152	105.4	0, 0, 0, 2, 1	3	123,136,138,146, 91	63.4	4.7
Pos. Control	78, 78, 73	64.9	28,25,22,18,14	107	89, 71, 76,123, 90	44.9	238.3[d]

[a]Relative cell survival (%) = $\frac{\text{Mean number of colonies/dish in the treated X 100}}{\text{Mean number of colonies/dish in the negative control}}$

[b]TG^r = 6-Thioguanine resistant

[c]CE(%) = $\frac{\text{Mean number of colonies/dish x 100}}{\text{No. of cells seeded/dish}}$

[d]Significantly (alpha ≤ 0.01) different from the negative control.

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Table 2A. Results of the Gene Mutation Assay in CHO Cells Treated with the Test Chemical in the Absence of S-9

Test Chemical: MDL 71,754; Cell Line: CHO-K_1-BH_4; Passage: 15
Pos. Control: 621 µg/ml EMS; Neg. Control: Culture Medium
Assay No. 1

Treatment (µg/ml)	Toxicity Assay: No. Colonies in Individual Dishes	Toxicity Assay: RCS(%)[a]	Mutation Assay: TG^r Colonies in Individual Dishes[b]	Mutation Assay: Total	Cloning Efficiency (CE): No. of Colonies in Individual Dishes	Cloning Efficiency (CE): CE(%)[c]	TG^r Mutants per 10^6 Clonable Cells
Neg. Control	161,140,141	100.0	1, 0, 1, 0, 0	2	151,151,165,129,156	75.2	2.7
625	170,164,166	113.1	5, 2, 5, 2, 2	16	146,149,131,139,134	69.9	22.9[d]
1250	146,165,130	99.8	0, 0, 1, 1, 5	7	163,153,128,135,149	72.8	9.6
2500	154,184,150	110.4	1, 1, 1, 0, 0	3	153,155,140,157,169	77.4	3.9
5000	136,133,155	95.9	0, 1, 0, 0, 1	2	161,129,129,135,137	69.1	2.9
Pos. Control	114,105,114	75.3	18,18,19,16,20	91	81, 80, 74, 66, 68	36.9	246.6[d]

[a] Relative cell survival (%) = $\frac{\text{Mean number of colonies/dish in the treated X 100}}{\text{Mean number of colonies/dish in the negative control}}$

[b] TG^r = 6-Thioguanine resistant

[c] CE(%) = $\frac{\text{Mean number of colonies/dish x 100}}{\text{No. of cells seeded/dish}}$

[d] Significantly (alpha ≤ 0.01) different from the negative control.

92

SEGMENT I REPRODUCTION IN RATS (MALE)

A) Dosage

20 M at 50, 100, or 150 mg/kg/day, in diet, for 84 days, after which drug was removed from diet and each M was mated to an untreated F for 1 week.

Dams sacrificed approximately 16 days from the midweek of the mating period.

Strain: CD (SD), [redacted]

Lot #: 71,754-49 b(4)

B) Results

1) Observed signs

No drug effects

2) Food consumption and bodyweight gain

Decreased at MD and HD, D-R (See attached figure)

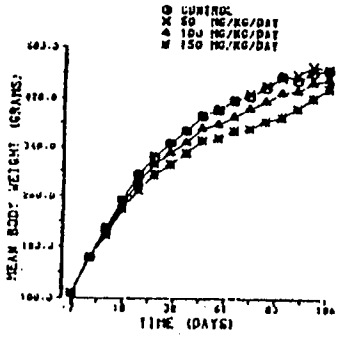
3) Reproductive data

No drug effects (See attached tables).

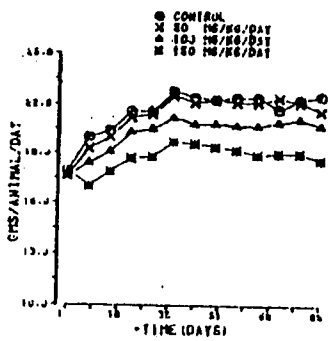
MALE FERTILITY STUDY WITH MDL 71,754 IN RATS

Plate I

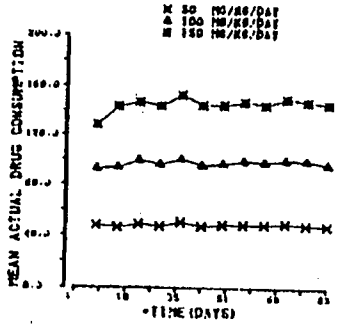
MALE MEAN BODY WEIGHTS (GMS)



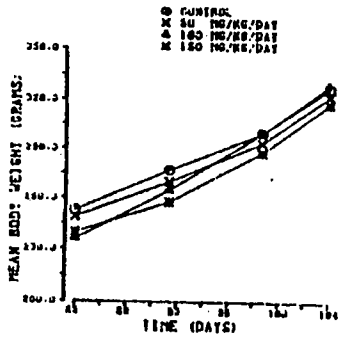
MALE MEAN FOOD CONSUMPTION (GMS/ANIMAL/DAY)
*POINTS PLOTTED END OF PERIOD



MALE MEAN ACTUAL DRUG CONSUMPTION
*POINTS PLOTTED END OF PERIOD



MEAN BODY WEIGHTS (GMS)
UNDOSED PREGNANT FEMALES ONLY



800C

MALE FERTILITY STUDY WITH MDL 71,754 IN RATS

Table I

Reproductive Data[a]

Dose Group mg/kg/day	No. Mated	Number Females Pregnant	Average Number/Litter				Pre-Implantation Loss (%)[b]	Post-Implantation Loss (%)[c]
			Corpora Lutea	Implants	Viable Fetuses	Resorptions		
Control	20	19	15.1	13.2	11.9	1.21	12.6	9.2
50	20	18	14.6	12.8	12.1	0.67	12.2	5.2
100	20	17	15.8	14.4	13.6	0.71	9.0	4.9
150	20	17	14.4	13.1	12.4	0.71	8.6	5.4

[a] Sacrificed approx. 16 days from mid week of cohabitation

[b] $\frac{\text{No. corpora lutea - No. implants}}{\text{No. of corpora lutea}} \times 100$

[c] $\frac{\text{No. implants - No. viable fetuses}}{\text{No. of implants}} \times 100$

SEGMENT I REPRODUCTION IN RATS (FEMALE)

A) Dosage

20 F at 0, 50, 100, or 150 mg/kg/day, in diet, from 2 weeks premating, through a 1 week mating period, and for 1 week thereafter. Males (1:1 mating ratio) were untreated except during the 1 week mating period when they were exposed to the female's diet.

Dams sacrificed approximately 16 days from the mid-week of the mating period.

Strain: CD (SD) BR, [redacted] b(4)

Lot #: 71,754-49

B) Results

1) Observed signs

Alopecia in several HD

2) Food consumption and bodyweight

Results shown in attached figure. Food consumption and weight gain were decreased at all doses, D-R, during the treatment period. (HD also had a transient weight loss). Food consumption and weight gain rebounded after stopping treatment.

3) Reproductive data

Results shown in attached table. No drug effects on pregnancy rate, dead fetuses, resorptions, or pre- and post-implantation loss. Numbers of CL, implantation sites, and viable fetuses were slightly decreased at HD.

SEGMENT I REPRODUCTION IN RATS (FEMALE)

A) Dosage

20 F at 0, 50, 100, or 150 mg/kg/day, in diet, from 2 weeks premating, through a 1 week mating period, and for 1 week thereafter. Males (1:1 mating ratio) were untreated except during the 1 week mating period when they were exposed to the female's diet.

Dams sacrificed approximately 16 days from the mid-week of the mating period.

Strain: CD (SD) BR, [redacted] b(4)

Lot #: 71,754-49

B) Results

1) Observed signs

Alopecia in several HD

2) Food consumption and bodyweight

Results shown in attached figure. Food consumption and weight gain were decreased at all doses, D-R, during the treatment period. (HD also had a transient weight loss). Food consumption and weight gain rebounded after stopping treatment.

3) Reproductive data

Results shown in attached table. No drug effects on pregnancy rate, dead fetuses, resorptions, or pre- and post-implantation loss. Numbers of CL, implantation sites, and viable fetuses were slightly decreased at HD.

~~5- 6932, V1.30~~

97

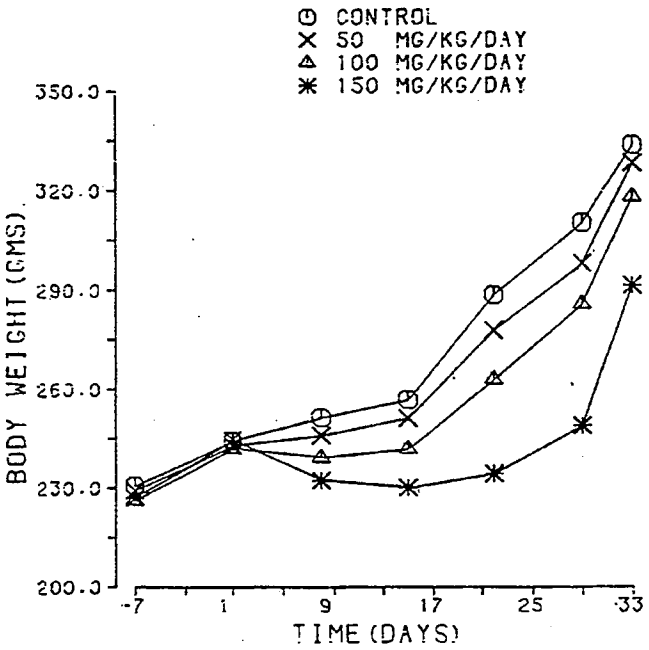
800C-9

REPRODUCTION STUDY WITH MDL 71,754 IN FEMALE RATS

PLATE 1

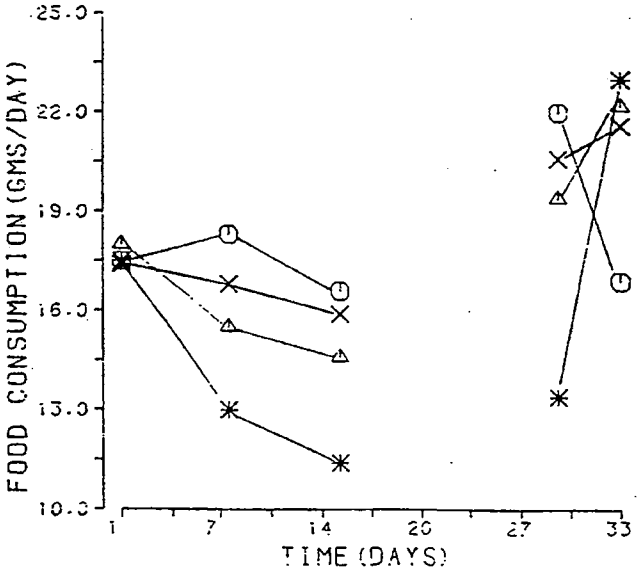
MEAN BODY WEIGHTS (GMS)

PREGNANT FEMALES ONLY



MEAN FOOD CONSUMPTION (GMS/DAY)

PREGNANT FEMALES ONLY



REPRODUCTION STUDY WITH MDL 71,754 IN FEMALE RATS

Table 1

Summary of Reproductive Results

Dose[1] Group mg/kg/day	No. Dams Sacrificed[2]	No. Dams Pregnant	Average no/female					Pre-implant Loss %	Post-implant Loss %
			Viable Fetuses	Dead Fetuses	Resorption Sites	Implantation Sites	Corpora Lutea		
0	20	17	13.4	.12	1.0	14.5	17.0	14.5	7.7
50	20	17	12.6	.12	1.3	14.0	15.7	10.9	10.1
100	20	19	12.8	0	.37	13.2	16.4	19.3	2.8
150	20	20	11.5[a]	.1	.6	12.2[a]	14.0[a]	12.9	5.7

[1] Male exposed to female diet during 1 week mating period
[2] Sacrificed approximately 16 days from midweek of mating
[a] $P < .05$

SEGMENT II REPRODUCTION IN RATS:

A) Dosage

20 F at 0, 50, 100, or 150 mg/kg/day, days 7-16 of pregnancy (day 1 = first 24 hrs. after mating), by gavage.

Dams sacrificed day 21 of pregnancy; all fetuses examined externally; 1/2 of fetuses from each litter examined for head (slicing method) and visceral (dissection) changes; remaining fetuses examined for skeletal changes (Alizarin Red S).

Strain: CD (SD), [redacted] b(4)

Lot #: 71,754-46

B) Results

1) Observed signs in dams

No information given.

2) Dam bodyweight and food consumption

Results shown in attached figure; no further data given. The text states that food consumption was decreased at MD and HD (5 and 9%, resp.) during the first week of treatment, which was reflected in a slightly decreased weight gain. (From the figures, it appears that an effect at LD was also possibly present).

3) Reproductive data

Results shown in attached table. No drug effects apparent except for a slight decrease in fetal weight at MD and HD.

4) Fetal exams

Results shown in attached table.

Multiple malformations seen in 3 LD fetuses and 2 HD fetuses; they were not considered drug-related by the sponsor since they were all of different types with a low incidence of each type. (Historical control values not given). Also note the lack of dose-relationship.

The incidence of enlarged ureters and renal pelves was equivocally increased at HD; this was not considered to be of significance by the sponsor due to the commonness of these variations. (Note that dilated renal pelvis or ureter was not increased in the behavioral teratology study in rats).

The incidence of sternal variations (small, pinpoint, incomplete ossification, or unossified) was equivocally increased at HD; this may be related to reduced fetal weight.

5. Rangefinding study

A non-GLP study was performed (apparently after the main study) in which 5 F received 0, 300, 400, or 500 mg/kg/day. (Number pregnant at term: 5, 4, 3, 4, resp.). A marked decrease in food consumption, accompanied by weight loss during the dosing period, was seen at all doses. Alopecia was seen at MD and HD. Decreased fetal weights seen at all doses. Fetuses were examined externally; 1 LD fetus was malformed (exencephaly and gastroschisis).

~~5-6996, v1.30~~

101

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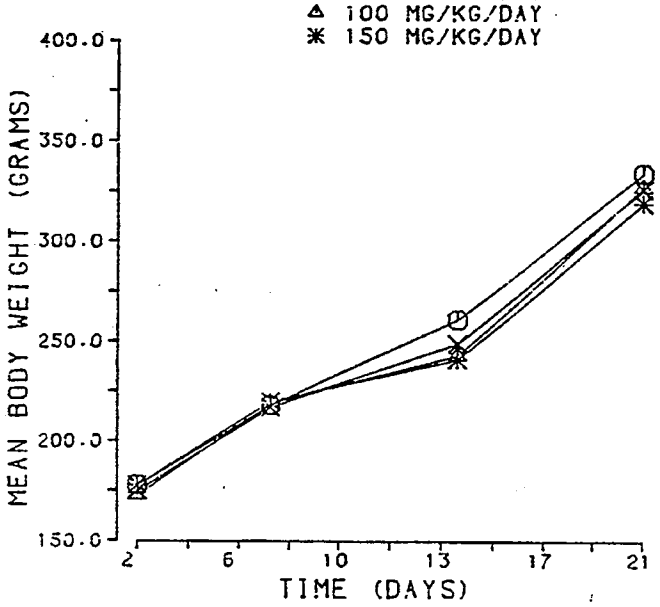
MORPHOLOGIC TERATOLOGY STUDY WITH MDL 71,754 IN RATS

Plate I

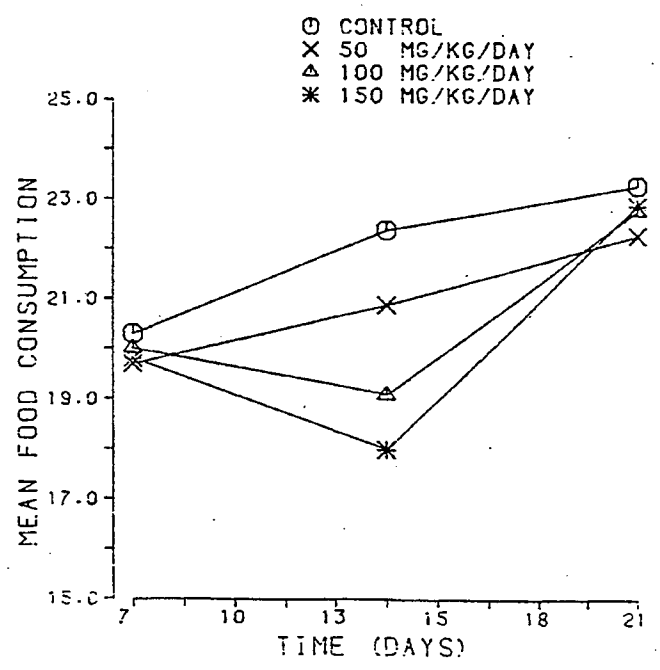
MEAN BODY WEIGHTS (GRAMS)

PREGNANT DAMS ONLY



MEAN FOOD CONSUMPTION (GMS/ANIMAL/DAY)

PREGNANT DAMS ONLY



MORPHOLOGIC TERATOLOGY STUDY WITH MDL 71,754 IN RATS

Table 1

Summary of Reproductive Data

	Dose Groups (mg/kg/day)			
	Control	50	100	150
No. inseminated (by supplier)	20	20	20	20
No. with corpora lutea	20	20	19	20
No. not pregnant (died)	0	0	0	0
No. not pregnant (lived)	3	4	2	2
No. pregnant (died)	0	0	0	0
No. pregnant (at term)	17	16	18	18

	Total	%	Avg.	Total	%	Avg.	Total	%	Avg.	Total	%	Avg.
Live fetuses	191	95.5	11.2	182	95.3	11.4	216	94.3	12.0	190	95.5	10.6
Dead fetuses	0	0	0	0	0	0	0	0	0	0	0	0
Incomplete resorptions	0	0	0	0	0	0	1	0.44	0.06	1	0.50	0.06
Complete resorptions	9	4.5	0.53	9	4.7	0.56	12	5.2	0.67	8	4.0	0.44
Implantations	200		11.8	191		11.9	229		12.7	199		11.1
Sex ratio		1.2/1			0.9/1			1/1			0.9/1	
Mean fetal wts.[a] (g)		3.6			3.7			3.4[b]			3.3[b]	

[a] Mean of litter means
[b] $P < .05$

MORPHOLOGIC TERATOLOGY STUDY WITH MDL 71,754 IN RATS

Table 2

Fetal Observations

	Dose Groups (mg/kg/day)			
	Control	50	100	150
Number examined externally (includes palate)	191	182	216	190
Hematomas	15	5	12	0
Number examined by dissection	91	87	104	90
No. with Malformations:				
Micrognathia*, microstomia*, microglossia, furrowed palate, abnormally shaped nasopharyngeal passageway	-	1	-	-
Horseshoe kidney, atresia ani*, small bilateral encephaloceles, tail thread like with enlarged end*	-	1	-	-
Microphthalmia, unilateral	-	-	-	1
Tracheoesophageal fistula, 2 small openings in anterior palate	-	1	-	-
Small opening in posterior palate	-	-	-	1
No. with Developmental Variations:				
Renal pelvis enlarged - unilateral	8	2	1	-
- bilateral	6	8	7	14
Ureters enlarged - unilateral	1	1	6	2
- bilateral	1	3	3	13

* Noted on external examination

MORPHOLOGIC TERATOLOGY STUDY WITH MDL 71,754 IN RATS

Table 2 (continued)

Fetal Observations

	Dose Groups (mg/kg/day)			
	Control	50	100	150
Number examined after clearing and staining	100	95	112	100
No. with Developmental Variations:				
Sternal				
- small, pinpoint, incomplete ossification and/or unossified	67 (67%)	51 (54%)	82 (73%)	88 (88%)
- asymmetrical	14 (14%)	21 (22%)	23 (21%)	10 (10%)
Ribs				
- only 12 ribs (12 thoracic vertebrae)	-	-	-	1
- 13th, 1 small, 1 full sized	5	2	5	2
- 13th bilaterally - small	-	1	1	-
- 14th unilateral present	-	4	-	1
bilateral present	1	2	-	-
Thoracic vertebrae				
- 14th present	-	2	-	-
- centra bilobed, bipartite and/or unossified	18 (18%)	29 (31%)	30 (27%)	22 (22%)
Lumbar vertebrae				
- centra bipartite and/or bilobed	-	1	-	-
- only 5 lumbar vertebrae	-	-	1	-
Pubis and/or ischium				
- incomplete ossification	2	-	1	1
Hyoid unossified	10	3	2	2

SEGMENT II (BEHAVIORAL TERATOLOGY) REPRODUCTION IN RATS:

A) Dosage

Two studies were performed, each using the following doses:

20 F at 0, 50, 100, or 150 mg/kg/day, days 7-16 of pregnancy (day 1 = first 24 hrs. after mating), by gavage

Dams were allowed to whelp and nurse their young. Litters were reduced to 4/sex/group (where possible) at 48 hr PP. Various developmental milestone (surface righting, auditory startle, pupil contraction, and eyelid opening) were assessed during the nursing period. Pups were weaned day 26-28 PP and 1/sex/litter/group were mated (to nonsiblings) at about 90 days of age. (In the first study, cohabitation was 1:1 for 1 week, and F_1 dams sacrificed approximately 16 days from midweek of cohabitation for reproductive evaluation. In the second study, 1:1 cohabitation continued until there was evidence of mating; if this did not occur by 2 weeks the dam was placed with a known breeder male from the same group for a similar length of time; dams were sacrificed on day 16 of gestation for reproductive evaluation). (There were no other apparent differences in methodology between the 2 studies).

