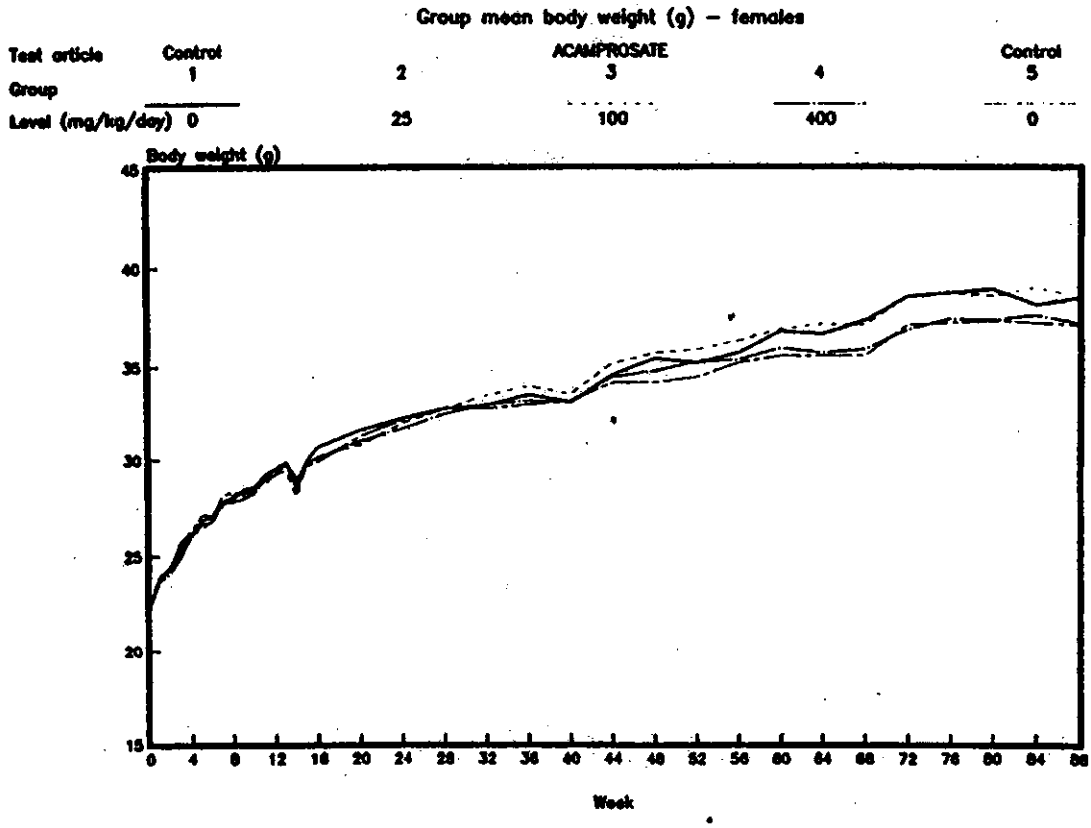
FIGURE 4



Food consumption: No treatment-related effects.

Hematology ,Blood Chemistry: Only data for white blood cell count submitted. No treatment-related effects.

Organ weights: Not submitted.

Gross pathology: No treatment-related effects.

Histopathology:

Non-neoplastic: Organs with increased non-neoplastic findings in male and female Acamprosate-treated mice are shown in the following table.

Mice Affected (Combined Decedents and Terminals, Percent)

	Acamprosate Dose (mg/kg/d, Males)					Acamprosate Dose (mg/kg/d, Females)				
Dose	**C1**	**25**	**100**	**400**	**C2**	**C1**	**25**	**100**	**400**	**C2**
Liver: Hepatocyte Vacuolation	8	10	14	16	14	4	8	9	12	8
Colon: Nematodes	17	20	11	33	15	47	22	29	33	39
Rectum: Nematodes	4	0	0	12	4	0	6	5	0	2
Testis Mineralization	16	11	6	21	16	-	-	-	-	-

Epididymis: Oligospermia	10	8	16	25	12	-	-	-	-	-
Heart: Arteritis	0	4	3	8	0	6	15	0	2	2
Heart: Arterial Thrombus	4	4	6	10	2	2	5	0	0	0

Neoplastic: In the trend-test, non-significant increase in benign pituitary adenoma, thyroid follicular adenoma, uterus stromal polyp and uterus hemangioma, skin subcutis malignant histiocytic sarcoma, and malignant lung carcinoma in female mice. In the pairwise test, marginally significant increase in malignant histiocytic sarcoma of the uterus in female mice ($p<0.0497$).

MOUSE TUMOR INCIDENCE

	Acamprosate Dose (mg/kg/d, male mice)					Acamprosate Dose (mg/kg/d, female mice)				
	C1	25	100	400	C2	C1	25	100	400	C2
Uterus (Total # Examined)	-	-	-	-	-	51	43	46	50	50
*B Stromal Polyp(#)	-	-	-	-	-	2	2	2	6	3
B Hemangioma (#)	-	-	-	-	-	0	0	0	3	1
*M Histiocytic Sarcoma (#)	-	-	-	-	-	0	0	0	3	1
M Leiomyosarcoma (#)	-	-	-	-	-	0	2	2	1	1
M granular Cell Myoblastoma (#)	-	-	-	-	-	0	0	0	1	0
Combined Incidence (%)	-	-	-	-	-	4%	16%	9%	28%	12%
Skin Subcutis (Total # Examined)	51	29	38	50	51	51	22	24	50	50
B hemangioma (#)	0	0	0	1	0	0	0	0	0	0
M Sarcoma(#)	3	1	2	2	1	1	1	4	0	0
M Osteosarcoma (#)	0	0	1	1	0	1	0	2	0	0
Combined Incidence (%)	6%	3%	8%	8%	2%	2%	4%	25%	0%	0%
Pituitary (Total # Examined)	51	25	31	50	51	50	20	24	49	50
B Adenoma (#)	0	0	0	0	0	2	0	2	5	3
B Pars Intermedia Adenoma (#)	1	0	0	0	0	0	0	0	1	0
M Carcinoma (#)	0	0	0	0	0	1	0	1	0	0
Combined Incidence (%)	2%	0%	0%	0%	0%	6%	0%	12%	12%	6%
Thyroid (Total # Examined)	51	23	31	51	50	50	20	23	49	51
B Follicular Adenoma (#)	1	0	0	0	1	0	0	0	2	0
Combined Incidence (%)	2%	0%	0%	0%	2%	0%	0%	0%	4%	0%
Lung (Total # Examined)	51	34	38	51	51	51	29	29	50	51
B Adenoma (#)	10	9	4	11	9	4	10	6	4	11
M Carcinoma (#)	1	1	3	2	3	1	1	3	4	1
Combined Incidence (%)	22%	29%	18%	25%	23%	10%	38%	31%	16%	23%

B: Benign; M: Malignant. Decedents and terminal combined. Tumors with increased incidence in Acamprosate-treated mice compared to controls shown, only.

NEOPLASM SUMMARY

Mice Affected (Percent)

	Males					Females				
Dose (mg/kg/d)	**C1**	**25**	**100**	**400**	**C2**	**C1**	**25**	**100**	**400**	**C2**
Decedents										
Primary Neoplasms	68	65	45	43	50	65	65	74	57	78
Benign Neoplasms	45	38	29	17	32	17	35	22	14	44
Malignant Neoplasms	50	27	32	26	36	57	40	70	43	44

Metastatic Neoplasms	0	0	0	0	0	0	0	0	0	0
Locally Invasive neoplasms	0	0	0	0	0	0	0	0	0	0
Other Neoplasms	0	0	0	0	0	0	0	0	0	0
Terminal Kill										
Primary Neoplasms	69	76	70	82	66	43	52	43	76	67
Benign Neoplasms	66	68	65	79	62	29	42	36	59	58
Malignant Neoplasms	3	8	15	14	17	14	19	11	30	18
Metastatic Neoplasms	0	0	0	0	0	0	0	0	0	0
Locally Invasive neoplasms	0	0	0	0	0	0	0	0	0	0
Other Neoplasms	0	0	0	0	0	0	0	0	0	0

Toxicokinetics: Drug absorption was verified in blood samples taken in weeks 90 (males) and 91 (females).