Strain: CD (SD), [redacted] b(4)

Lot #: 71,754-46 (both studies)

B) Results

1) Observed signs in F_0 dams

No drug effects.

2) F_0 dam bodyweight and food consumption

Results shown on attached figures, no further data were given. According to the text, the following were statistically significantly decreased : food consumption during first week of treatment at all doses in 1st study and at MD and HD in 2nd study; bodyweights at MD and HD in the 1st study and HD in the 2nd study

3) Reproductive data (through weaning)

Results shown in attached tables. There were no clear adverse drug effects on post-implantation loss, pup survival, pup weights, appearance of developmental milestones, and necropsy of dead or culled pups. (Number of implants and live pups were slightly decreased at MD only; probably incidental).

4) Reproductive data (post-weaning)

Results shown in attached figures/tables. There were no clear drug effects on F_1 food consumption or bodyweight (note that no data shown other than in the figures), clinical signs, reproductive performance, or necropsy. (There was no apparent explanation for the unusually low conception rate in controls in the first study; it was apparently because of this finding that the second study was performed).

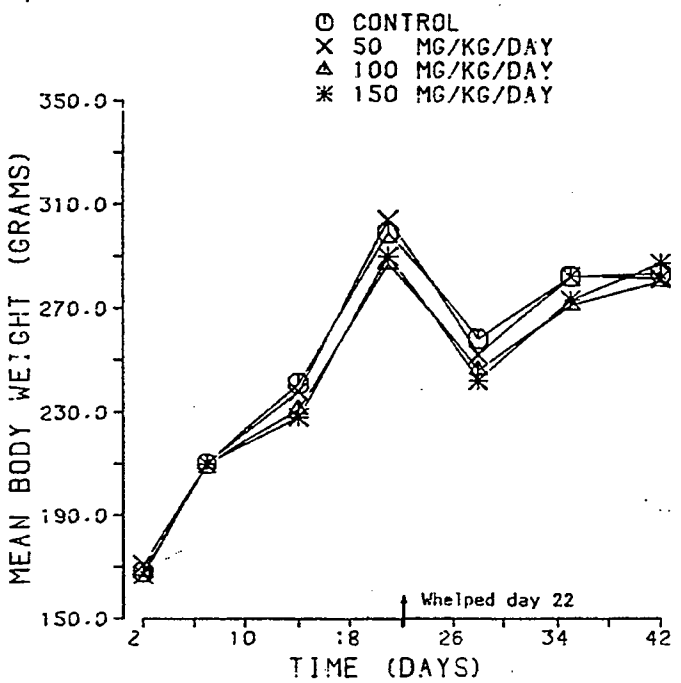
~~5-7202-W130~~

107

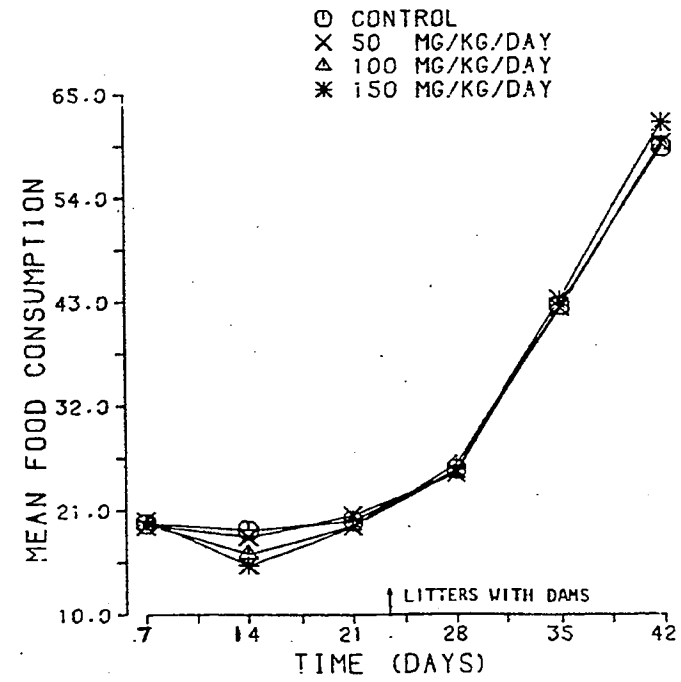
~~8~~ B00C-5

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS
(F_0 Generation)
First Study
Plate 1

MEAN BODY WEIGHTS (GRAMS)



MEAN FOOD CONSUMPTION (GMS/ANIMAL/DAY)



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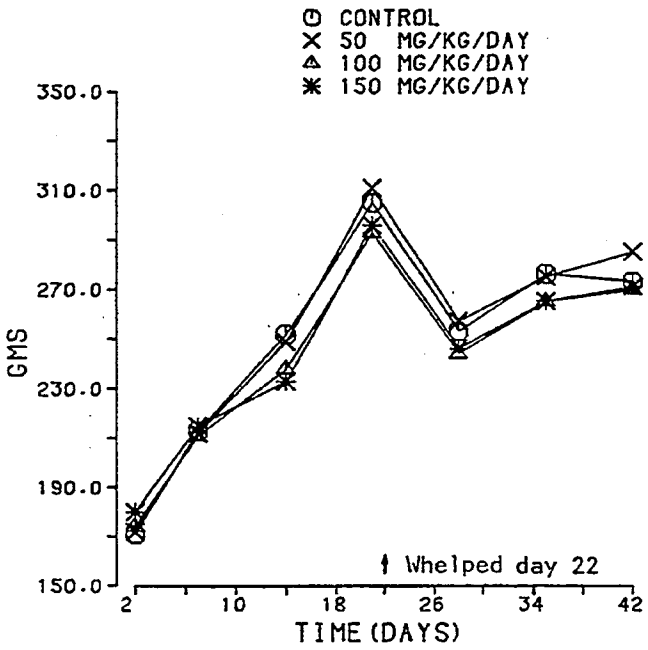
108

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS ~~8MG/KG~~

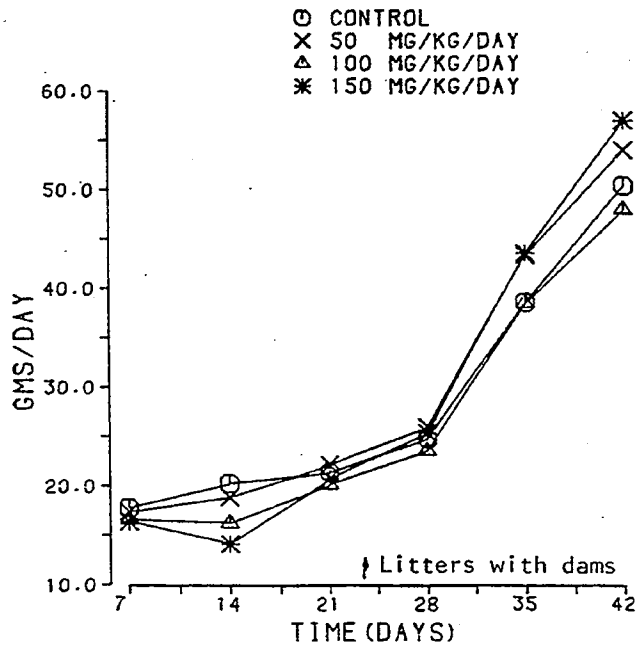
Second Study

Plate II

MEAN BODY WEIGHTS
DAMS WITH LIVE FETUSES ONLY



MEAN FOOD CONSUMPTION
DAMS WITH LIVE FETUSES ONLY



BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 1

Summary of Reproductive Data (F_0), Pup Survival Rate

First Study

Dose Group mg/kg/day	Number Litters	Implants	Birth Live	Birth Dead	48 Hours Survival Remain	48 Hours Survival %	48 Hours Survival Retained	21 Days Survival Remain	21 Days Survival %	No. retained days 26-28 Males	No. retained days 26-28 Females
0	17	12.0	11.5	0	11.5	100	7.8	7.8	99.2	17	17
50	18	11.8	11.3	0	11.3	100	7.8	7.7	98.6	18	18
100	18	10.7	9.9[a]	0.22	9.6	97.2	7.4	7.4	100	18	18
150	18	12.1	10.9	0.17	10.8	99.0	7.9	7.9	100	18	18

a $P < .05$

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 2

Summary of Reproductive Data (F_0), Pup Survival Rate

Second Study

Dose Group mg/kg/day	Number Litters	Implants	Birth		48 Hours			21 Days		No. retained days 27-29	
			Live	Dead	Remain	Survival %	Retained	Remain	Survival %	Males	Females
0	14	10.5	9.4	0	8.5	90.2	7.0	6.9	98.0	13	13
50	18	10.2	8.7	.11	8.7	100.0	7.2	6.8	93.8	17	17
100	16	9.1	7.8	.69	7.7	99.2	6.7	6.7	100.0	16	14
150	12	11.4	10.3	.25	10.0	97.6	7.8	7.8	100.0	12	12

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 3

Mean Pup Weights

First Study

Dose Group mg/kg/day	Number Litters	Mean Pup Weight (g) Birth	48 Hr. Remain	48 Hr. Retain	21 Days Remain
0	17	5.9	7.6	7.8	48.7
50	18	5.9	7.4	7.5	47.6
100	18	6.0	7.8	7.9	48.8
150	18	6.0	7.8	7.9	48.3

800C-10

Table 4

Mean Pup Weights

Second Study

Dose Group mg/kg/day	Number Litters	Mean Pup Weight (g) Birth	48 Hr. Remain	48 Hr. Retain	21 Days Remain
0	14	6.0	7.9	8.0	49.2
50	18	6.3	8.4	8.5	52.9
100	16	6.3	8.3	8.4	49.6
150	12	6.4	8.3	8.3	53.6

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 5

Pup Observations

No. Pups with Postive Response

First Study

	Dose Group (mg/kg/day)			
	Control	50	100	150
Number litters	17	18	18	18
Number pups tested	133	141	134	143
Surface righting (day B5)	78 (59%)	84 (60%)	95 (71%)	88 (62%)
Auditory startle (day B15)	129 (97%)	139 (99%)	133 (99%)	142 (99%)
Eyelids open (day B16)	131 (98%)	136 (96%)	123 (92%)[a]	130 (91%)[a]
Pupil contraction (day B17)	133 (100%)	141 (100%)	133 (99%)	141 (99%)

[a] $P < .05$

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 6

Pup Observations

No. Pups with ~~Positive~~ Response
Second Study

	Dosa Groups (mg/kg/day)			
	Control	50	100	150
No. of litters	13	17	16	12
No. of pups tested	97	122	107	94
Surface righting (Day B5)	47 (48.5%)	57 (46.7%)	45 (42.1%)	41 (43.6%)
Auditory startle (Day B15)	96 (100%)	122 (100%)	103 (96.3%)	93 (98.9%)
Eyelids open (Day B16)	92 (95.8%)	122 (100%)	105 (98.1%)	94 (100%)
Pupil contraction (Day B17)	96 (100%)	122 (100%)	107 (100%)	94 (100%)

800C-5
800C-6

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 7

Necropsy Findings on F_1 Generation

First Study

	Dose Groups (mg/kg/day)							
	Control		50		100		150	
	A	B	A	B	A	B	A	B
No. examined	162	34	167	36	143	36	162	36
Brain - lateral ventricles moderately dilated	-	-	-	-	1*	-	-	-
Kidney - dilated pelvis (one or both)	10	4	12	3	17	3	8	-
- cyst	3	-	3	1	2	1	2	-
- pale	-	1	-	-	-	-	-	-
Parovarian cyst	-	-	-	2	-	1	-	-
Uterus - distended with fluid	-	3	-	-	-	2	-	-
Urinary bladder - thickened wall with yellowish material	-	-	-	-	-	-	1	-
Testicles - 1 small, 1 large	-	-	-	-	-	2	-	-
Accidental trauma	-	-	1	1	-	2	-	-
Front legs bowed	-	-	-	-	1*	-	-	-

A = birth to weaning (includes all culled and dead pups)
B = weaning to sexual maturity
* = same pup

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS.

Table 8

Necropsy Findings on F_1 Generation

Second Study

	Dose Groups (mg/kg/day)							
	Control		50		100		150	
	A	B	A	B	A	B	A	B
No. examined	91	26	116	34	105	30	97	24
Eyes - chromodacryorrhea	-	1	-	1	-	1	-	-
- microphthalmia	-	-	-	1	-	-	-	-
Liver - pale	-	-	-	-	-	1	-	-
Spleen - 2 mm cyst	-	1	-	-	-	-	-	-
Kidney - dilated pelvis (one or both)	4	2	12	7	4	2	2	-
- white areas cortex	-	-	-	-	-	1	-	-
- cortex rough	-	-	-	-	-	1	-	-
- pale	-	1	-	-	-	1	-	-
Ureters - dilated	-	-	-	1	-	-	-	-
Parovarian cyst	-	1	-	-	-	1	-	-
Urinary bladder - thickened wall/enlarged with calculi	-	1	-	-	-	-	-	-
- distended	-	-	-	-	-	1	-	-
Accidental trauma	-	1	-	-	-	-	-	-
Severe PMC/Autolysis	-	1	-	-	1	-	-	-

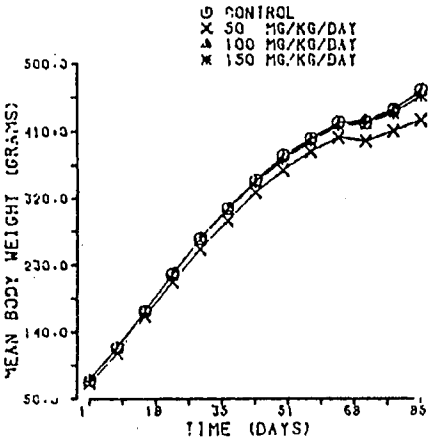
A = birth to weaning (includes all culled and dead pups)
B = weaning to sexual maturity

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

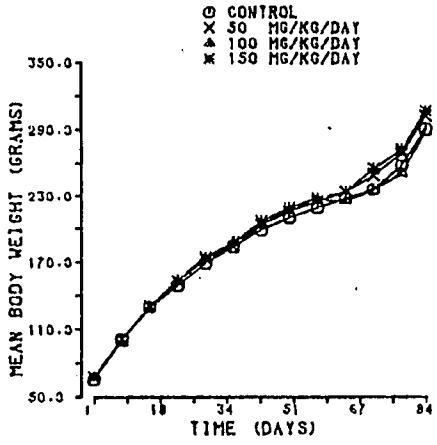
Plate III

First Study

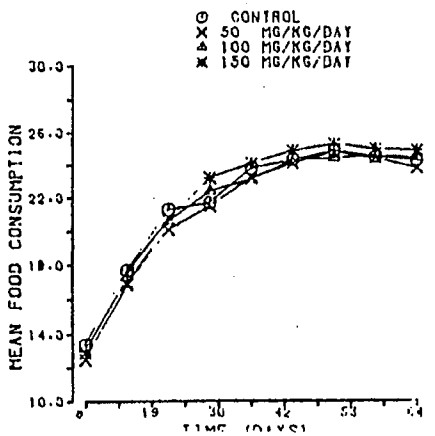
MEAN BODY WEIGHTS (GRAMS)
MALES (UNDOSED F-1 GENERATION)



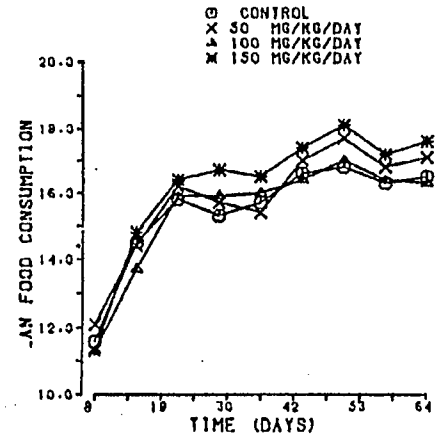
MEAN BODY WEIGHTS (GRAMS)
FEMALES (UNDOSED F-1 GENERATION)
PREGNANT FEMALES ONLY



MEAN FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)
MALES (UNDOSED F-1 GENERATION)



MEAN FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)
FEMALES (UNDOSED F-1 GENERATION)



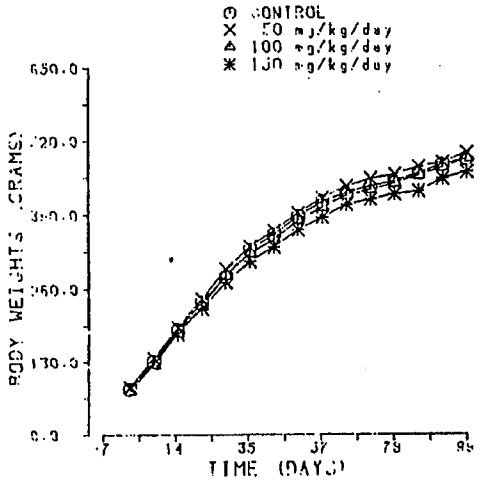
BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

PLATE IV

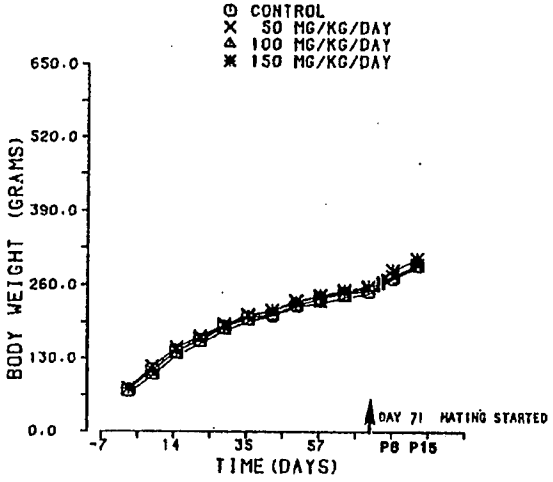
SECOND STUDY

MEAN BODY WEIGHTS (GRAMS)

MALES (UNDOSED F-1 GENERATION)

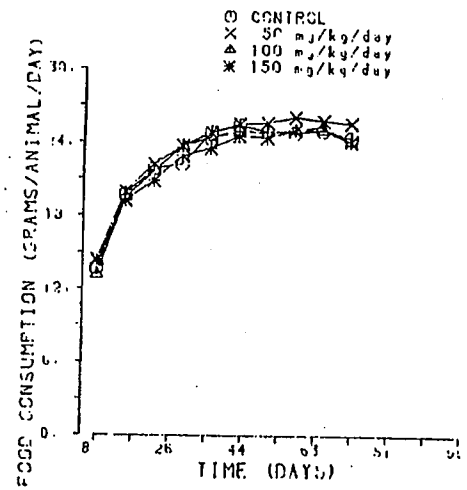


FEMALES (UNDOSED F-1 GENERATION)
DAMS WITH LIVE FETUSES ONLY

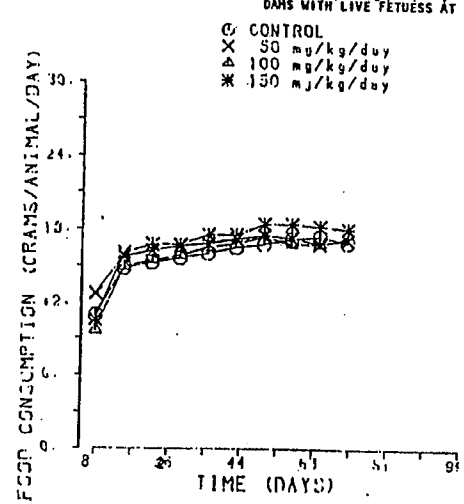


MEAN FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)

MALES (UNDOSED F-1 GENERATION)



FEMALES (UNDOSED F-1 GENERATION)
DAMS WITH LIVE FETUSES AT TERM ONLY



BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 9

Summary of Reproductive Data (F_1) Females

First Study

Dose Group[a] mg/kg/day	Number Retained	Died	Number Mated	Number Females Pregnant	Corpora Lutea	Implants	Viable Fetuses	Dead Fetuses	Resorptions	Pre-implantation[b] loss (%)	Post-implantation[c] loss (%)
Control	17	0	17	6	14.7	10.8	10.2	0	0.67	26.1	6.2
50	18	1	17	11	14.7	11.8	10.2	0	1.64	19.8	13.9
100	18	2	16	11	13.6	11.6	11.4	0	0.27	14.7[d]	2.3
150	18	0	18	12	14.8	12.1	10.9	0	1.17	18.5	9.7

[a] F_1 generation never dosed

[b] $\frac{\text{No. corpora lutea - No. implants}}{\text{No. corpora lutea}} \times 100$

[c] $\frac{\text{No. implants - No. viable fetuses}}{\text{No. implants}} \times 100$

[d] $P < .05$

8/1

BEHAVIORAL TERATOLOGY STUDIES WITH MDL 71,754 IN RATS

Table 10

Summary of Reproductive Data (F_1) Females

Second Study

Dose Group[a] mg/kg/day	Number Retained	Number Died	Number Mated	Number Females Pregnant	Corpora Lutea	Implants	Viable Fetuses	Dead Fetuses	Resorptions	Pre-implantation loss (%)[b]	Post-implantation loss (%)[c]
Control	13	2	11	11	15.7	14.1	13.1	0	1.00	10.4	7.1
50	17	0	17	16	16.4	15.1	13.3	0.06	1.75	8.0	12.0
100	14	1	13	13	16.3	14.5	14.2	0	0.23	11.3	1.6
150	12	0	12	12	19.5	14.8	13.5	0	1.25	24.4[d]	8.5

[a] F_1 generation never dosed

[b] $\frac{\text{No. Corpora lutea - No. implants}}{\text{No. corpora lutea}} \times 100$

[c] $\frac{\text{No. implants - No. viable fetuses}}{\text{No. implants}} \times 100$

[d] $P < .01$

SEGMENT II REPRODUCTION IN RABBITS:

A) Dosage

Two studies were performed, with dosing by gavage from days 7-19 of pregnancy (day 1 = day of insemination) at the following doses:

1) First study: 20 F at 0, 50, 100, or 200 mg/kg/day

2) Second study: 20 F at 0, 100, 150, or 200 mg/kg/day

Dams were sacrificed on day 29 of pregnancy. In the first study, fetuses were examined externally; heads were fixed in Bouins for exam by gross sectioning; thoracic and abdominal cavities examined grossly by dissection; bodies were macerated in KOH, stained with Alizarin Red S and examined for skeletal abnormalities. In the second study, nothing is stated in the "Materials and Methods" section about fetal exams other than external and head (as above); however results indicating visceral and skeletal exams are shown.

Strain: New Zealand White

Lot #s: 71,754-40 (1st study)
71,754-49 (2nd study)

B) Results

1) Observed signs in dams

Nothing stated

2) Mortality in dams

A few deaths were seen in the drug groups (Tables 1 and 2); the text states that these were "usually from dosing accidents or pneumonia". (In a rangefinding study in pregnant rabbits, 300 mg/kg caused 4/5 deaths; these were apparently considered to be drug-related).

3) Dam bodyweight and food consumption

Results shown in attached "plates" I and II. Large decreases in food consumption (followed by rebound after drug discontinuation) were seen at 100 mg/kg and above. Transient weight loss was seen at 150 mg/kg and above.

4) Reproductive data

(Results in tables 1 and 2 [first and second studies, resp.]).

a) A few abortions were seen in all drug groups, not D-R; not considered to be drug-related by the sponsor.

b) In the first study, the number of F having total resorptions was increased at HD; however this was not seen in the second study.

c) Mean number of resorptions was increased at HD in both studies; smaller, equivocal increases also seen at the lower doses.

5) Fetal exams

(Results shown in tables 3 [first study] and 4 [second study]).

Cleft palate was the only clearly drug-related adverse effect. It was seen in both studies; overall incidence was 9/103 fetuses and 4/17 litters at 200 mg/kg and 3/131 fetuses and 2/17 litters at 150 mg/kg. (None in other groups).

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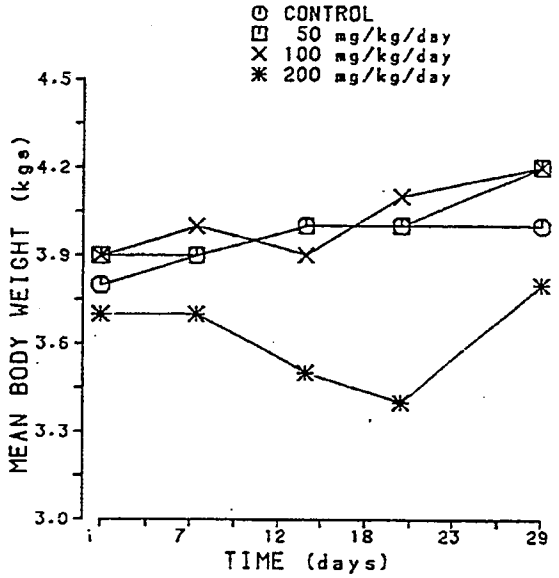
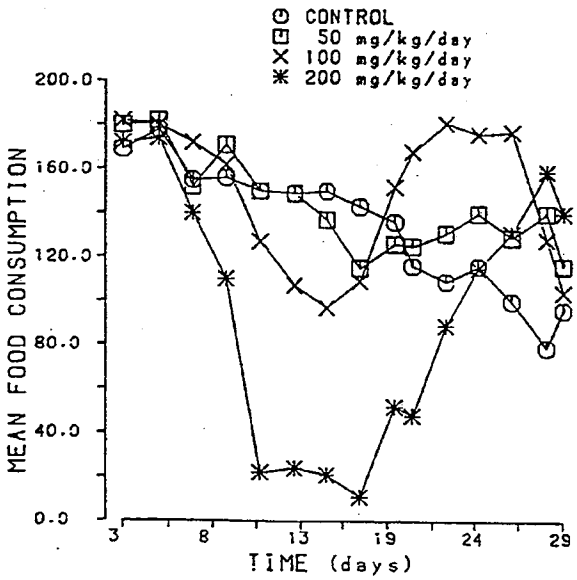
TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Plate I (First Study)

MEAN BODY WEIGHTS (kgs)
ONLY PREGNANT FEMALES
WITH LIVE FETUSES



MEAN FOOD CONSUMPTION (gms/animal/day)
ONLY PREGNANT FEMALES
WITH LIVE FETUSES



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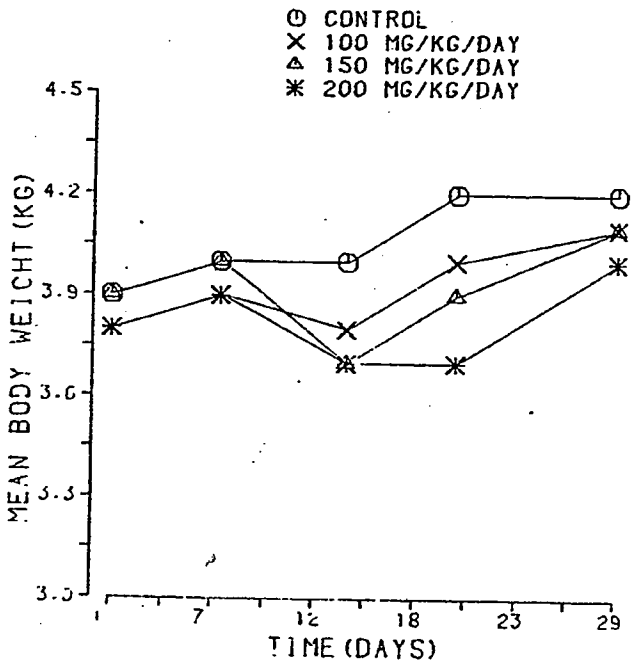
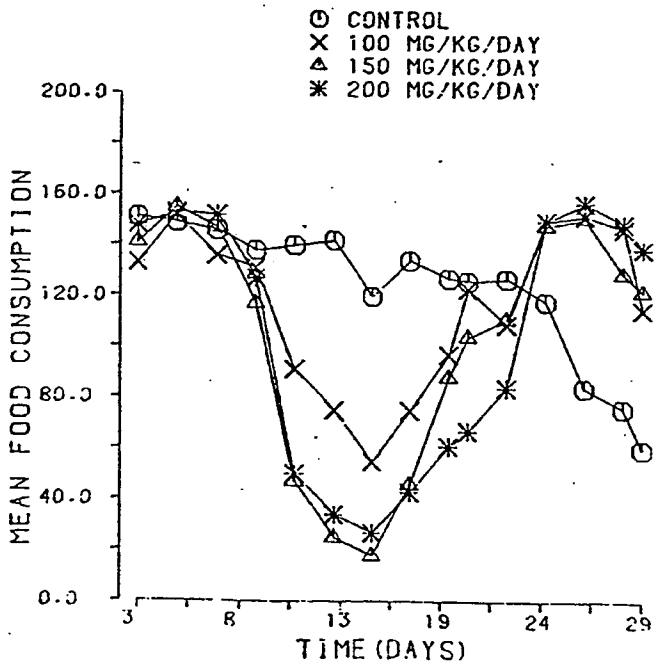
TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Plate II (Second Study)

MEAN BODY WEIGHTS (KG)
ONLY PREGNANT FEMALES
WITH LIVE FETUSES



MEAN FOOD CONSUMPTION (GMS/ANIMAL/DAY)
ONLY PREGNANT FEMALES
WITH LIVE FETUSES



TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Table 1

Summary of Reproductive Data (First study)

	Dose Groups (mg/kg/day)											
	Control			50			100			200		
No. inseminated	20			20			20			20		
No. not pregnant (lived)	1			2			4			4		
No. pregnant (died)	0			0			0			1		
No. aborted	0			2			1			2		
No. totally resorbed (term)	2	(1.5)		1	(1)		2	(2.5)		8	(4.1)	
No. pregnant (term)	17			15			13			5		
	Total	%	Avg.	Total	%	Avg.	Total	%	Avg.	Total	%	Avg.
Live fetuses	116	90	6.8	88	81	5.9	68	87	5.2	32	74	6.4
Dead fetuses	0	-	-	1	.92	.07	0	-	-	0	-	-
Incomplete resorptions	2	1.6	.12	3	2.8	.20	3	3.8	.23	7	16.3[a]	1.4
Complete resorptions	11	8.5	.65	17	15.6	1.1	7	9.0	.54	4	9.3[a]	.8
Implantations	129		7.6	109		7.3	78		6.0	43		8.6
Mean of mean fetal wts. (g)	33.9			33.1			38.0[b]			26.7[b]		

( ) = Average number of resorptions

[a] = Combined resorptions (postimplant loss) $P < .05$

[b] = $P < .05$

TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Table 2

Summary of Reproductive Data (Second Study)

	Dose Groups (mg/kg/day)											
	Control			100			150			200		
No. inseminated	20			20			20			20		
No. with corpora lutea	20			18			20[a]			19		
No. not pregnant (died)	0			0			1[a]			0		
No. not pregnant (lived)	1			3			0			1		
No. pregnant (died)	0			3			0			3		
No. aborted	0			2			2			2		
No. with totally resorbed litters	3	(5)		2	(10)		0			2	(9.5)	
No. pregnant at term (with live fetuses)	16			10			17			12		
	Total	%	Avg.	Total	%	Avg.	Total	%	Avg.	Total	%	Avg.
Live fetuses	134	89	8.4	91	90	9.1	131	80	7.7	71	74	5.9
Dead fetuses	0	-	-	0	-	-	1	1	0.1	0	-	-
Incomplete resorptions	3	2	0.2	7	7	0.7	11	7	0.6	13	14[b]	1.1
Complete resorptions	14	9	0.9	3	3	0.3	20	12	1.2	12	13[b]	1.0
Implantations	151		9.4	101		10.1	163		9.6	96		8.0
Mean of mean fetal wts.- gm	30.9			30.5			29.8			30.8		

[a] Died day 5

[b] Combined resorptions (post implant loss) $P<.01$

( ) Average number of resorptions

TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Table 3

Examination of Fetuses (First Study)

	Dose Groups (mg/kg/day)			
	Control	50	100	200
No. examined/no. litters	116/17	88/15	68/13	32/5
Malformations:				
Clubbed hind feet, no tail, fused kidneys, multiple vertebral and rib anomalies	-	1/1	-	-
Rhinencephaly, small mouth, no external nares, intranasal septum and turbinates absent, brain small	1/1	-	-	-
Hydrocephalus	1/1	-	-	1/1
Cleft palate, absence of corpus callosum with hydrocephalus	-	-	-	1/1[a]
Cleft palate	-	-	-	2/1[a]
Vertebral and/or rib malformations (scoliosis)	3/3	3/2	-	-
Totals	5/5	4/3	--	4/2

[a] Same litter

TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Table 3 (continued)

Examination of Fetuses (First Study)

	Dose Groups (mg/kg/day)			
	Control	50	100	200
No. examined/no. litters	116/17	88/15	68/13	32/5
Developmental Variations:				
Front paws flexed	1/1	-	-	-
Hematoma on head and snout	1/1	-	-	-
Sternal				
- small, pinpoint, unossified, bipartite, uneven ossification, bilobed and/or notched	111/17 (96%)	85/15 (97%)	66/13 (97%)	25/5 (78%)
- pinpoint ossification centers anterior to 1st sternebra	1/1	-	-	-
- shifted ossification centers	6/4 (5%)	6/6 (7%)	2/2 (3%)	6/3 (19%)
- fused	-	1/1	-	-
- xiphoid cartilage forked	-	2/1	-	-
Ribs				
- 12th small	-	1/1	-	-
- Bilateral 13th	42/14	25/12	18/9	14/4
- Unilateral 13th	21/13	16/9	8/7	1/1
- Cervical (7th)	2/2	2/2	1/1	1/1
Cervical vertebrae				
- unev sification of bodies	-	-	-	1/1

TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Table 4

Examination of Fetuses (Second Study)

	Dose Groups (mg/kg/day)			
	Control	100	150	200
No. examined/no. litters	134[a]/16	91/10	131[b]/17	71/12
Malformations:				
Cleft palate	-	-	3/2	6/3
Presacral vertebra missing with rib malformation	1/1	-	-	-
Cyclopia (2 eyes) with proboscis above orbit, small mouth with no tongue, oral cavity blind posteriorly, no apparent air passage way anterior to larynx	-	-	1/1	-

[a] Skeleton of one fetus not examined - disintegrating during processing
[b] Skeleton of one malformed fetus not examined - fixed in Bouin's solution
[c] Body of one fetus not dissected - dehydrated during processing

TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Table 4 (continued)

Examination of Fetuses (Second Study)

	Dose Groups (mg/kg/day)			
	Control	100	150	200
No. examined/no. litters	134[a]/16	91[b]/10	131[b]/17	71[b]/12
Developmental variations:				
Front paws flexed	-	3/2	3/3	-
Hematoma on shoulder and/or abdomen	-	2/1	-	-
Dilated pelvis of kidney	3/3	5/3	8/5	-
Sternal:				
- unossified, incomplete ossification, small, pinpoint, bipartite, irregular notched, bilobed	122/16	79/10	118/17	60/10
- asymmetrical	5/4	5/3	8/6	2/2
- fused	-	1/1	-	-
- one thoracic vertebra body 1/2 ossified (arches normal)	1/1	-	-	-
- one presacral vertebra missing	1/1	-	-	-
- one extra presacral vertebra	5/4	2/2	8/5	6/3

[a] Skeleton of one fetus not examined - disintegrated during processing
[b] Skeleton of one malformed fetus not examined - fixed in Bouin's solution
Body of one fetus not dissected - dehydrated during processing

TERATOLOGY STUDIES WITH MDL 71,754 IN RABBITS

Table 4 (continued)

Examination of Fetuses (Second Study)

	Dose Groups (mg/kg/day)			
	Control	100	150	200
No. examined/no. litters	134[a]/16	91/10	131[b]/17	71/12
Developmental variations (continued):				
Ribs				
- 12th one small	1/1	-	-	-
- bilateral 13th	41/10	31/8	70/12	30/10
- unilateral 13th	17/9	10/6	8/6	7/5
- cervical (7th)	-	-	2/2	-
- distal ends flattened (lt 11 & 12)	-	-	1/1	-
Pubic bones unossified, pinpoint	5/3	3/1	10/4	1/1
Dead fetus - one fore paw flexed, one rear foot rotated, not included in no. examined	-	-	1/1	-

[a] Skeleton of one fetus not examined - disintegrated during processing
[b] Skeleton of one malformed fetus not examined - fixed in Boulin's solution
[c] Body of one fetus not dissected - dehydrated during processing

SEGMENT III REPRODUCTION IN RATS:

A) Dosage

20 F at 0, 50, 100, or 150 mg/kg/day, in diet, from day 15 of gestation (day 1 = first 24 hrs. after mating) through weaning of pups.

F_1 litters culled to 8 at 2 days PP. At weaning, 1/sex/litter was retained and raised to sexual maturity for mating (at 99 days of age; cohabitation was 1:1 [to nonsibling] for 1 week); these F_1 pups received drug in diet at the above doses. Mated F_1 females were sacrificed approximately 16 days from midweek of cohabitation for evaluation of reproductive parameters. Histopathological exams were performed on F_1 dams and sires.

Strain: CD (SD), [redacted] b(4)

Lot #: 71,754-46

B) Results

1) Observed signs in F_0 dams

No drug effect

2) F_0 dam bodyweight and food consumption

Results shown in attached figure; no further data shown. The text states that a D-R decrease in food consumption occurred during the first week of treatment; from the figure it appears that consumption also remained below controls during the 2nd week of dosing, and also at HD at subsequent times. The text states that bodyweights were statistically significantly less than control at MD and HD after the first week of treatment, and remained decreased throughout the nursing period.

3) Reproductive data (through weaning)

(Results summarized in attached tables.)

a) According to the text, numbers of implants and live fetuses were slightly decreased at LD and HD; this was considered to be drug related at HD. (No explanation was given for this; it is not clear how decreased implantations could be drug related since treatment was begun day 15 of gestation.)

b) Pup weights were decreased at MD and HD on day 21 PP (but not at birth or day 2 PP); note that it is not clear how much drug the pups received via the dams' diet.

c) One LD cannibalized all pups on day of parturition, one HD showed evidence of dystocia, and one MD and one HD developed agalactia and lost their entire litter. These findings were considered to be incidental.

d) No drug effect or number of dead pups at birth, pup survival through day 21 PP, or necropsy findings on dead or discarded pups.

4) Reproductive data (post-weaning)

(Results summarized in attached tables/figures)

a) Convulsions seen in most HD and 1 MD. First seen at approximately 43 days of age. One MD F and 1 HD M died from undetermined causes.

b) Bodyweights at MD and HD remained below control through time of mating. (Note that weights at weaning were decreased at these doses as noted above; it is not clear how much of the subsequent decrease was due to this vs due to further drug effects. [Individual and group mean weights used to compose plate II were not given]). Food consumption appeared to be decreased at all doses but LD F. (Again, no data provided aside from the figure).

c) Reproductive results of F_1 matings shown in attached table 5. Numbers of CL, implants, and viable fetuses were decreased at HD. (CL also slightly decreased at MD). There were no drug effects on pre- or post-implantation loss.

5) Histopathology in F_1 parents

Results shown in attached Table 7. Brain vacuolation (primarily in hippocampus; it is not clear what other areas were examined beside hippocampus and cerebellum) seen at all doses; however incidence (and possibly also severity in F, not shown in table) was inversely D-R. (This is curious in that convulsions were mainly seen at HD.)