Mean Acamprosate Plasma Levels (ng/ml)

	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d
Males	254.5	227.9	3798.1
Females	160.3	477.1	2477.1

In the 2-week study in mice (see Appendix 3) plasma acamprosate was below the level of detection (approximately <0.2 mg/l) in 67% of samples at the low dose (target 100 mg/kg/d). In the remaining 32% samples at the low dose, the maximum mean plasma levels (across timepoints) were 0.36 mg/l in the males (range [redacted]) and 0.63 mg/l in the females (range [redacted]. Maximum mean plasma levels (across timepoints) at the targeted high dose (400 mg/kg/d) were 1.4 mg/l (range [redacted] in males, and 2.14 mg/l (range [redacted]) in females at 23 and 21 hours respectively. Sampling every 2-6 hours demonstrated steady plasma levels over 19 hours.

MOUSE MTD/HUMAN MRD: Not determined in this study
MOUSE/HUMAN BSA RATIO: (400mg/kg/d)(3)/(1998mg/50kg/d)(37) = 1200/1479 = 0.8X
MOUSE/HUMAN AUC RATIO: Not determined in this study

* No correction for protein binding: unknown in mice

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:

The carcinogenicity study in mice used an adequate number of animals, and showed adequate survival, parameters evaluated and duration of treatment, which was limited by the end of study survival rate normal for this species. The sponsor demonstrated stability and homogeneity of acamprosate in the animal chow and drug absorption was verified in blood samples. However, the study is considered invalid based on several criteria. The dosing is deemed inadequate based on lack of toxicity, including changes in body weights, at the highest dose. CAC concurrence on the protocols, including the doses used, was not received prior to conducting the study. The results may have been confounded by nematode infestation. Inadequate numbers of animals were

evaluated for tumor incidence in the low and mid-doses, and, therefore, the trend test was not appropriate. The CAC committee concluded that the carcinogenicity study in mice is unacceptable, and recommended that the sponsor repeat a mouse carcinogenicity study.

Evaluation of tumor findings:

No significant treatment-related increases in neoplastic effects were observed. However, the high incidence of mortality as well as inadequate histologic assessment of the low- and mid-dose groups prevent a definitive conclusion on the carcinogenic potential of this drug.

ACAMPROSATE: 104 WEEK ORAL (DIETARY ADMINISTRATION) CARCINOGENICITY STUDY IN THE RAT

Key study findings:

- No significant treatment-related neoplastic findings were noted.
- Dosing in males was deemed adequate as the high-dose was at or near the MTD based on decreased body weight, tail sores and renal effects.
- Dosing in females was deemed marginally acceptable as it is consindered to approximate one-third of the MTD based on renal effects observed in a 13-wk dietary study.

Study number: 7062-537/26
Volume # 25-29
Conducting laboratory and location: [redacted]
Date of study initiation: August 7, 1989
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #

Acamprosate Lot Numbers used in the Carcinogenicity Study in Rats

Lot Number	Batch Number	Quantity Received (kg)	Receipt Date at HUK
3	OTA 3011	[redacted]	June 13, 1989
6	OTA 3011		March 13, 1991
7	OTA 3011		July 23, 1991

% purity: [redacted]
CAC concurrence: No

Study Type: 2 year bioassay
Species/strain: CD(SD)BR Rats, [redacted]
Number/sex/group; age at start of study: 50; 28 days
Animal housing: Caged in groups of 5 in stainless steel mesh cages in a single, exclusive room air-conditioned (15 air changes/hour), temperature 19-25 degC, humidity 40%-70%, with fluorescent lighting in a 12-hour light-dark cycle