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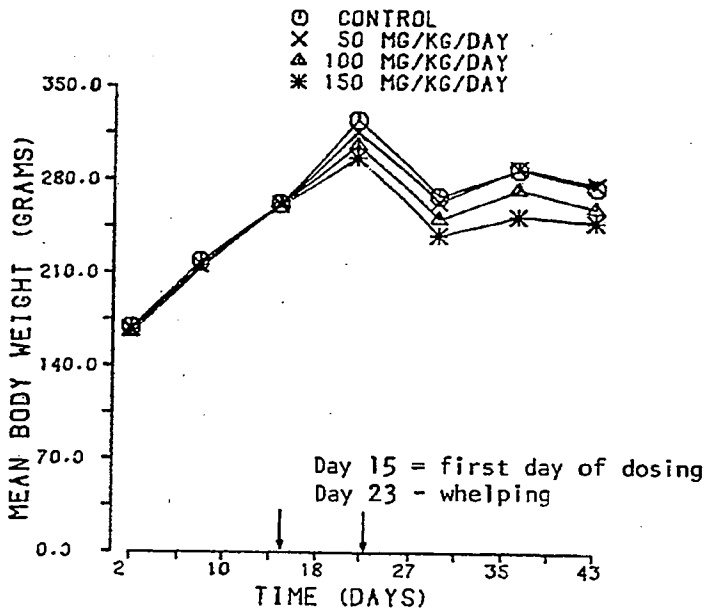
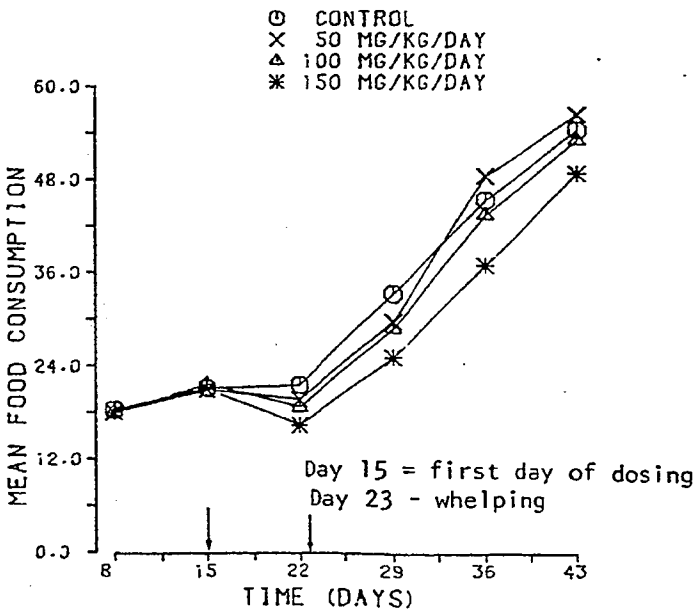
PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS
(F_0 GENERATION)

Plate I

MEAN BODY WEIGHTS (GRAMS)
(F_0 Pregnant Dams)



MEAN FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)



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PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 1

Summary of Reproductive Data (F_0)

Dose Group mg/kg/day	Number Litters	Mean Implants	Mean Number of Pups: Birth Live	Birth Dead	48 hours Remain	48 hours Retained	21 days Remain
Control	20	12.6	11.2	.05	10.8	7.8	7.8
50	19	11.7*	10.8	--	10.4	8.0	7.9
100	19	12.0	10.9	.05	10.7	8.0	7.4
150	19	11.1*	10.0	.16	9.6	7.5	7.2

* = $P < .05$

Table 2

Dose Group mg/kg/day	Mean pup weight (g[a]): At Birth	48 hours Remain	48 hours Retained	21 days Remain
Control	6.1	7.9	7.9	48.8
50	6.1	8.2	8.3	47.1
100	6.1	7.7	7.9	42.1**
150	6.2	7.9	7.9	39.5**

[a] Mean of litter means

** = $P < .01$

PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 3

Pup Survival

Dose Groups mg/kg/day	No. Live Birth	No. Live 48 hrs.	% Survival 48 hrs.	No. Retained 48 hrs.	No. Live 21 days	% Survival 21 days	No. Retained 21 days M	F
Control	224	216	96	155	155	100	20	20
50	205	198	97	152	151	100	19	19
100	206	203	99	152	141	93*	18	17
150	190	183	96	142	137	96	18	18

* P <.01

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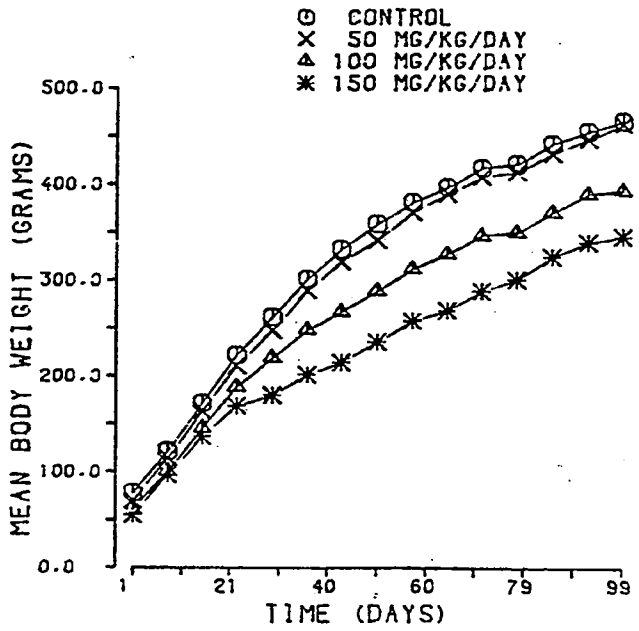
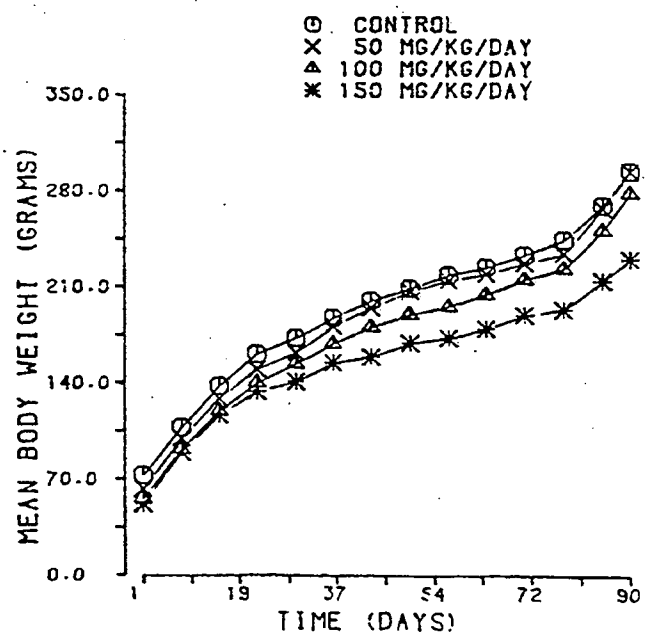
PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS
(F_1 GENERATION)

Plate II

MEAN BODY WEIGHT (GRAMS)
MALES



FEMALES



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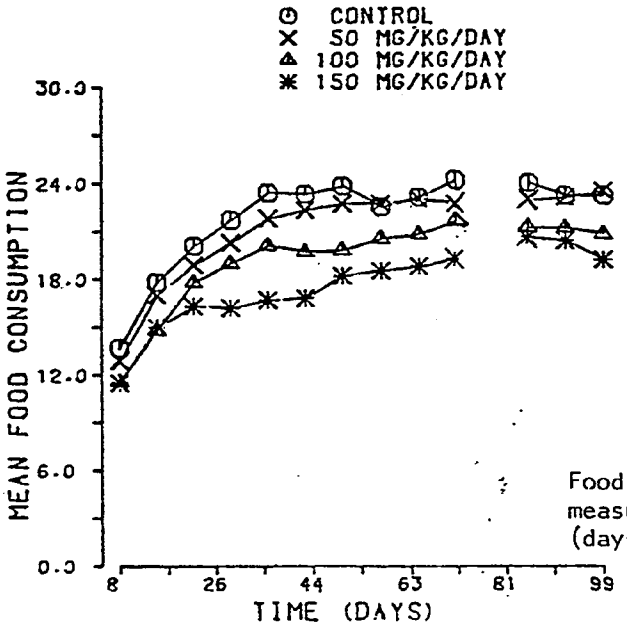
PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS
(F_1 GENERATION)

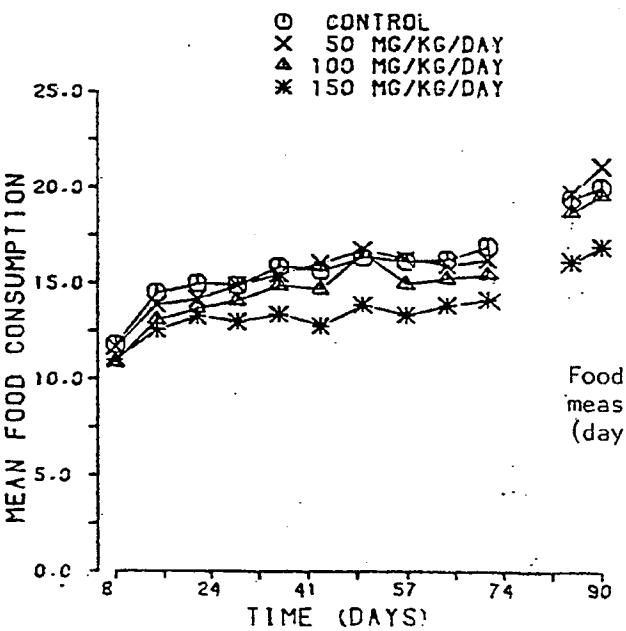
Plate II (Continued)

MEAN FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)
MALES



Food consumption not measured during cohabitation (days 71-78)

FEMALES



Food consumption not measured during cohabitat (days 71-78)

PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 4

Summary of Clinical Observations (F_1)

Weaning to Maturity

	Dose Group (mg/kg/day)							
	Control		50		100		150	
	20M	20F	19M	19F	18M	17F	18M	18F
Convulsions	-	-	-	-	1	-	16	12
Alopecia	-	-	-	-	-	3	-	-
Eye -- hemorrhage/cloudy cornea	-	-	-	-	-	-	1	-
Tip of tail missing	1	-	-	1	1	-	1	2
Abrasions	-	-	1	-	-	-	-	-
Dried blood around mouth and nose	-	-	-	-	-	-	1	-
Not eating or drinking due to accidental trauma	1	-	-	-	-	-	-	-
Reddish discharge from vagina	-	-	-	-	-	-	-	1
Missing from cage (1-2 days)	-	1	3	4	-	3	-	4
Died	-	-	-	-	-	1	1	-
Nothing significant	19	19	15	14	16	10	1	3

PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 5

Reproductive Data F_1 [a]

Dose Group mg/kg/day	No. Paired	No. Pregnant	Corpora Lutea	Implants	Viable Fetuses	Dead Fetuses	Resorptions	Pre-implant [b] Loss %	Post-implant [c] Loss %
Control	20	18	16.2	12.7	12.1	0	.61	22	4.8
50	19	19	16.2	12.9	12.4	0	.53	20	4.1
100	16	16	14.5*	12.3	11.6	.06	.63	16	5.6
150	17	14	11.4*	8.7*	8.3*	0	.43	23	4.9

[a] Sacrificed approximately 16 days from midweek of cohabitation

[b] $\frac{\text{No. corpora lutea - no. implants}}{\text{No. corpora lutea}} \times 100$

[c] $\frac{\text{No. implants - no. viable fetuses}}{\text{No. of implants}} \times 100$

* $P < .05$

PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 7

Summary Incidence of Microscopic Observations

TISSUES WITH FINDINGS	Animal SEX Dosage Group No. in Group	ANIMALS AFFECTED Males 1	Males 2	Males 3	Males 4	Females 1	Females 2	Females 3	Females 4
		20	19	18	18	20	19	17	18
Brain	Number examined	20	19	18	17	20	19	17	18
- Vacuolation – HIPPOCAMPUS		0	13	6	2	0	12	7	2
- Vacuolation, cerebellum		0	0	0	0	0	0	2	0
Peripheral nerve	Number examined	20	0	0	16	18	0	0	18
Eye	Number examined	20	0	0	17	20	0	0	18
- Iridocyclitis		0	0	0	1	0	0	0	0
Pituitary	Number examined	20	0	0	17	18	0	0	17
Thyroid	Number examined	18	0	0	16	18	0	0	18
- Ultimobranchial cyst		3	0	0	0	2	0	0	1
Adrenal(s)	Number examined	20	0	0	17	20	0	0	18
- Cortical vacuolization		0	0	0	1	0	0	0	0
Lacrimal gl. Ex.	Number examined	20	0	0	17	20	0	0	18
- Vacuolization		1	0	0	0	0	0	0	0
Lacrimal gl. Pos.	Number examined	20	0	0	17	20	0	0	18
- Mononuclear dacryoadenitis		0	0	0	0	1	0	0	0
Salivary gland	Number examined	20	0	0	17	20	0	0	18
Lymph N., Mand.	Number examined	19	0	0	16	18	0	0	18
Spleen	Number examined	20	0	0	17	20	0	0	18
Thymus	Number examined	20	0	0	17	20	0	0	17
Lung	Number examined	20	0	0	17	20	0	0	18
- Lymphoid follicular hyperplasia		15	0	0	16	18	0	0	12
- Interstitial pneumonia		1	0	0	4	6	0	0	6
Esophagus	Number examined	20	0	0	17	20	0	0	18

PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 7 (continued)

Summary Incidence of Microscopic Observations

TISSUES WITH FINDINGS	Animal Dosage Group / No. in Group	ANIMALS AFFECTED Males 1	Males 2	Males 3	Males 4	Females 1	Females 2	Females 3	Females 4
		20	19	18	18	20	19	17	18
Diaphragm	Number examined	20	0	0	17	20	0	0	18
Heart	Number examined	20	0	0	17	20	0	0	18
- Mononuclear interstitial myocarditis		3	0	0	2	0	0	0	0
Aorta	Number examined	20	0	0	17	20	0	0	17
Tongue	Number examined	19	0	0	16	20	0	0	16
Liver	Number examined	20	0	0	17	20	0	0	18
- Vacuolization		1	0	0	0	0	0	0	0
- Necrosis		0	0	0	1	0	0	0	0
Stomach	Number examined	20	0	0	17	20	0	0	18
Pancreas	Number examined	20	0	0	17	20	0	0	18
Small intestine	Number examined	20	0	0	17	20	0	0	17
Large intestine	Number examined	17	0	0	15	19	0	0	17
Lymph N., Mesen.	Number examined	20	0	0	17	17	0	0	18
Kidney (s)	Number examined	20	0	0	17	20	0	0	18
- Chronic interstitial nephritis		6	0	0	6	4	0	0	1
- Dilated pelvis		2	0	0	1	1	0	0	1
- Mineralization of the medulla		0	0	0	0	3	0	0	0
- Cyst		0	0	0	0	0	0	0	1
Urinary bladder	Number examined	20	0	0	17	20	0	0	16
Mammary gland	Number examined	20	0	0	17	20	0	0	18
Skeletal muscle	Number examined	20	0	0	17	20	0	0	18
Skin/Subcutis	Number examined	20	0	0	17	20	0	0	18

PERINATAL AND POSTNATAL TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 7 (continued)

Summary Incidence of Microscopic Observations

TISSUES WITH FINDINGS	Animal SEX / Dosage Group / No. in Group	ANIMALS AFFECTED Males 1	2	3	4	Females 1	2	3	4
		20	19	18	18	20	19	17	18
Testis	Number examined	20	0	0	17				
- Suppurative orchitis		1	0	0	0				
- Aspermatogenesis		1	0	0	1				
Epididymis	Number examined	20	0	0	17				
- Lymphocytic interstitial epididymitis		2	0	0	3				
Prostate	Number examined	20	0	0	17				
- Chronic prostatitis		3	0	0	2				
Sem. Ves.	Number examined	19	0	0	17				
Ovary(s)	Number examined					20	0	0	17
Uterus	Number examined					20	0	0	18
- Placentation						18	0	0	14
Vagina	Number examined					20	0	0	18
- Mucin-cell hyperplasia						18	0	0	13
Bone	Number examined	20	0	0	17	20	0	0	18
Bone marrow	Number examined	20	0	0	17	19	0	0	18

SUMMARY

A) ADME/PK

The salient results of the ADME/PK studies, performed in rats, dogs, and monkeys, are as follows:

1) In rats and dogs, absorption of drug after oral dosing was nearly complete, with no apparent first pass effect.

2) Oral absorption in monkeys was poor; based on urinary excretion data the degree of absorption ranged from 10-40% of the dose across studies, with some evidence of decreasing % absorption with increasing dose.

3) Excretion of label after dosing with labelled drug was almost entirely urinary after p.o. and i.v. dosing in rats and dogs and i.v. dosing in monkeys.

4) Plasma T½ for parent drug was short (1-4 hr.) in all 3 species (although one monkey study showed values of 7 and 12 hr. after p.o. doses of 50 and 260 mg/kg, resp); plasma T½ for total label in rats was 25-30 hr. (Note that the duration of action of the drug, at least those actions mediated by GABA-T inhibition, will be determined more by the resynthesis time of the enzyme than by drug levels, since the enzyme inhibition is irreversible).

5) GVG is excreted almost entirely unchanged in all 3 species.

6) GVG did not bind to human serum proteins at drug concentrations of 20 and 100 μM; protein binding was apparently not studied in other species.

7) GVG does not appear to induce hepatic drug metabolizing enzymes in rats.

8) Some discussion of comparative drug exposure across species in relation to GVG-induced intramyelenic edema is in the Evaluation section of this review, below.

B) ACUTE TOXICITY

In studies done in mice and rats using p.o. and i.p. dosing, the most consistent sign was a long-lasting (several days) sedation. Piloerection was seen in rats, and convulsions were seen in 1 of 3 i.p. mouse studies at higher doses. Deaths were generally delayed with most occurring within 4 days after dosing. The LD_{50} values were in the 1-3 g/kg range.

C) SUBACUTE/CHRONIC TOXICITY/CARCINOGENICITY

The following oral studies were performed (daily doses in mg/kg in parentheses):

1) Mouse

   a) 1 month dietary rangefinding (10/sex at 100, 200, 300, 500)

   b) 18 month dietary carcinogenicity (50/sex at 50, 100, 150)

2) Rat

   a) 2 week gavage rangefinding (5-10/sex at 10, 25, 50, 100, 200, 500, 1000)

   b) 3 month gavage (20/sex at 30, 100, 300)

   c) 1 year dietary (20-40/sex at 30, 100, 200, 300)

   d) 2 year dietary carcinogenicity (50/sex at 50, 100, 150)

3) Dog

   a) 2 week rangefinding (1/sex at 100, 300, 600, 1000)
   b) 3 month (3/sex at 30, 100, 300)
   c) 1 year (4-5/sex at 50, 100, 200)

4) Monkey

   a) 1 month rangefinding (2/sex at 500, 750, 1000 [+ 2/sex at 300 b.i.d. for 1 week]).

   b) 6 year (5/sex at 50, 100, 300)

Several additional, specialized studies were performed to further elucidate or characterize selected aspects of the toxicity of GVG; these will be summarized after the following summaries of the routine studies by species:

1) MOUSE

In the 18 month carcinogenicity study, sporadic convulsions were seen in a few males at MD (100 mg/kg) and HD (150 mg/kg) starting at month 10. (No convulsions seen in the 1 month rangefinding study; HD = 500 mg/kg). There were slightly more deaths in all treated groups compared to controls in the carcinogenicity study although this was not D-R (and not statistically significant by the sponsor's analysis). Weight gain was slightly decreased in the carcinogenicity study at MD and HD; final weight at HD was about 90% of control. It was implied that food consumption was slightly decreased in the carcinogenicity study at MD and HD although this was difficult to discern from the graphs provided. (No decrease in food consumption was seen in the rangefinding study up to 500 mg/kg despite decreases in weight gain). Lab tests were not done in either study. There were no drug-related increases neoplastic lesions in the carcinogenicity study; the incidence of total primary and total benign tumors was decreased across all doses (with greatest effect at HD). Drug-

related non-neoplastic findings in this study were in brain and eye. The incidence of brain vacuolation was increased, in males only, in some areas at all doses, but not clearly dose-related. The primary areas affected included cerebellum, reticular formation, and thalamus. Although vacuolation was not increased in F, several MD and HD F (as well as several MD and HD M) had foci of mineralization in the area of the cerebellar roof nuclei. (In the rangefinding study, cerebellar and/or midbrain vacuolation were seen at 200 mg/kg + in F and at all doses in M). There were no drug effects on sciatic nerve in either the rangefinding or carcinogenicity study. An effect in eye was seen in the carcinogenicity study only, where the incidence of "retinal degeneration" was increased in HD F and equivocally in LD and MD F (incidence = 1/47, 3/38, 4/39, and 8/39 in control, LD, MD, and HD, resp.). It was described as mild, involving focal loss of rods and rod nuclei from outer retinal layers, and as "somewhat similar to that previously reported in albino rats treated with Vigabatrin." (The finding of "outer nuclear layer dysplasia" was decreased in treated groups of both sexes; this was not discussed in the text).

2) RAT

The most prominent toxic sign was frequent convulsions. They were seen at all but the lowest dose (30 mg/kg) in the 1 year dietary study and at all doses (LD = 50 mg/kg) in the 2 year study. They were not seen until after 3-6 months of treatment. Reversibility was assessed in the 1 year study; the convulsions regressed very slowly, lasting at least 3-4 months after cessation of treatment. (After 12 month's treatment, complete reversibility was not established). Ataxia was seen in association with the convulsions, and various traumatic lesions were seen which were considered to be caused by the convulsions. In the shorter term gavage studies, convulsions were only reported in 2 HD (300 mg/kg) rats in the 3 month study.

In the 2 year study, a few LD and HD rats were "unable to move hind quarters". Some of these rats showed histopathology in sciatic nerve, but this was apparently similar to that seen in unaffected animals, including controls. Histological exam of spinal cord (done in these animals only) was said to show only artifactual/autolytic changes. (No concurrent controls examined). This clinical sign is pertinent in that the intramyelinic edema caused by GVG, unlike that caused by hexachlorophene, has only been demonstrated histologically in the CNS. This was the only report of this clinical sign in any of the animal studies of GVG.

Many HD (300 mg/kg) rats in the 1 year study became moribund and were prematurely sacrificed. Mortality was also slightly increased at 200 mg in this study. There were no drug effects on mortality in the 2 year study (HD = 150 mg/kg).

Bodyweight gain and food consumption were decreased at most doses across all studies. In the 2 year study, final weights were about 93, 85, and 75% of control at LD, MD, and HD, resp.

Several slight lab test changes were seen; these were generally not consistent across studies with the exception of a decrease in blood calcium. Decreased ALT and

AST were seen at the higher doses in the 2 week study; this was attributed to transaminase inhibition by GVG.

The primary drug-induced histological changes were in brain and eye. Brain vacuolation was increased in all studies but the 2 week study. (Note that this does not preclude this change occurring after 2 weeks' treatment since it is not clear that brain was as extensively examined in the 2 week study as in the other studies). Vacuoles were seen primarily in white matter in several areas of brain; most affected were cerebellum, reticular formation, optic tract, anterior commissure, columns of fornix, colliculus, hippocampus, thalamus, cerebral peduncle, and corpus callosum. (Peripheral nerve was not affected). EM exam showed the vacuoles split the myelin sheath at the intraperiod line, similar to the intramyelenic edema produced by drugs such as hexachlorophene. Vacuolation was seen at HD (300 mg/kg) in the 3 month study and eventually at all doses in the 1 and 2 year studies. (In the 1 year study, vacuolation at LD was not seen at the 6 month interim sacrifice). The sponsor considered the overall vacuolation in the 2 year study to be slightly more widespread than in the 1 year study. Reversibility of the vacuolation was examined in the one year study; it was not seen after a 3 month recovery period (but note that reversibility at HD following 12 month's treatment was not studied). However, other lesions, i.e., eosinophilic spheroids (said to be suggestive of swollen or degenerated axons; gave positive response to staining with antibodies to neurofilament protein) and calcium-containing mineralized microbodies were seen (primarily in cerebellum) which were not reversible (and in fact appeared to become more pronounced [regarding incidence and size] during the recovery period). These changes were seen at all doses in the 1 and 2 year studies with the exception of the LD (30 mg/kg) in the former (and at 100 mg/kg, they were seen at 12 but not 6 months). It was the sponsor's impression that the mineralized microbodies in the 2 year study had a greater incidence and were somewhat larger and more widespread than in the 1 year study. (Also note that various other findings were described by individual consultants as noted earlier. Also, as discussed later, gliosis as demonstrated by increased GFAP staining was seen.)

Limited luxol fast blue staining and EM exams in brain led to the sponsor's conclusion that despite possible indications of slight myelin disruption or degradation, there was no evidence of classical segmental demyelination.

In the 3 month study, the incidence of "retinal degeneration" was increased at HD (7/30 vs 0/30 controls); lower doses were not examined. (Further discussion of retinal changes is made below under "Special Studies".)

In the 2 year study the incidence of pituitary adenomas was decreased at all doses (D-R); the incidence of total benign and total malignant tumors was also decreased (but not always D-R).

3) DOG

In dogs, daily doses of 300 mg/kg and above were generally not well tolerated; drug effects included emesis, diarrhea, anorexia, decreased bodyweight, and emaciation. Both dogs given 1000 mg/kg died during the second week of treatment. Doses of up to 200 mg/kg were well tolerated in the 1 year study; no drug effects on observed signs, bodyweight, or food consumption were seen. Ophthalmoscopic and EKG exams, done in the 3 month and 1 year studies, showed no drug effects. There were no clearly drug-related effects on laboratory parameters in the 1 year study (HD = 200 mg/kg) aside from a decreased ALT in all groups but LD M; this was considered to be due to transaminase inhibition (although it is not clear why the decrease was primarily manifest at the 3 month time point). In the 3 month study there were moderate decreases in RBC, Hb, and Hct in a few HD (300 mg/kg) and slight (10-20%) decreases in blood Ca in most dogs at MD (100 mg/kg) and HD. (Also, decreases in ALT were seen in the 2 week and 3 month studies as was seen in the 1 year study). Several lab test changes were seen among the dogs which died or became emaciated in the 2 week and 3 month studies; the direct relationship of these changes to drug is not clear.

The primary histopathological finding in the dog studies was brain vacuolization. This was seen at MD (100 mg/kg) and HD (300 mg/kg) in the 3 month study; in the 1 year study it was seen at MD (100 mg/kg) and HD (200 mg/kg) at 6 months and at all doses (LD = 50 mg/kg) at 1 year; thus a no-effect dose was not established. (Note that the lack of a reported effect in the 2 week study does not preclude an effect at this time since brains were apparently not as extensively evaluated. In fact, a subsequently performed special study in dogs given 300 mg/kg/day in which animals were sacrificed weekly and numerous areas of brain examined, slight effects in some areas were apparent by 2 weeks). The primary areas affected were columns of fornix, optic tract, and thalamus; also affected were hypothalamus, fimbria of fornix, and inferior colliculus. (Note: Other areas were also identified in the evoked potential and MRI studies, summarized later, in which dogs received 300 mg/kg subacutely. These areas included anterior and posterior commissure, hippocampus, median forebrain bundle, stria medullaris, cerebellar periventricular area, lateral geniculate body, mamillothalamic tract, corpus callosum, optic chiasm, habenular nucleus, and pretectal nucleus.) The increased vacuolation was not apparent after 4-6 month recovery periods in the 1 year study. No other changes in brain were seen aside from a mild gliosis in optic tract of one HD at 12 months. (However, note that in a special study on somatosensory evoked potentials discussed later, microgliosis was seen in dogs receiving 300 mg/kg for 12 weeks. Also, as noted later, astrogliosis as demonstrated by increased GFAP staining has been seen in dogs.) Limited myelin staining (mainly in 3 month study) showed no evidence of segmental demyelination. There were no drug effects in peripheral nerve or spinal cord.

Other drug-related histological changes in dogs were splenic hematopoiesis and sternal marrow hyperplasia seen in a few HD (300 mg/kg) dogs in the 3 month study; this was considered to be a compensatory response to the anemia seen in this group. Several pathological changes were seen in the dogs which died or became emaciated in the 2 week and 3 month studies (including thymic atrophy, stomach ulcerations/hemorrhage, myocardial hemorrhage/degeneration/necrosis); however, these changes likely represent indirect/agonal effects.

4) MONKEY

In the rangefinding study loose stools/diarrhea was seen at all doses (LD = 500 mg/kg) beginning within the first 3 days and lasting throughout the study; in the 6 year study the only clearly drug-related sign was occasional, transient instances of loose stools/diarrhea at HD (300 mg/kg). There were no drug effects on food consumption or weight gain in the 6 year study; these were said to be reduced at 750 and 1000 mg/kg in the rangefinding study but the data presented were not convincing. Ophthalmoscopic exams were said to be negative in both studies. CSF pressures were measured in a few controls and HD at 16 months; there was no apparent drug effect but the inter-animal variation was large. The only clearly drug-related lab test change was a decrease in ALT in the 6 year study, mainly at MD and HD, thought to be due to transaminase inhibition. (Equivocal effects included very slight, sporadic decreases in RBC, Hb, and Hct in the rangefinding study and primarily at HD in the 6 year study, and sporadic elevations in triglycerides in the 6 year study). The only histopathological effect considered to be possibly drug-related was vacuolization in brain. Conclusions by the sponsor's pathologists and several consultants regarding the 6 year study ranged from no drug effect to possible drug effect to slight drug effect, with the latter 2 only pertaining to HD monkeys. Where a possible or slight drug effect was concluded the vacuolization was generally in the optic tract; one consultant also concluded possible effects in corpus callosum, septum, and cerebellum. In the rangefinding study vacuolization in the corpus callosum was seen in 1 monkey each at 500 and 750 mg/kg but the relation to drug was equivocal in view of the vacuolization seen in some controls in the 6 year study. No drug effects were apparent in sciatic nerve or spinal cord.

Plasma, CSF, and urine levels of drug were assayed occasionally in these studies; specific results are shown earlier in this review. Although conclusions are difficult to reach because of the small Ns, it appears that plasma levels may become saturated with increasing dose over the range studied. It was also seen that the % of dose excreted as unchanged drug averaged about 10-20%. Brain GABA, GABA-T, and GAD, and CSF GABA, were measured 6 hr. after the final dose in the 6 year study; brain GABA-T was decreased by 30-37% and CSF GABA was equivocally increased about 20-30%; brain GABA level was unaffected.

5) SPECIAL STUDIES

Several studies were performed to try to further characterize the intramyelenic edema produced by GVG in animals, and to try to develop a method for noninvasive detection of this lesion which could be used to monitor for the lesion in humans. In addition, retinal lesions were seen in some rodent studies and were further evaluated. The sponsor's summary of these studies, which is generally accurate, is attached. The salient findings are discussed below:

a) Evoked potential and MRI studies in dogs

Several studies were done in dogs to try to establish a non-invasive method for monitoring intramyelinic edema in brain. Attached is my previous review of one of these studies, as well as several published studies, which contain some details of the methods used and results obtained. In these studies dogs were given 300 mg/kg/day, for 3-4 months, usually followed by recovery periods of 3-4 months. Methods evaluated for monitoring the brain lesion included evoked potentials (somatosensory, visual, and auditory) and MRI. A summary of the main findings are as follows:

1) Changes in somatosensory evoked potentials (SEP), manifest as increased central transmission time (i.e., difference between latency of sensorimotor cortical response and latency of cervical spinal cord response to electrical stimulation of forelimb), and in visual evoked potential (VEP; also referred to as flash evoked potential, or FEP), manifest as an increased latency of the initial cortical potentials (A measure of purely central transmission, analogous to that made for SEPs, was not assessed due to difficulty in accurately measuring the peripheral component), were seen beginning at 4-10 weeks of treatment. Both the SEP and VEP changes as well as the brain vacuolation reversed during the recovery periods, although in one SEP study mild microgliosis persisted.

It should be noted that initially performed VEP studies failed to show the above-noted drug effects, apparently due to methodological difficulties/differences. One of these studies, while not showing an effect on the early components of the VEP, did appear to show a slowing of late components (waveforms with latency $\geq$ 75 m sec); however this was concluded based on a comparison of VEPs obtained after 12 week's treatment with those obtained after recovery periods; i.e., valid pre-drug baselines were not obtained.

2) Changes in MRI, manifest as increased T_2 and decreased T_1 weighted signal (with the former being apparently more sensitive to drug effect), were seen in columns of fornix and surrounding hypothalamic structures, and in thalamus. (It was stated that the clearest signal changes were found in these areas, and thus these areas were chosen for further semi-quantitative characterization of drug effect; other areas were apparently not studied further). In one study, changes were seen after 15 week's treatment, with earlier times not examined. In another study MRIs were examined only when weekly SEPs or VEPs showed a change; such changes occurred weeks 4-7 and all affected dogs had changed MRIs (and showed presence of vacuoles on histological exam performed at these times). In another study in which ex vivo MRIs were done weekly, MRI changes were seen starting at week 7; vacuoles were seen histologically in these areas starting at 2-4 weeks in this study. In an in vivo MRI study the MRI changes decreased over the 12 week

recovery period although some changes were still present at the end; vacuolization at this time was indistinguishable from controls. In the ex vivo study vacuolization decreased over the 16 week recovery period but was still marginally elevated at 16 weeks; the report states that the MRIs demonstrated "a definite trend toward reversal" although my interpretation of the data provided is that it is hard to conclude any clear reversal of MRI changes in hypothalamus and thalamus after the 16 week recovery period.

As occurred in the case of VEPs, an early study failed to show drug-induced MRI changes, indicating the effect of methodological refinement on detectability of change.

3) A brief published abstract (copy attached) was included which claims that MRI can detect brain vacuolization in rats.

4) One study examining auditory evoked potentials in dogs failed to find any drug effects.

b) GFAP staining of brain

Brain sections from rats, dogs, and monkeys were examined by GFAP staining, and the results were correlated with the presence of vacuolation as shown by H and E staining. (Sections from various studies were used, including rats treated orally for 90 days at 30, 100, and 300 mg/kg, rats treated for 6-12 months at 200-300 mg/kg followed by 3-6 month recovery periods, dogs treated orally for 15 weeks at 300 mg/kg with and without 12 week recovery periods, and monkeys treated for 16 months at 300 mg/kg or 6 years at 100 mg/kg; see attached table for numbers of animals used). Increased GFAP staining (considered to represent reactive astrocytosis) was seen in rats and dogs (at all doses but the lowest in rats). In some areas it was said that the astrocytosis was in "close association" with the drug-induced vacuoles, although in some areas drug-induced vacuoles were present without astrocytosis, (e.g., hippocampus of rats) and in some areas astrocytosis was present without vacuoles (e.g., thalamus and medulla of rats, and cerebellum and pons of dog). After the recovery periods vacuolation regressed or disappeared; there were fewer reactive astrocytes but "a more quiescent, residual gliotic reaction was seen". ("The astrocytes became less hypertrophic and the GFAP positive processes became compact. The overall appearance was of a more dense matrix of fibrillary processes in areas that were previously occupied by the large prominent reactive astrocytes, and myelin vacuolation.").

The production of intramyelenic edema by GVG in monkeys is equivocal, as discussed earlier; the GFAP results were likewise equivocal. It

GFAP study

TABLE 1

SUMMARY OF ANIMALS INCLUDED IN THE INVESTIGATIONS

SPECIES	DOSE	TOTAL NUMBER	TREATMENT PERIOD	RECOVERY	REFERENCE
Rat	0	14	90 days	-	Butler et al 1987 (7)
	30	12	90 days	-	
	100	13	90 days	-	
	300	24	90 days	-	
	300	6	6 months	3 months	Gibson et al 1990 (3)
	200	3	12 months	3 months	
	200	3	12 months	6 months	
Dog	0	2	15 weeks	-	Schroeder et al 1991 (8)
	300	4	15 weeks	-	
	0	2	15 weeks	12 weeks	
	300	4	15 weeks	12 weeks	
Primate	100	2	6 years	-	Lippert et al 1986 (9)
	300	3	16 months	-	
	0	2	16 months	-	Gerbig et al 1989 (10)

was stated that there was a minor increase in GFAP staining in the 2 monkeys treated for 6 years at 100 mg/kg compared to the 2 controls (although note the controls were sacrificed with the animals treated for 16 months).

c) Changes in soluble proteins in rat brain

Published studies in rats showed that GVG given in a liquid diet at 300 mg/kg/day (but not 50 mg/kg every other day) decreased the synthesis of total soluble proteins in brain, and changed the isoelectric focusing pattern of soluble proteins in brain and CSF. The authors hypothesize that the change in protein pattern might reflect an increased glia-to-neuron ratio in brain.

d) Retinal lesions

Retinal lesions, generally denoted as "retinal degeneration", were reported in 2 rat studies done in Europe. In a study done at [redacted] Europe, the lesion was seen in 80-100% of albino (Sprague-Dawley) rats of both sexes treated with 300 mg/kg GVG by gavage for 3 months (lower doses not used). The lesion was described as "focal, multifocal, or occasionally diffuse disorganization of the outer nuclear layer, with displacement of the nuclei into the area of the rods". (This study also showed, as did another rat study, that pyridoxine does not reverse GVG-induced toxicity).

In the other European study, done at [redacted] both albino (Sprague-Dawley) and pigmented (Lister-Hooded) rats were used, as was both gavage and dietary dosing. Doses used and results obtained are shown in Table 5-36 of the sponsor's summary. It can be seen that retinal degeneration (described here in a similar way to the lesion seen in the Hazelton study; it is further stated here that "there was no clear evidence of necrosis, although there appeared to be fewer nuclei per unit area" and that "no inflammatory infiltrate was present and no other layer of the retina was affected") was seen in albino rats at all gavage doses (LD = 30 mg/kg; although only 1 animal affected at this dose) and at 100 and 300 mg/kg (NOEL = 30 mg/kg) dietary doses; both incidence and severity were greater with increasing doses and with gavage (vs dietary) dosing. As in the Hazelton study, a majority of animals were affected at the higher doses. The lesion was not seen in pigmented rats given 300 mg/kg by either route. (It is noted, however, that toxic signs and brain vacuolation were also less in pigmented than in albino rats). It is noted that routine ophthalmoscopic exams showed no drug effects in this study.

b(4)

In view of the above findings, the sponsor re-evaluated retinal slides from many of their previously conducted routine and special toxicity studies with GVG. In the 3 month rat gavage study, retinal degeneration was found in 7/30 rats at 300 mg/kg (0/30 in controls; lower doses not evaluated). In rat dietary studies (ranging from 3-12 month's treatment with 300 mg/kg to 2 year's treatment with 150 mg/kg), there was no drug-effect in the 2 year study but a hint of a possible slight effect in the other studies (with overall incidence in the latter ranging from approximately 1% in controls to 9% at 300 mg/kg). (In one of these studies, the lesion was described as "loss of the rod and cone layer [RCL] and disorganization of the outer nuclear layer and its extrusion in the RCL"; it was further stated that it appeared to be similar to the lesion seen in the Hazelton study. Also, the description of the lesion in the 1 year study included loss of rods and cones and thinning of the outer nuclear layer). In the 18 month mouse dietary study the incidence of retinal degeneration (said to be "somewhat similar" to the lesion seen in rats) was increased, but in females only (incidence = 2, 8, 10, and 21% in controls, 50, 100, and 150 mg/kg groups, resp.; incidence in control males = 2%). No retinal effects were apparent in the dog and monkey studies.

In summary, GVG produced retinal degeneration in albino rats of both sexes, and in female mice, at doses as low as 30-50 mg/kg p.o. It was not seen in 1 study of pigmented rats; however it is noted that the pigmented rats were less sensitive to other toxic effects of GVG, raising the possibility of an overall lower sensitivity to drug in this strain, or possible PK-ADME differences between strains, as alternatives to the conclusion that pigmented animals are particularly resistant to the retinal lesion. The incidence and severity of the lesion is greater in rats dosed by gavage as opposed to diet. (In mice, only dietary dosing was studied). Reversibility of the lesion was apparently not examined. The lesion was apparently not manifested in routine ophthalmoscopic exams; traumatic and inflammatory lesions in eye were seen in rats which were considered secondary to convulsions. The sponsor states that the histological appearance of the lesion is similar to that following excessive exposure to light, and hypothesizes that GVG "somehow increases light exposure or sensitivity of the already overly-sensitive albino retina". It was not seen in dogs or monkeys.

e) Differences between S and R enantiomers

Studies in rats showed that only the pharmacologically active S enantiomer caused typical GVG-induced toxicity, including brain intramyelenic edema and retinal degeneration. The sponsor also notes that other GABA-T inhibitors, e.g., gamma allenyl GABA, ethanolamine-o-sulfate, and BW 357 U, have also caused intramyelenic edema. These observations support the possibility that the mechanism of the brain and retinal toxicity might involve GABA T inhibition/elevated GABA levels. (In addition to brain, GVG also inhibits GABA-T and elevates GABA levels in rat retina). However, one cannot exclude the existence of other differences between the R and S isomers besides GABA-T inhibition, importantly including pharmacokinetic differences,

e.g., levels of the S isomer in rat brain are several fold greater than those of the R. Also, in one published study (John, et.al., Biochem. Pharmacol. 36: 1467, 1987) both GVG and the GABA-T inhibitor ethanolamine-0-sulfate elevated rat brain GABA to a similar degree but the brain vacuolation was greater with the former, suggesting the existence of additional mechanisms.

D) GENOTOXICITY

The following genotoxicity assays were performed:

1) Ames Tests

2) Point mutation and gene conversion assays in yeast

3) CHO/HGPRT forward mutation assay

4) In vitro chromosomal aberration assay in rat lymphocytes

5) Micronucleus test in mice

GVG was negative in these assays. All assays were responsive to positive controls. All in vitro assays were done both with and without a metabolic activation system. The micronucleus test may be criticized on the grounds that signs of drug toxicity were not reported; however, the high dose was over 50% of a historically cited LD50 value. The Ames Tests and yeast assays did not contain GLP statements and were not QAU inspected.

E) REPRODUCTION

The following studies were performed (oral daily dose in mg/kg in parenthesis):

1) Segment I rat (separate studies in M and F): 50, 100, 150 (dietary)

2) Segment II rat: 50, 100, 150

3) Segment II rat (behavioral teratology) (2 separate studies performed): 50, 100, 150

4) Segment II rabbit (2 separate studies performed):

a) 50, 100, 200

b) 100, 150, 200

5) Segment III rat: 50, 100, 150 (dietary)

In the segment I studies, decreased parental weight gain and food consumption were seen in all treated groups but LD M. Alopecia was seen in several treated HD dams. The only effects on reproductive parameters were slight decreases in numbers of CL, implantation sites, and viable fetuses (without effect on pre- or post-implantation loss) in treated HD dams.

Three segment II studies were done in rats: one with standard fetal morphological exams and 2 behavioral teratology studies. Slight decreases in food consumption and weight gain seen, primarily at the higher doses. (In a non-GLP rangefinding study using doses of 300, 400, and 500 mg/kg a marked decrease in food consumption accompanied by weight loss was seen at all doses; alopecia was seen at the 2 higher doses.) Slightly decreased fetal weights were seen in the morphological study, but no effect on pup weights were seen in the behavioral study. There were no drug-related teratological effects (including F_1 developmental milestones and reproductive performance in the behavioral study); increases in enlarged ureters and renal pelves and in sternal variations at HD in the morphological study were equivocal, and if real likely represent a slight developmental delay. (Dilated renal pelvis or ureter was not increased in the behavioral teratology studies.)

Two segment II studies were done in rabbits. Large decreases in food consumption were seen at 100 mg/kg and above and transient weight loss was seen at 150 mg/kg and above. (In a non-GLP rangefinding study, 300 mg/kg was lethal to 4 of 5 rabbits). The number of resorptions was increased at HD; smaller equivocal increases were seen at the lower doses. The incidence of cleft palate was increased in both studies, with an overall incidence of 9/103 fetuses (4/17 litters) at 200 mg/kg and 3/131 fetuses (2/17 litters) at 150 mg/kg. (None in other groups).

In the segment III study slight decreases in dam food consumption were seen at all doses and slight decreases in dam bodyweight compared to control were seen at MD and HD. Pup weights were decreased at MD and HD on day 21 PP (but not at birth or on day 2 PP); it is not clear how much drug the pups received via the dam's diet. (In the rat behavioral teratology studies, where dams received the drug by gavage days 7-16 of pregnancy, there was no drug effect on pup weights.) There were no drug effects on pup survival through weaning. After weaning, these F_1 pups received drug in diet until mating (99

days of age); convulsions were seen in 1 MD and most HD (beginning approx. 43 days of age); one MD and one HD died from undetermined causes. Food consumption was decreased in all groups but LD F; bodyweight, which was decreased at weaning at MD and HD as noted above, remained below control in these groups until mating. Results of this F_1 mating showed decreased numbers of CL, implants, and viable fetuses at HD (CL also slightly decreased at MD), but no drug effect on pre- or post-implantation loss. (A similar effect was seen in the segment I study as noted above). Histopathological exams on F_1 dams and sires showed brain vacuolation at all doses (although the incidence [and possibly also the severity in F] of this finding was inversely dose-related).

6. Special Toxicity Studies

a. Summary of Special Toxicity Studies

The following conclusions can be drawn from the series of investigative special toxicity studies:

Evoked potentials and MRI are good non-invasive techniques for detecting vigabatrin-induced intramyelinic edema. In the dog, a species susceptible to this pathological effect, an increase in the central transmission latency of the somatosensory evoked potential and the flash visual evoked potential has been observed in several different studies after about 4-8 weeks of treatment with 300 mg/kg/day of vigabatrin. These latency increases correlate with the presence of intramyelinic edema (IME). As with the pathological change, these latency increases reversed when vigabatrin treatment was stopped. The MRI data indicate that this imaging technique was also capable of detecting the IME induced by vigabatrin and appeared to be equally sensitive to EP studies.

Retinal changes associated with vigabatrin treatment have occurred only in non-pigmented (albino) rats. The observed lesion is similar to that seen in albino animals exposed to light. Similar effects have not occurred in any of the pigmented species studied to date (dog, monkey, or pigmented rat).

Supplementing the diet with pyridoxine, a co-enzyme for GABA transaminase, did not appear to protect rats from the toxic effects of repeated administration of vigabatrin.

In the rat, 300 mg/kg/day vigabatrin for 3 months produced a reduction in body weight, brain weight, and the synthesis of soluble proteins as determined by isoelectric focusing, but no effects were seen at 50 mg/kg/day. Similar changes have not been observed in CSF of epilepsy patients treated with 50 mg/kg/day vigabatrin for up to 24 months.

Individual vigabatrin-induced intramyelinic edema vacuoles in rat brain ranged from 0.9μ-17μm (mean 4.7μ) with coalesced vacuoles ranging from 6.2μM -24.5μM (mean 12.7μM). Special immunocytochemistry stains for glial fibrillary acidic protein (GFAP) have demonstrated reactive astrocytes in close association with the microvacuoles of rat and dog. These GFAP changes persist after the vacuolation disappears, and may, therefore, be useful in differentiating fixation-artifact vacuolation from vigabatrin-induced vacuolation in human brain tissue.

b. Table of Special Toxicity Studies

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b(4)

Table 5-35 (Page 1 of 2), Special Toxicity Studies

Duration	Species/Strain	Number of Animals	Dose (mg/kg) and Drug Batch No.	Route of Administration	Laboratory	Reference Number
6 Months	Rat/Sprague-Dawley	32/Group (Males)	50, 100, 300 C36680 & C37708	Oral (Gavage)	Marion Merrell Dow Inc., Cincinnati	238 on page 5-5989, v1.28
Variable	Rat/Sprague-Dawley Rat/Lister-Hooded Dog/Beagle Monkey/Cynomolgus	Variable	Variable	Oral	Outside Investigator sponsored by: Marion Merrell Dow Inc., Winnersh [redacted]	239 on page 5-6022, v1.28
12 Weeks Plus 16-Week Recovery	Dog/Beagle	2/Sex/Group/Wk	300 C41832	Oral (Capsule)	Marion Merrell Dow Inc., Cincinnati	240 on page 5-6060, v1.28
6 Months	Rat/MRC Hooded	4/Sex	3 g/L (average daily dose = 312 mg/kg)	Oral (Drinking Water)	Publication/Outside Investigator	241 on page 5-6430, v1.28
3 Months	Rat/Lister-Hooded Rat/Sprague-Dawley	15 Males/Group 15 Males/Group	300 30, 100, 300	Oral (Half dietary, half gavage) Oral (Half dietary, half gavage)	[redacted]	243 on page 5-6453, v1.29
4 Months	Rat/Sprague-Dawley	10/Sex/Group	100, 300 C33672 & C33977	Oral (Diet)	Marion Merrell Dow Inc., Cincinnati	244 on page 5-6588, v1.29
12 Weeks Plus 17-Week Recovery	Dog/Beagle	8 Males	300 C37708	Oral (Capsule)	Albert Einstein College of Medicine	245 on page 5-6631, v1.29
12 Weeks Plus 8-Week Recovery	Dog/Beagle	4 Males	300 C41835	Oral (Capsule)	The Dow Chemical Company, Midland MI	246 on page 5-6683, v1.29
15 Weeks Plus 12 Weeks Recovery	Dog/Beagle	12 Males	300 C37709	Oral (Capsule)	Albert Einstein College of Medicine	247 on page 5-6722, v1.29
7 Weeks	Dog/Beagle	7 Males	300 C41832	Oral (Capsule)	Albert Einstein College of Medicine	248 on page 5-6817, v1.29

C. Toxicology
6. Special Toxicity Studies

b(4)

Table 5-35 (Page 2 of 2). Special Toxicity Studies

Duration	Species/Strain	Number of Animals	Dose (mg/kg) and Drug Batch No.	Route of Administration	Laboratory	Reference Number
3 Months	Dog/Beagle	2 Males	300	Oral (Capsule)	Albert Einstein College of Medicine	249 on page 5-6845, v1.29
12 Weeks	Dog/Beagle	2/Sex/Group/Wk	300	Oral (Capsule)		250 on page 5-6862, v1.29
2 Months	Rat/Sprague-Dawley	6 Males	250	Not given in abstract, but presumed oral		251 on page 5-6904, v1.29
3 Months	Sprague-Dawley	6 Males/Group	50, 300 every other day	Oral (Liquid Diet)		252 on page 5-6908, v1.29, 253 on page 5-6912, v1.29
2 Weeks	Rat	N/A	500 nmol Vigabatrin 1000 nmol GABA	*In Vitro* study		254 on page 5-6915, v1.29

c. Individual Special Toxicity Study Report Summaries

i. Sequential Neuropathology Study with Vigabatrin in Dogs

(240 on page 5-6060, v1.28)

This neuropathology study was conducted to obtain detailed information on the time course of the onset and recovery of vigabatrin-induced brain microvacuolation (intramyelinic edema) in the Beagle dog. This data was needed to determine the significance of evoked potential and magnetic resonance imaging data generated in the dog.

Four treated (2/sex) and two control (1/sex) dogs were necropsied weekly during a 12-week treatment period and also 1, 2, 4, 8, 12, and 16 weeks after treatment was stopped. Vigabatrin was administered orally by gelatin capsule in single dose of 300 mg/kg/day. A detailed neurohistopathologic examination was conducted in a blinded fashion by two different pathologists. Four different coronal brain sections were examined on each dog as well as spinal cord (lumbar), sciatic nerve, eyes (retina and optic nerve) and several ganglia (dorsal root, stellate, caudal cervical and celiac). A standard set of hematology and clinical chemistry tests were conducted pretest and terminally. Samples of terminal plasma, cerebrospinal fluid and brain tissue were collected and analyzed for vigabatrin levels, vigabatrin and GABA levels and GABA concentration, and GABA-T and GAP activities, respectively. In addition, an *ex vivo* T_2-weighted MRI evaluation was obtained on the brain of each dog.

During the first four weeks a number of treated dogs became anorexic and lost weight and three either died or had to be sacrificed. The anorexia and associated signs regressed after supplementation with canned dog food and adaptation to the drug. Lowered erythrocytic values were detected in 5 treated dogs after 5 weeks of treatment. Cholesterol levels were also elevated in 50% of the treated dogs terminated during the first 3 weeks, and was possibly related to the anorexia and dietary changes. Serum glutamic-pyruvic transaminase values were also somewhat reduced during the treatment period.

Histopathologically vigabatrin-treated dogs had varying degrees of brain vacuolation, which was reversible upon cessation of dosing. The most sensitive areas included the thalamus, columns of the fornix and the hypothalamus, all of which showed notable effects after about 4 weeks and reached their maximum effects after 8-12 weeks of treatment and returned to background levels within 12-16 weeks of cessation of treatment.

Vigabatrin concentrations (24-hour post-dose) in the cerebrospinal fluid (CSF) reached steady state within one week, and were higher than comparable plasma concentrations, reflecting slower clearance. Total GABA concentrations (free and conjugated) also reached steady state within one week. Both vigabatrin and GABA concentrations in the CSF were unusually high during the first three weeks of the study, but then appeared to stabilize, suggesting some adaptation. They returned to background within one week after dosing was stopped. Brain GABA levels tended to parallel CSF GABA levels. Brain GAD and GABA-T activity were below control values during the treatment period.

In conclusion, brain microvacuolation induced by vigabatrin (300 mg/kg/day) in Beagle dogs was first noticeable after about 4 weeks of treatment, and reached maximum levels after 8-12 weeks. The microvacuolation showed noticeable regression within 1-2 weeks after treatment was stopped and had returned to background within 12-16 weeks.

ii. The Use of Non-invasive Techniques for Detecting Intramyelinic Edema in Animals

Studies have been carried out in dogs in an attempt to find non-invasive techniques for detecting vigabatrin neurotoxicity which could be used in clinical trials. Two techniques have been investigated: magnetic resonance imaging (MRI) and evoked potentials (visual, auditory, and somatosensory). An increase in the latency of somatosensory and visual evoked potentials has been correlated with the occurrence of brain microvacuolation (intramyelinic edema) in several different studies. In addition MRI studies have also shown changes which are correlated with the intramyelinic edema. Therefore both techniques appear to be useful for clinical monitoring.

iii. Magnetic Resonance Imaging (MRI) Studies in Dogs Treated with Vigabatrin

To date, several studies have been conducted evaluating the ability of MRI to detect neuroanatomical changes produced by vigabatrin (247 on page 5-6722, v1.29, 248 on page 5-6817, v1.29, 249 on page 5-6845, v1.29, 250 on page 5-6862, v1.29, 251 on page 5-6904, v1.29). In the first investigation (249 on page 5-6845, v1.29), two male Beagle dogs from the initial evoked potential study (245 on page 5-6631, v1.29) were used, with each animal serving as its own control. MRI scans (T_1 and T_2 weighted images) were taken prior to the initiation of vigabatrin administration and again after three months of oral treatment with 300 mg/kg/day of vigabatrin. In both dogs, there were no differences between the baseline scans and the scans done after three months of drug treatment. The dogs were necropsied at the end of the treatment period, and their brains were examined by light and electron microscopy. Both dogs showed extensive microvacuolation. Thus, the MRI procedure used in this study was not capable of detecting pathological changes in dog brain resulting from three months treatment with vigabatrin.

More recently, the ability of MRI to detect neuropathological changes induced by vigabatrin in male Beagle dogs was re-evaluated (247 on page 5-6722, v1.29). This was done due to the number of advances that had occurred in MRI technology since the original investigation discussed above. In this study, in which evoked potentials were also evaluated (247 on page 5-6722, v1.29), MRIs (both *in vivo* and *ex vivo*) were obtained for each dog (8 treated, 4 control) at baseline, after 15 weeks of dosing with vigabatrin (300 mg/kg/day), and repeated in 3 treated and 2 control dogs 5 and 12 weeks after discontinuation of dosing. MRI was performed at 1.5 Tesla on a GE Signa magnet, using the GE extremity coil. Sagittal and coronal images were obtained with T_1 and T_2 weighting. After dosing week 15, all treated dogs showed increased T_2 and decreased T_1 weighted signal, prominent in and surrounding the columns of the fornix, and less obvious in discrete areas extending throughout the thalamus and hypothalamus. As noted above, these regions are known to show microvacuolation in dogs dosed chronically with vigabatrin and also showed microvacuolation in this study. Control dog MRIs were unremarkable. Signal abnormalities improved markedly after vigabatrin withdrawal.

In an additional *ex vivo* study (250 on page 5-6862, v1.29) done in conjunction with the sequential neuropathology study in dogs (240 on page 5-6060, v1.28), Beagles were administered vigabatrin orally at a dose of 300 mg/kg/day. Animals were sacrificed and the formalin-fixed brains examined at weekly intervals during the 12 weeks of treatment and at 1, 2, 4, 8, 12 and 16 weeks after discontinuation of drug treatment. Myelin microvacuolation in the thalamus, hypothalamus and fornix were noted histologically after 4 weeks of treatment. *Ex vivo* increases in MRI T_2 intensity were observed in the hypothalamus after week 4 and in the thalamus and columns of the fornix after 7 weeks. MRI T_2 intensity and microvacuolation continued to increase during the 12 weeks of vigabatrin treatment. When vigabatrin treatment was stopped after week 12, both MRI T_2 intensity and microvacuolation began to decrease. Sixteen weeks after discontinuation of vigabatrin, MRI examination demonstrated a near complete reversal of microvacuolation.

Thus, as a result of advances in MRI technology, this technique now appears to be capable of detecting intramyelinic edema produced by vigabatrin in dogs and should be a valuable non-invasive techinque for clinical monitoring.

iv. Evoked Potential Studies in Dogs Treated with Vigabatrin

To date, four separate electrophysiological studies have been completed in dogs dosed repeatedly with vigabatrin. In the first investigation (245 on page 5-6631, v1.29), eight male Beagle dogs were treated orally with vigabatrin at a dose of 300 mg/kg/day for 12 weeks; two dogs treated with placebo capsules served as controls. At the end of 12 weeks of treatment, two drug-treated animals were sacrificed for histological examination. The remaining animals were allowed to recover for an additional 17 weeks, at which time they were sacrificed for histological examination. Electrophysiological data (flash visual, auditory, and somatosensory evoked potentials) were obtained weekly during the baseline and the drug treatment phase, and every two weeks during the recovery phase. During all test sessions, animals were tranquilized using a combination of ketamine (25 mg/kg) and xylazine (22 mg/kg). One treated dog died within 2.5 weeks of initiation of drug treatment and was excluded from the study. The dogs were also evaluated by MRI both pretest and just prior to termination.

A summary of the results for each of the types of evoked potentials tested in this study is given below:

a) <u>Brainstem Auditory Evoked Potential (AEP)</u>

AEPs were recorded to binaural, high intensity (80 db/SPL), 100 μsec compression clicks delivered at a rate of 1/sec. The recording montage consisted of vertex to right mastoid and vertex to mid-inion configurations. End points assessed included: 1) peak latency of wave I (measured in mastoid to mastoid data); 2) peak latency of wave V (measured in vertex to mastoid data); and 3) total central transmission (i.e., inter-peak latency between waves I and V).

AEP data failed to demonstrate a consistent alteration in peripheral or central transmission latency throughout the course of the study. In the Beagle, peripheral auditory transmission is approximately 1.2 msec, while the central delay (V-I) is approximately 2.5 msec. These are

extremely rapid responses that reflect activity in the VIII cranial nerve as well as the brainstem auditory pathways. The AEP waveform was well recorded in the Beagle and the data were generally within 10% of the baseline for all dogs across all weeks of the study. There was also no AEP amplitude effect associated with vigabatrin treatment.

b) Somatosensory Evoked Potential (SEP)

SEPs were recorded to 100 μsec constant current electrical square waves delivered to the right forelimb (median nerve) at a rate of 1/sec. Stimulating electrodes were positioned with the anode 1.0 cm distal to the cathode, and current was adjusted to supra-maximal levels (between 3.0 and 6.0 mA). Positioning of the stimulating electrodes was facilitated by observing a twitch of the distal paw extensors. Recording electrodes were positioned overlying the cervical spinal cord and the contralateral sensorimotor region. End points assessed included: 1) peak latency of response at the cervical spinal cord; 2) onset and peak latency of the initial cortical component; and 3) total central transmission time (defined as the difference between the latency overlying the cervical cord and the onset latency of the initial cortical negativity).

A significant increase in the somatosensory central transmission latency was associated with vigabatrin treatment (Figure 5-18 on page 5-200, v1.12). This effect began between the fifth and seventh week of drug administration. The prolongation in latency relative to baseline ranged from 22 to 55% in drug treated dogs, while in control animals most values were within 5% of the baseline mean. The prolongation of SEP latency began to reverse within two weeks after treatment was stopped. By the end of the eleventh week of the recovery period, the SEP latencies had returned to baseline values. Histological examination showed that after 12 weeks of treatment with vigabatrin, there was extensive microvacuolation in the brain. The microvacuolation showed a marked degree of recovery after 17 weeks off treatment. The intramyelinic edema in these dogs was also characterized by electron microscopy.

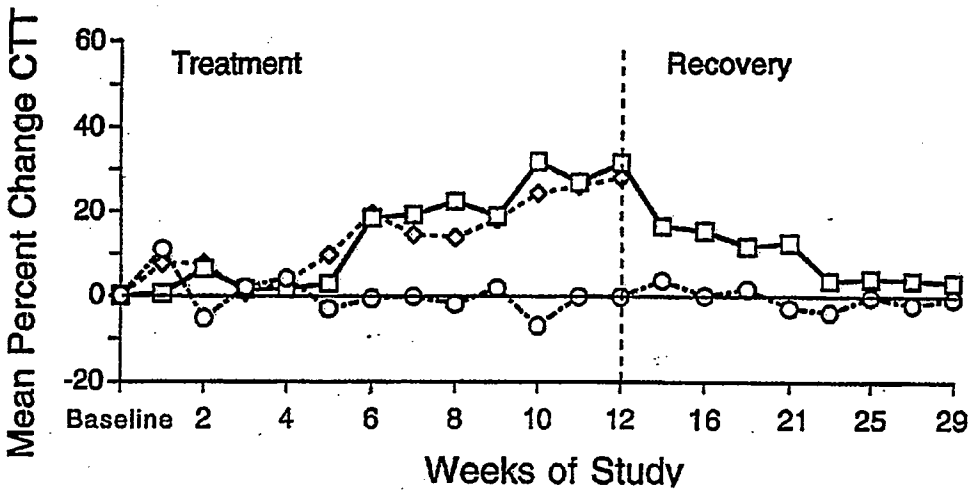


Figure 5-18. Effect of vigabatrin on somatosensory evoked potential central transmission time (C.T.T.) in male Beagle dogs. Animals received either placebo or vigabatrin 300 mg/kg/day PO for 12 weeks. At the end of the treatment period, two dogs given vigabatrin were sacrificed, and their brains examined for the presence of microvacuolation. The remaining dogs were allowed to recover for 17 weeks prior to sacrifice. Somatosensory evoked potentials were determined weekly during the treatment phase of the study, and every two weeks during the recovery phase of the study.

◇ Dogs treated with vigabatrin for 12 weeks and sacrificed at the end of the dosing period (n=2).

□ Dogs treated with vigabatrin for 12 weeks and allowed to recover for 17 weeks prior to sacrifice (n=5).

o Control animals (n=2).

c) Flash Visual Evoked Potential (FEP)

Short latency FEPs were recorded to binocular high intensity stroboscopic visual stimuli delivered at a rate of 1/second. Recording electrodes were placed at empirically determined optimal locations over the left and right visual cortices. The electroretinogram (ERG) was recorded from surface electrodes positioned approximately 45 degrees above the inner canthus of each eye. End points assessed included: 1) onset and peak latency of the initial cortical negativity; 2) the latency of the B wave of the ERG; 3) total central delay (defined as the difference in latency between the onset of the B wave of the ERG and the initial cortical activity); and 4) the amplitude of the initial cortical negativity.

Short latency FEP data proved to be extremely difficult to record in these unanesthetized Beagle dogs. The "A wave" of the ERG, which reflects receptor depolarization, was broad in needle

surface recordings, and it often obscured the onset of the "B wave", which reflects activity in the optic nerve. Consequently, central transmission latencies were unobtainable, and only the onset of the initial cortical response (approximately 17.5 msec) was measured. The cortical activity was also variable in waveform and difficult to score in the Beagle. No consistent alterations in the amplitude or latency of FEP activity were found. However, this negative finding appears to have been related principally to technical difficulties with the FEP methodology used in this study.

As noted above, FEP data proved to be extremely difficult to record in this initial electrophysiological study investigating the effects of daily administration of vigabatrin to dogs. However, since microvacuolation produced by vigabatrin is consistently apparent in the optic tract of the dog, several additional studies were performed with the goals of determining whether it was possible to reliably measure FEPs in the dog and, if so, to study the effect of chronic administration of vigabatrin. The next investigation (246 on page 5-6683, v1.29) was a preliminary study in which six male Beagle dogs were used. Four were dosed orally with vigabatrin 300 mg/kg/day. The other two dogs were treated orally with placebo capsules, and served as controls. FEPs were measured every two weeks during the 12 week treatment phase and 12 week recovery phase of this study. Results from this preliminary study showed that it is possible to reliably measure FEPs in the dog. In addition, it appeared that vigabatrin produced a significant slowing of the late components (waveforms with a latency of $\geq$75 msec) of the FEP; early components of the FEP were not affected by drug treatment. This slowing of the late components of the FEP was apparent by treatment week 12 and was found to be progressive throughout the waveform. Due to experimental difficulties prior to the 12th week of treatment, the time of onset of late latency FEP slowing could not be determined. Posttreatment waveforms in dogs given vigabatrin gradually accelerated after 2, 4 and 6 weeks of recovery; no differences were noted in FEP latencies in treated dogs between 6 and 8 weeks of recovery. FEP peak latencies of treated dogs were similar to those in the two control dogs after 6 weeks of recovery. This pattern of slowing, combined with an inability to detect a change in early components of the FEP, suggests a drug-induced effect that is distributed at multiple points along the visual pathway rather than an effect focused at the optic tract or other discrete locations. Alternatively, this pattern of slowing could also be due to increased GABA inhibiting cortical processing (277 on page 5-7765, v1.31, 278 on page 5-7775, v1.31). No evidence was obtained in this study for vigabatrin having an effect on the shape of the FEP. Due to intra- and inter-animal variability, it was not possible to determine if vigabatrin had an effect on the ERG in this study.

More recently, another study was done with the goals of confirming the earlier SEP findings with vigabatrin and to investigate again whether the compound (when given chronically) has any effect on the short latency FEP (247 on page 5-6722, v1.29). Prior to the initiation of this study the FEP techniques were improved and evaluated in several additional dogs. For this study, seven Beagle dogs received vigabatrin 300 mg/kg/day for 15 weeks; (one other dog that became anorexic and died in the first few weeks was excluded) 4 additional dogs were given placebo and served as controls. SEPs and short latency FEPs were recorded at baseline and every 2 weeks throughout treatment and recovery. Dogs given vigabatrin showed an increase in central latencies beginning at 6 weeks, attaining significance ($p < 0.05$) at 8 and 10 weeks for the SEP and FEP, respectively. No changes occurred in peripheral or spinal conduction in treated dogs, or in any measure in control dogs. Four treated and 2 control dogs were necropsied after 15 weeks of treatment, while 3 vigabatrin-treated and two control dogs were followed for an additional 12 weeks during the recovery period. Both electrophysiological measures returned to baseline values within

5 weeks. Thus, in this study the SEP and the short latency FEP were delayed by subacute administration of vigabatrin, and returned to baseline within 5 weeks following drug discontinuation.

v. *Histopathologic and MRI Correlates of Initial Electrophysiologic Changes*

An additional evoked potential study (248 on page 5-6817, v1.29) was conducted in male Beagle dogs to determine the status of the brain histopathology and MRI at the time when the initial delay in evoked potential transmission time was observed. Two controls were used while 7 treated dogs were given vigabatrin (300 mg/kg/day single oral doses) daily until the weekly SEP central transmission time showed an increase greater than 0.5 msec and/or the flash VEP showed a 1.1 msec increase in absolute cortical onset latency. As soon as possible thereafter a brain MRI was conducted and the dog necropsied for neurohistopathologic evaluation. A control dog was terminated at the same time as the first and last treated dog. Initial evoked potential changes were noted after the 4th through 7th weeks (5 showing changes after 4 or 5 weeks). Two dogs were identified by SEP and 5 by VEP. *In vivo* MRI's and histopathology were both positive at the time of the initial evoked potential changes. These findings indicate that intramyelinic edema must be present for alteration of evoked potentials or MRI, which appear to be about equally sensitive to these changes.

The timing of these effects on evoked potential latency and their reversibility suggests that it is correlated with the histopathological lesion produced by long-term administration of vigabatrin. Thus, SEP and VEP latency appears to be a useful non-invasive technique for monitoring both the formation and the disappearance of microvacuolation induced by vigabatrin. These histopathologic alterations also correlate with MRI changes, which are as sensitive as electrophysiologic techniques.

vi. *Retinal Studies*

As noted above, in studies with albino rats conducted at [redacted] (220 on page 5-3499, v1.21), retinal degeneration was noted microscopically in animals given large doses of either vigabatrin or its pharmacologically active S-enantiomer, administered by gavage. By contrast, from a much larger series of subacute and chronic dietary studies in dogs, monkeys, and albino rats conducted at [redacted] definitive evidence of drug-related retinopathy was not apparent (see 5.C.6.vi.a. Review of [redacted] Studies on page 5-203, v1.12). b(4)

In attempting to reconcile these disparate findings, a comprehensive histologic review was made by [redacted] staff pathologists of the eyes of all animals entered in these studies, as well as a representative series obtained from the [redacted] study. In addition, studies were initiated by the [redacted] in which the compound was administered via the diet and by gavage to both albino and pigmented rats. The rationale for this test design was 1) to establish whether different modes of drug administration (gavage vs. diet) might influence the occurrence of retinal changes, and 2) equally important, to determine whether routine exposure of control and treated albino and pigmented rats to identical lighting and other laboratory conditions would reveal any differences regarding retinal changes. b(4)

a) Review of [redacted] Studies (215 on page 5-3020, v1.20, 217 on page 5-3123, v1.20, 218 on page 5-3173, v1.20, 219 on page 5-3181, v1.21, 221 on page 5-3571, v1.22, 223 on page 5-3693, v1.22, 225 on page 5-3759, v1.22, 226 on page 5-3874, v1.22, 228 on page 5-4010, v1.23, 229 on page 5-4101, v1.23, 230 on page 5-4233, v1.23, 231 on page 5-4245, v1.23, 232 on page 5-4476, v1.24, 233 on page 5-4756, v1.24, 244 on page 5-6588, v1.29). b(4)

An in-depth histologic review of the eyes of all animals from [redacted] studies with vigabatrin revealed no retinal degenerations in dogs receiving doses up to 200-300 mg/kg/day for 3-12 months or monkeys given 300 mg/kg/day for 16 months or 50 or 100 mg/kg/day for six years. In addition, no retinal degeneration was noted in pigmented Long-Evans rats given the drug in the diet at dosages of 150 mg/kg/day or less for 2 years (carcinogenicity study). Among albino rats treated for 3-12 months at doses up to 300 mg/kg/day via the diet, retinal degeneration was only occasionally noted, and without evidence of a dose-relationship.

b) [redacted] Study (243 on page 5-6453, v1.29)

In this study, both Lister-Hooded (pigmented) and Sprague-Dawley (albino) rats were used. Albino rats were given doses of 0 (control), 30, 100, and 300 mg/kg/day of vigabatrin, either by gavage or via the diet, for a period of three months. Pigmented rats were similarly administered 0 (control) and 300 mg/kg/day. At termination of the study, the eyes from all animals were fixed in Davidson's solution and prepared for histologic evaluation. b(4)

As shown in Table 5-36 on page 5-204, v1.12, retinal lesions were not found in any of the pigmented rats, whereas alterations similar to those found in the [redacted] study occurred in the albino rats. The incidence and severity of these changes were dose-related, and occurred to a considerably greater extent in the groups treated by gavage rather than via the diet.

Table 5-36. Comparative Incidence of Retinal Degeneration in Pigmented and Albino Male Rats Given Vigabatrin by Different Routes for 3 Months. (Data from Reference 243 on page 5-6453, v1.29)

Dose Group (mg/kg/day)	Route of Administration	
	Dietary[a]	Gavage[a]
Pigmented		
0 (Control)	0/15	0/15
300	0/15	0/15
Albino		
0 (Control)	0/15	0/15
30	0/15	1/15
100	2/15	8/15
300	10/15	14/15

[a] Data are expressed as number of animals with retinal changes/number of animals examined.

In the wide array of experiments conducted to date, retinal degeneration associated with vigabatrin treatment has consistently occurred only in nonpigmented (albino) rats. Similar effects have not occurred in any pigmented species (dog [one-year], monkey [six-year], Lister-Hooded rat [3-month], or Long-Evans rat [2-year]). Retinal degeneration was also rare and not dose related in [redacted] studies in albino rats, including the one-year chronic study with doses up to 300 mg/kg/day. Since the only differences in the albino rat studies [redacted] and [redacted] appear to be associated with room light and/or caging (clear plastic vs. stainless steel), it appears that vigabatrin somehow increases light exposure or sensitivity of the already overly-sensitive albino retina (274 on page 5-7693, v1.31, 275 on page 5-7711, v1.31, 276 on page 5-7736, v1.31). These studies clearly demonstrate that pigmentation of ocular structures protects the retina from the degeneration associated with vigabatrin administration in albino rats. Such findings are quite consistent with the absence of ophthalmologic pathology reported to date in vigabatrin-treated patients. b(4)

vii. Effect of Pyridoxine on Vigabatrin Toxicity

A total of 60 male and 60 female CD [redacted] rats were divided into 6 groups and given vigabatrin and/or pyridoxine hydrochloride in the diet for 4 months as illustrated in Table 5-37 on page 5-205, v1.12. b(4)

Table 5-37. Schedule for PO Dosing of Pyridoxine and Vigabatrin in Male and Female CD Rats for 4 Months. (Data from Reference 244 on page 5-6588, v1.29).

	Dosing Schedule					
Treatment Groups	**1**	**2**	**3**	**4**	**5**	**6**
Vigabatrin (mg/kg/day)	0	0	100	300	100	300
Pyridoxine HCl (mg/kg/day)	0	300	100	300	0	0

The principal clinical sign observed in these studies was convulsions in rats receiving 300 mg/kg/day of vigabatrin (Groups 4 and 6) after three months of treatment. Slightly fewer convulsions were observed in rats given pyridoxine; the difference did not suggest any protection against development of convulsions by the vitamin. Animals treated with vigabatrin occasionally experienced alopecia. Administration of pyridoxine alone caused little toxicity.

Decreased body weight gain and food consumption were also observed in animals given 100 and 300 mg/kg/day vigabatrin. Supplementation with pyridoxine resulted in little improvement in body weight gain.

Microscopic observations of the brain and eyes revealed microvacuolation (intramyelinic edema) in the brains of rats treated with either 100 mg/kg/day (Group 5) or 300 mg/kg/day (Group 6) of vigabatrin. As with previous studies, the microvacuolation was located primarily around the roof nuclei and in the folia of the cerebellum as well as to a lesser extent in such diverse structures as the reticular formation, thalamus, hippocampus, optic tract and anterior commissure. Results of this study further demonstrate that supplementation with doses as high as 300 mg/kg/day of pyridoxine had no apparent effect on the development, incidence, distribution or degree of severity of the microvacuolation in the brains of rats treated with vigabatrin at doses up to 300 mg/kg/day.

Histopathologic examination also revealed a low incidence of retinal degeneration in these albino rats (Table 5-38 on page 5-206, v1.12). The exact significance of this finding is not obvious, but pyridoxine treatment did not prevent this effect and pyridoxine alone did not produce any brain or retinal lesions.

In a previously reported study at [redacted] 241 on page 5-6430, v1.29), pyridoxine 10/mg/kg/day was administered in conjunction with 300/mg/kg/day of the vigabatrin racemate. In that study neither the retinal degeneration nor the intramyelinic edema were prevented by the administration of 10 mg/kg/day of pyridoxine in conjunction with 300 mg/kg/day of the vigabatrin racemate. b(4)

Table 5-38. Incidence of Retinal Degeneration in CD Rats After PO Administration of Vigabatrin and/or Pyridoxine for 4 Months. (Data from Reference 244 on page 5-6588, v1.29)

Dosing Schedule						
Group Number	**1**	**2**	**3**	**4**	**5**	**6**
Treatment (mg/kg/day)	**Control**	**300 PHCl**	**100 VGB 100 PHCl**	**300 VGB 300 PHCl**	**100 VGB**	**300 VGB**
Male	0	0	1	0	0	0
Female	0	0	0	2	0	2

Male and female CD rats (10/sex/group) were given vigabatrin and/or pyridoxine hydrochloride in the diet for 4 months.

VGB Vigabatrin

PHCl Pyridoxine hydrochloride

viii. Electron Microscopic Evaluation of Brain and Peripheral Nerves

(238 on page 5-5989, v1.28)

Rats from a 6-month pharmacokinetic study (Reference numbers 289 on page 5-8170, v1.33 and 290 on page 5-8189, v1.33) were used to examine by transmission electron microscopy (TEM) perfusion-fixed tissues, and to measure the size of vacuoles resulting from this treatment. Cerebellum (2 rats) and sural and tibial nerves (2 rats) were examined from rats treated with 300 mg/kg/day for 6 months.

TEM confirmed previous light microscopic results, *i.e.*, that vacuoles in the cerebellar roof nuclei area were due to splitting of the myelin sheath at the intraperiod line. Diameter of individual vacuoles ranged from 0.9 μm - 17.0 μm (mean 4.7 μm) and coalesced vacuoles ranged from 6.2 μm - 24.5 μm (mean 12.7 μm). Examination of sural and tibial nerves did not reveal signs of intramyelinic edema.

ix. Special Immunohistopathological Investigation of Vigabatrin-Induced Intramyelinic Edema in Animals

(239 on page 5-6022, v1.28)

In several animal species chronic treatment with vigabatrin gave rise to a reversible non-progressive intramyelinic edema in specific white matter tracts of the brain. The pathogenesis of the lesion remains unknown although the lesions are similar in appearance to neurotoxicological changes induced by triethyltin and hexachlorophene. Because of this effect in animals, an extensive program has been carried out to assure safety during clinical trials. Evoked potential monitoring as well as magnetic resonance imaging have been shown to be effective non-invasive techniques for demonstrating the presence of this lesion in dogs, and have been used clinically. Another important means of determining whether vigabatrin-induced intramyelinic edema occurs in man has been the histopathologic examination of human post-mortem and surgical brain speci-

mens. However, because of occasional post-mortem fixation artifacts, questions concerning the presence of brain microvacuolation (intramyelinic edema) may be difficult to evaluate. For this reason special immunocytochemical staining (immunoperoxidase method) has been used to study the tissue responses associated with the myelin vacuolation in animals. Using antibody stains to glial fibrillary acidic protein (GFAP), reactive astrocytes with increased glial fibers have been shown to occur in association with the focal microvacuolation present in treated rats and dogs. Limited study of monkey tissues was inconclusive. This reactive glial change is not easily recognized using conventional histologic stains. It was found that these GFAP changes in rats and dogs persisted, even after the myelin vacuolation disappeared when treatment was stopped. Thus, this GFAP immunohistochemical stain can be used on human post-mortem material to differentiate fixation artifact vacuolation from drug-induced intramyelinic edema. The results of the human post-mortem and surgical histopathologic evaluations are reported in the clinical section, but have not revealed any evidence of intramyelinic edema in man.

x. Comparative Study of Vigabatrin and Ethanolamine-O-Sulphate

(241 on page 5-6430, v1.29)

This study compared the degree of brain microvacuolation produced by VGB to that produced by ethanolamine-O-sulphate (EOS), another inhibitor of GABA-aminotransferase.

Assessment by computerized image analysis revealed that both the number of vacuoles and the area occupied by them was twice as high in the VGB-treated rats. This was due to the fact that in VGB-treated rats the vacuoles extended into the white matter tracts between the cerebellar folia whereas in the EOS-treated rats it was confined to the roof nucleus.

xi. Effect of Vigabatrin on Soluble Brain Proteins in Rat

(252 on page 5-6906, v1.29, 253 on page 5-6912, v1.29)

Five groups of six male rats were treated for three months with vigabatrin (300 mg/kg once a day or 50 mg/kg every other day), sodium valproate (100 or 300 mg/kg/day) or vehicle. Parameters examined included body weight, brain weight, the assay of total soluble proteins over a pI range of 4.5 to 7.4 in the cerebral cortex, hippocampus, and cerebellum, and the pattern of total soluble proteins in the brain and CSF by isoelectric focusing and 2-dimensional electrophoresis.

Rats given 300 mg/kg/day vigabatrin showed a 30% reduction in body weight and a 6% reduction in brain weight compared to the other groups. The synthesis of total soluble proteins in the cerebral cortex, hippocampus, and cerebellum was reduced in rats treated with the higher dose of vigabatrin. These alterations were not considered to be due to nutritional factors, as the fluid diet of all groups was adjusted to that of the group with the lowest intake, however, the permanent reductions in body and brain weight raise questions as to the exact significance of these differences. Isoelectric focusing revealed changes in the pattern of brain and CSF soluble proteins in the 300 mg/kg/day vigabatrin group compared to controls and other treated groups.

Similar techniques have been applied to the CSF of patients treated with vigabatrin (50 mg/kg/day) for up to 24 months without any significant changes.

In summary, these studies indicate that, in the rat, repeated administration of a high dose of vigabatrin (300 mg/kg/day) is associated with changes in the pattern of brain and CSF soluble proteins. Similar changes have not been observed in the CSF of humans to date.

xii. MRI Study in Rats Treated with Vigabatrin

(251 on page 5-6904, v1.29)

This study was done to determine whether quantitative MRI could detect white matter vacuolation in rats.

MRI was shown to be a sensitive method to detect this lesion. In addition, VGB was shown to cause a general increase in T2 decay in the cortex and brainstem, which is not explained by the vacuolation.

xiii. Effects of Vigabatrin and GABA on Rat Cerebellar Cultures

(254 on page 5-6915, v1.29)

A study was done to evaluate the effects of high concentrations of vigabatrin and GABA on myelinated of rat central nervous system cultures. Cerebellar cortex and deep nuclei from newborn Wistar rats were cultured on collagen-coated cover slips and exposed for up to 14 days to 500 nmol/ml vigabatrin or 1000 nmol/ml GABA.

No differences between control and experimental cultures exposed for 5-6 days were observed by light microscopy and only slight differences were observed in cultures exposed for 14 days. Although no changes could be seen in living cultures, mild toxicity, characterized by more numerous irregularities in the diameters of myelinated fibers, were seen in Sudan black B-stained cells. No clear-cut differences could be seen between the two compounds although vigabatrin appeared to be slightly more toxic than GABA. Some degenerating myelinated fibers and astrocytic gliosis were seen by electron microscopy in both control and experimental cultures, but appeared to be milder in controls. No marked primary demyelination, and no obvious intramyelinic edema were seen. Neuronal cell bodies did not show any patent degeneration. It was concluded that the toxicity of vigabatrin and GABA was very mild in this sensitive test system.

Barry N. Rosloff, Ph.D.
January 20, 1987

155 Q

PHARMACOLOGIST REVIEW OF IND 17,213
SUBMISSION OF OCTOBER 9, 1987

SPONSOR: Merrell Dow Research Institute
2110 E. Galbraith Road
Cincinnati, Ohio

DRUG: gamma-vinyl GABA

CATEGORY: anticonvulsant

CONTENT OF SUBMISSION:

Results of evoked potential study in dogs.

SUMMARY:

The purpose of this study was to try to develop a method for noninvasively monitoring the occurrence of the intramyelenic edema in brain which has been shown to occur in 4 species (rat, mouse, dog, monkey) and is presumed to occur in humans. The study was performed by Dr. Joseph C. Arezzo in conjunction with Dr. Herbert Schaumberg at Albert Einstein College of Medicine. Nine beagles were used: 7 drug treated and 2 controls. Dose was 300 mg/kg/day, 7 days per week, in capsules. The dogs were treated for 12 weeks, at which time 2 drug-treated dogs were sacrificed for histological exam of the CNS. The remaining dogs (2 controls, 5 drug-treated) were kept drug free for an additional 17 weeks at which time they were sacrificed for histological exam. Evoked responses (visual, auditory, and somatosensory) were measured weekly during the 12 week treatment period and every 2 weeks during the 17 week recovery period.

Histological exam of the 2 treated dogs sacrificed at 12 weeks showed a picture similar to that seen previously in animals treated with this drug. Extensive vacuolation was seen in the fornix, septum, optic tract, hypothalamus, and hippocampal endplates; less extensive vacuolation occurred in midbrain, thalamus, and cerebral cortex. Some of these regions showed microglial proliferation. The cervical spinal cord was normal. After the 17 week recovery period no vacuolation was seen; however, dosed animals displayed a mild degree of hippocampal endplate microgliosis that was not seen in control dogs. Somatosensory evoked potential (SEP) data (see graphs on following pages) showed a significant increase in central transmission time due to drug; this effect began at weeks 5-6 for 4/7 treated dogs with all 7 affected by week 12. At 12 weeks, the mean prolongation of central latency relative to baseline was 30% with a range of 22-55%. The prolongation of SEP latency reversed during the drug-free recovery period, with return to baseline by 11 weeks. In contrast to the SEP, auditory evoked potentials failed to

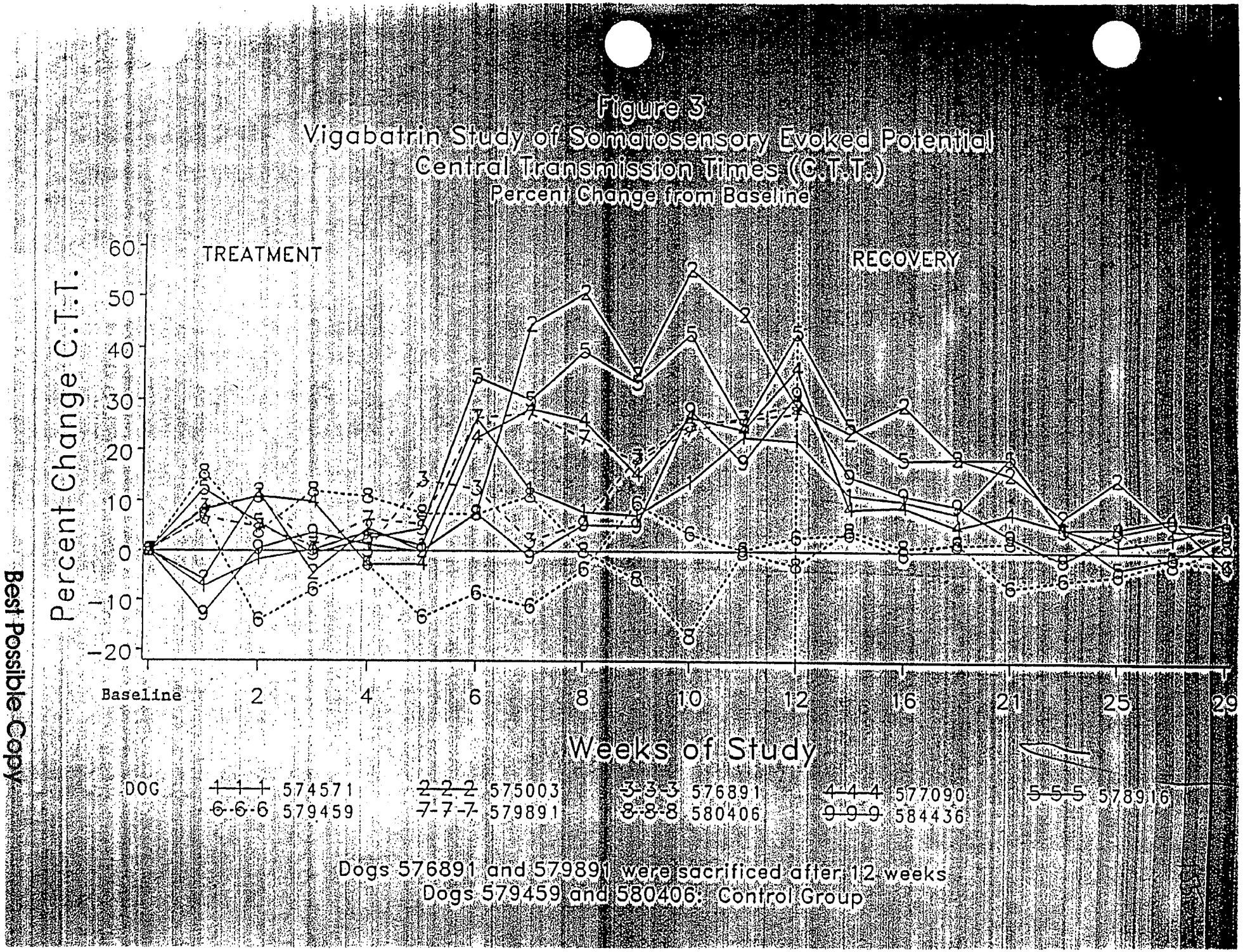
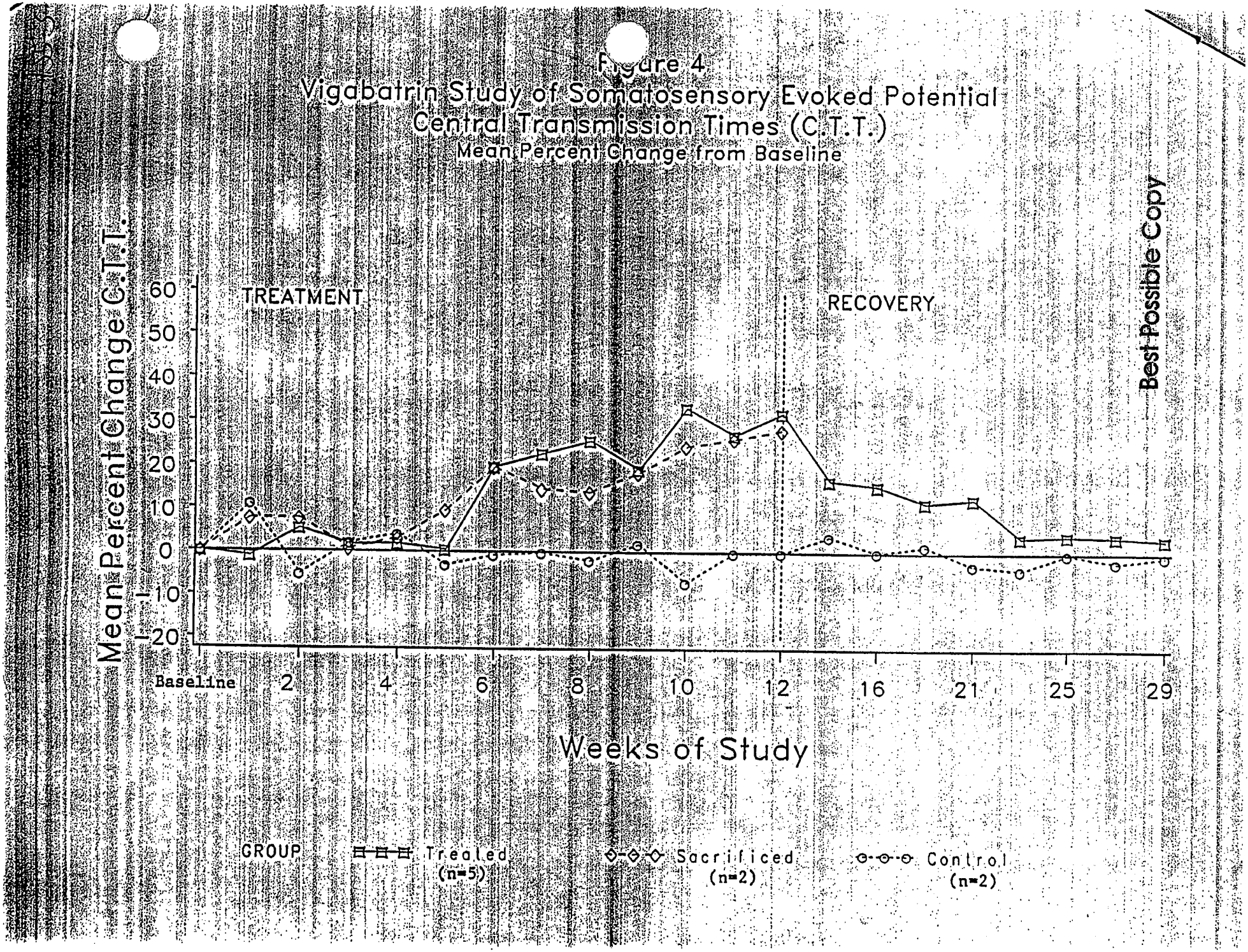


Figure 4

Vigabatrin Study of Somatosensory Evoked Potential
Central Transmission Times (C.T.T.)
Mean Percent Change from Baseline



demonstrate consistent alterations in peripheral or central transmission latencies throughout the study. Technical difficulties in obtaining visual evoked potential data in unanesthetized dogs precluded evaluation of this parameter.

EVALUATION:

The brain vacuolation produced by GVG appears to be potentially monitorable as shown by the SEP results obtained in this study. SEP changes were produced by a dosage regimen which caused vacuolation, and these changes disappeared during a time span over which the vacuoles also disappeared. However, it should be noted that the design of this study did not permit a precise correlation between the onset of SEP changes and the onset of vacuolation; e.g., the vacuolation may have been present (but not detected) prior to the onset of SEP changes. The SEP changes did begin to occur at a time when the vacuolation was reversible, which is an essential criterion for a useful monitoring procedure. However, an apparently non-reversible change occurred (hippocampal micogliosis in this study; also note that various other apparently nonreversible changes have been seen in previous animal studies) which did not correlate with the recovery of the SEP changes, and thus SEP was not able to monitor all brain changes produced by GVG. NB - maybe wouldn't have gotten irreversible changes if Tx had been stopped sooner (eg when SEP changes first seen

The importance of monitoring visual evoked potentials (VEP) in subjects receiving GVG has been previously noted, since the optic tract is prominently affected by GVG in animals. (Hexachlorophene, which produces a similar lesion, also prominently affects the optic tract in animals and in humans as well). The author of the report suggests that it is "reasonable to assume that in species which display robust and reliable VEPs (i.e., man, monkeys and rabbits) these data would provide a robust and sensitive index of [GVG] induced pathology." The use of monkeys to obtain VEP data was encouraged by the Neurology Advisory Committee (Meeting of October 18, 1985), where it was suggested that parenteral dosing be used to produce a more pronounced lesion (Lesions observed so far in monkeys have been limited to mild vacuolation in optic tract. It is not known if this represents an intrinsic resistance of monkeys to the toxic effects of GVG, or the fact that monkeys have not received adequate systemic exposure to the drug due to GI intolerance and/or poor absorption). It should be noted that hexachlorophene has been shown to alter VEP in monkeys (cited in Towfighi, Experimental and Clinical Neurotoxicology, ed. by Spencer and Schaumberg, p. 446, 1980).

In summary, it is still not possible to say that there exists a noninvasive method which can detect all of the brain pathology produced by GVG or which can detect the pathology at a time when it is still completely reversible. However, the SEP changes were shown to roughly correlate with the appearance and disappearance of vacuoles which are a prominent and early sign of GVG toxicity. As suggested by the authors of this study and by the Neurology Advisory Committee, a similar study should be done in monkeys or rabbits, using VEPs. Not only would this test the visual system, an area where GVG produced prominent lesions in all species studied, but it would also be a measure of the robustness of the method of evoked response measurement as a monitoring method for GVG across species.

It is also noted here that magnetic resonance imaging, done in 2 of the dogs treated for 3 months at 300 mg/kg/day, was not capable of detecting the brain lesion as seen with light microscopy.

RECOMMENDATIONS:

It is recommended that a study be performed in monkeys or rabbits to determine whether visual evoked potentials can be used to detect the appearance of GVG-induced brain lesions, especially at a time when these lesions might still be reversible.

Barry Rosloff

Barry N. Rosloff, Ph.D.

cc:
IND ORIG.
HFN-120
HFN-120/JContrera:1/20/87
HFN-120/BNRosloff:1/20/87
FT:1/28/87:dt:1/21/87:jgj
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EVALUATION:

Following This NDA is approvable. Suggestions regarding the proposed labelling are made in ~~a previous~~ the section of this review.

The following findings from the animal studies are of potential relevance to the human use of gamma-vinyl GABA (GVG):

A) Brain intramyelenic edema (IME).

The lesion is of concern because (1) it was produced in 3 (rat, mouse, dog) and possibly 4 (equivocal in monkey) species, (2) although the vacuoles are reversible after cessation of drug treatment, residual lesions, likely indicative of axonal damage, persist, (3) the incidence and distribution of the lesions appear to increase with increasing duration of administration, and in addition the lesions occur at lower doses which were previously unaffected by shorter term treatment, (4) doses producing the lesion in animals are within the range of those needed to increase brain GABA and produce anticonvulsant effects in animals and are within the human therapeutic range; also note that no-effect doses for producing the lesion in animals have not been established, and (5) similar lesions are produced by compounds such as hexachlorophene, which are known to produce neurotoxic effects in humans (although it is noted that unlike hexachlorophene, the GVG-induced lesion did not progress to severe central axonal destruction, and did not appear to involve peripheral nerves; however, note that peripheral nerve exams, especially using plastic fixation, were limited in the GVG studies; in addition, in the 2 year rat study a few rats were said to be unable to move their hind quarters).

Absorption of GVG after oral dosing in monkeys was low compared to that in rat and dog; it would be worthwhile to examine comparative drug exposure in these species as a possible explanation for the insensitivity of monkeys to GVG-induced intramyelenic edema. However, such a comparison encounters numerous difficulties, including (1) the number of animals evaluated was generally small, and mainly males, (2) plasma and CSF levels of drug were generally taken at only 1 or 2 time points after dosing, with these times not always the same across species, (3) it is not known which measure of exposure (e.g., C_{max}, C_{min}, AUC, duration above some threshold level, etc.) is most critical for production of the lesion, (4) in rats, all of the exposure data was obtained after gavage dosing whereas most of the data regarding the brain lesion, especially at lower doses, was obtained using dietary dosing, (5) in rats and dogs, exposure data was only obtained in studies of 4 month's duration or less, (6) drug protein binding was apparently not studied in animals (it did not occur in humans); any cross-species differences in binding could affect the interpretation of comparative plasma levels, and (7) it was shown in rats that after administration of racemic GVG, levels of the R isomer, which does not cause intramyelenic edema, were several fold lower than those of the S isomer in brain (despite being equal in plasma, although note that in humans plasma levels of R are greater than those of S); if this enantiomeric difference does not

occur or is quantitatively different in other species, it would make cross-species comparisons based on levels of racemic GVG in plasma, CSF, or brain problematic. Keeping these problems in mind, the data seem to indicate that although at the same mg/kg dose plasma levels in monkeys are less than those in rats and dogs, plasma levels in monkeys at the highest dose used in the chronic toxicity study (300 mg/kg) were at least as great as those in rats given 50 mg/kg by gavage; 30 mg/kg in diet caused intramyelenic edema in rats, with lower doses not tested. Although the dog data are somewhat more limited it appears likely that plasma levels in monkeys given 300 mg/kg would also be at least as great as those in dogs given 50 mg/kg, the lowest dose shown to cause intramyelenic edema (and, as in rat, a no-effect dose was not determined in dogs). Levels of GVG in brain of monkeys given 300 mg/kg/day were also in the same ballpark as those in brain of rats given 50 mg/kg, although it is not clear if the same brain area was assayed in both species. (Brain levels of GVG in dog were apparently not studied.) In CSF, in contrast to the situation in plasma and brain, levels of drug in monkeys were similar to or greater than those in rats given the same mg/kg dose. (CSF drug levels were much greater in dogs at the same mg/kg doses; even at the highest dose used in the monkey toxicity study [300 mg/kg] levels were below those in dogs given 50 mg/kg, which is the lowest dose shown to cause intramyelenic edema in that species [although note lower doses were not tested]). Thus, keeping in mind the caveats discussed earlier, there is some evidence that exposure to drug in monkeys, at least at the highest dose used in the chronic toxicity study, was at least as great as that occurring at lesion-associated doses in rats (again, assuming valid extrapolation between gavage and dietary dosing in rats), and thus the reason for the insensitivity of monkeys to GVG-induced intramyelenic edema is not necessarily due to inadequate exposure to drug. A possible reason for this insensitivity, in view of the evidence of the association of GVG-induced intramyelenic edema with the pharmacologic activity of the drug (i.e., GABA-T inhibition/GABA level elevation) (and the fact that since GVG is an irreversible inhibitor of GABA-T the levels of GVG may be less relevant than the extent of this pharmacological action in this case), are the observations that (1) despite CSF GVG levels at least as great as those in rats given the same doses, increases in CSF levels of GABA were only marginally and equivocally increased in monkeys, compared to increases of approximately 3-6x baseline in rats given the same doses (even greater increases in CSF GABA were seen in dogs, associated with relatively high levels of CSF GVG), and (2) levels of GABA in brain were not increased in monkeys, despite a modest inhibition of brain GABA-T; other studies indicate that at doses within the range of those used in the toxicity studies, GVG causes elevation of brain GABA in rats, mice, and dogs. It is thus tempting to speculate that the failure of GVG to sufficiently inhibit GABA-T and increase GABA levels in monkey brain, rather than inadequate exposure to drug per se, may be the reason for (or a marker for) the relative insensitivity of monkeys to GVG-induced intramyelenic edema.

(It would also be instructive to compare human exposure to drug with that in animals, although as discussed above there are numerous caveats to consider. It appears that plasma levels of GVG in humans receiving the

maximum clinical dose are greater than those of rat and dog at the same mg/kg dose, and CSF levels of GVG in humans receiving the maximum clinical dose are greater than those of rat but less than those of dog at the same mg/kg dose. This dose in rat and dog is somewhat above the lower end of the range shown to cause intramyelenic edema in these species; thus humans cannot be "exempted" from the likelihood of lesion production based on these exposure comparisons, with the exception of comparison to dog CSF levels, although note that a no-effect dose in dogs was not established. Perhaps more relevantly, CSF levels of GABA were increased in humans receiving therapeutic doses of GVG [increases ranged from about 1.5-10 x baseline across studies], and the sponsor of a recently submitted individual investigator IND [47,137] indicates that preliminary NMR studies in humans show that brain GABA levels are also increased. As noted above increases in central GABA, which occurred in rats, mice, and dogs, but not in monkeys, may be associated with the production of intramyelenic edema in brain).

The sponsor has performed studies in dogs to attempt to develop a non-invasive method for monitoring for IME which might be usable in humans. It was shown that changes in somatosensory evoked potentials (SEP), visual evoked potentials (VEP), and MRI images roughly paralleled the appearance and reversal of brain vacuolation in subacute studies (involving daily dosing at 300 mg/kg). These three measures were roughly equally sensitive, i.e., changes were seen in all 3 generally beginning at 4 weeks of treatment. (This is equal to or slightly later than the earliest times that vacuoles were histologically demonstrable at this dose, i.e., 2-4 weeks. It is also noted that these monitoring techniques were not sensitive to the residual changes, e.g., gliosis, which persisted after drug discontinuation. It is further noted that although it was shown that the monitoring can detect vacuoles at a time when they are still reversible, the data were inadequate to determine if stopping treatment at the time of the earliest change in monitoring parameters would prevent residual changes such as gliosis.).

It may be questioned whether the observed changes in monitoring parameters are directly due to the presence of the histological lesion, or are just epiphenomena (i.e., possibly resulting from other actions of the drug aside from lesion production, e.g., GABA levels are known to normally exert some influence on the VEP). However, even if only epiphenomena, changes in the monitoring parameters could be useful for monitoring for the lesion of they co-vary extensively with the latter. The data on this point is suggestive but not extensive. For example, changes in monitoring parameters roughly paralleled the times of appearance and regression of the vacuoles with subacute treatment of dogs. On the other hand, there is no information on monitoring sensitivity at lower doses (only 300 mg/kg was used in the dog study); i.e., it would have been useful to see if threshold dosing regimens for production of the lesion were the same as those for production of changes in monitoring parameters. Regarding the MRI data, although in some cases there was some correlation between brain areas with vacuolation and those with MRI changes, this correlation was not always very tight. Examination of correlations across

individual animals, such as between degree of change in MRI vs SEP vs VEP, or between degree of change of any of these with degree of vacuolation, revealed no clear evidence of correlation (in some cases due to absence of evidence, in some to evidence of no correlation). Furthermore, it should be noted (1) that there is no information on how or if GVG-induced changes in these monitoring parameters co-vary with GVG-induced IME in humans, and (2) that early attempts at VEP and MRI monitoring in dogs were not successful and only became so after refinement of the monitoring techniques. Thus, although there is some evidence that evoked potential changes occur in association with myelinopathies in humans (Arezzo, et.al., Br. J. Clin. Pharmac.27: 535, 1989), a specific method shown to be able to detect the GVG-induced brain lesion in humans has not been established, and thus one cannot conclude the absence of the lesion in humans until a method which has been validated in humans is employed. On the other hand, evoked potential or MRI monitoring can be relevant in their own right, i.e., not necessarily as manifestations of or markers for GVG-induced intramyelenic edema, but as independent manifestations of GVG-induced neurotoxicity. (However, note that possible changes in evoked potentials or MRI associated with epilepsy per se may complicate interpretation of any effects seen.)

Based on correlations between increased GFAP staining and the presence of GVG-induced brain vacuolation in rats and dogs, the sponsor suggests that GFAP staining could be used in humans to differentiate drug-induced vacuoles from "artifactual" vacuoles. There are some problems with this; e.g., while in some brain areas drug-induced vacuoles and increased GFAP staining were co-localized, they were dissociated in others (i.e., vacuoles present without increased GFAP staining and vice-versa). Thus, for example, the presence of vacuoles in the absence of increased GFAP in humans might falsely lead to the conclusion that they were artifactual. (Also, I am not sure if the vacuoles which have been seen in control animals [the incidence of which was often considerable] or in humans are truly artifacts, as the sponsor seems to suggest, or represent a background spontaneous incidence of vacuolation/intramyelenic edema [or possibly are of both types]. I am not aware of any accepted histological criteria for distinguishing between these, including the absence or presence of a GFAP response. In the rat GFAP study some vacuoles were seen in controls associated with normal GFAP staining; if, for example these vacuoles were not artifacts but true, background vacuolation of insufficient severity to result in increased GFAP staining, then it is possible that a mild drug-induced vacuolation in humans, also of insufficient severity to result in increased GFAP staining, would be falsely concluded to be artifactual. Another scenario might be if the control rat vacuoles were artifacts, then true background vacuolation in humans, which might be associated with increased GFAP staining [this possibility not having been ruled out by the rat results], would lead to the false conclusion of a drug effect in humans). The reported occurrence of gliosis in epileptic patients might further compromise the usefulness of this approach.

B) Retinal lesions

"Retinal degeneration" (see earlier for more specific descriptions) was seen in albino rats of both sexes and in female mice at doses as low as 30-50 mg/kg/day p.o. It was not seen in dogs and monkeys. Reversibility was not examined. It was not detected by routine ophthalmoscopic exams. It has been noted that there is some similarity between the lamellar organization of the membranes of the affected retinal components (rod outer segments) and myelin, suggesting a possible connection between the retinal lesions and the brain intramyelenic edema. In this regard it is notable that hexachlorophene, which also causes intramyelenic edema, also causes retinal lesions in rats (Towfighi, et.al., Laboratory Investigation32: 330, 1975); this retinal lesion may be similar to that caused by GVG although the degree of similarity is not clear from the descriptions provided; hexachlorophene also caused additional changes thought to be secondary to optic nerve lesions (idem.).

C) Cleft palate in rabbits

The incidence of cleft palate was increased in each of 2 segment II studies in rabbits. The overall incidence was 9/103 fetuses (4/17 litters) at 200 mg/kg and 3/131 fetuses (2/17 litters) at 150 mg/kg. (None in other groups; next lowest dose = 100 mg/kg). Large decreases in food consumption and transient bodyweight loss were seen at the affected doses. Resorptions were increased at 200 mg/kg. No teratogenic effects were seen in segment II studies in rats.

D) Transaminase decreases

Decreases in ALT and less prevalently AST were seen in the animal toxicity studies. This was attributed to the pharmacological action (i.e., transaminase inhibition) of the drug. This should be kept in mind when using transaminase levels to evaluate potential toxic effects of GVG (or any other cause) in humans.

4 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 15

RECOMMENDATIONS:

This NDA is approvable. Recommendations concerning proposed labelling are made in the previous section of this review.

Barry Rosloff

Barry N. Rosloff, Ph.D.

cc: NDA 20-427
HFD-120
HFD-120/GFitzgerald GG 3/20/95
/BRosloff
/RPitts