Formulation/vehicle: Test article as a white powder for admixture with the diet/vehicle was powdered diet

Drug stability/homogeneity: Samples were taken from diet mix for each group, from top, middle and bottom of supplies, in weeks 1, 8, 10, 18, 23, 36, 49, 62, 75, 88, and 101 for analysis of stability and homogeneity. Mean concentration differed from theoretical values by >10% in 1 sample only (Grp 2 male, week 10), homogeneity differed from theoretical concentration by >15% in 6 samples only throughout study (range -17.4% - +38.8%); Certificates of Analysis provided.

Methods:

Doses: 0, 25, 100 and 400 mg/kg/day

Basis of dose selection: The sponsor did not provide a rationale for dose selection. A 3-month dietary study in rats was performed and the MTD was determined to be 1000 mg/kg/day (see Appendix 2). Dosing is considered to be adequate in males due to observed body weight decreases, renal effects and tail sores; dosing in males is considered marginally acceptable since the high-dose of 400 mg/kg approximates one-third of the MTD identified in the 13-week dietary study.

Restriction paradigm for dietary restriction studies: Rat Maintenance Diet No. 1, expanded, ground fine [redacted] *d libitum*, fasted overnight before necropsy. Drinking water filtered tap, changed daily, *ad libitum*.

Route of administration: Oral by admixture with the diet

Frequency of drug administration: Continuous in diet

Dual controls employed: Yes: both untreated powdered diet

Interim sacrifices: No

Satellite PK or special study group(s): No

Deviations from original study protocol:

-Temperature range (16-27degC) and humidity (range 36-76%) in animal room deviated from protocol limits on a few occasions

-Animals moved to different room for 5h in week 22 due to construction work

-Additional diet samples taken for stability/homogeneity in weeks 8, 18, 23

-Sublingual salivary gland taken with submaxillary at necropsy

-Sponsor changed address

Statistical methods: Sponsor:

ANOVA, Regression and Dunnett's: Body weight gain, food consumption, total white blood count

ANOVA and t-test: Body weight

Kruskal Wallis, Terpstra Jonckheere and Wilcoxon rank sum test: Total white blood cell count

Survival: Kaplan-Meier technique, survival curves compared by the log-rank procedure

Tumor analysis: Treated group against control: 1-sided risk testing for increasing risk with increased dose and 1-sided risk for testing decreasing risk with increased dose; Incidences in 2 control groups compared using 2-sided risk, where significant comparisons with other groups carried out using separate controls, where not different, analysis performed using combined controls; uncertain tumors analyzes as if all fatal and as if all non-fatal: In fatal context analyzed by log-rank procedure, in non-fatal context

analyzed with IARC annex (fixed intervals 1-50 wks, 51-80 wks, 81-104 wks, and terminal kill); Permutation tests used to establish significance of findings where fatal and non-fatal tumors observed with total incidence of at least 3 but less than 10, or where combined incidence of fatal and non-fatal tumors less than 10 but at least 3.

Agency Statistical Reviewer: statistical test on the survival data to compare the survival curves of the four dosage groups. Then, this reviewer performed two statistical tests on the tumor incidence. The first was a trend test intending to identify any significant positive dose-response linear trend for the tumor incidence. The second was a pairwise test intending to identify any significant difference between the high dose and placebo groups.

Two control groups were combined in both tests. They were stratified on properly divided time intervals to adjust for intermittent mortality. For the rat study, time intervals were 0-50, 51-80, 81-104, and 105-106 (terminal sacrifices) weeks.

Observations and times:

Clinical signs: Daily
Body weights: Baseline, Day 1, weekly to week 16, every 4 weeks thereafter
Food consumption: Baseline, Day 1, weekly to week 16, every 4 weeks thereafter
Hematology: At necropsy
Clinical chemistry: At necropsy
Organ weights: Not done
Gross pathology: At necropsy
Histopathology: At necropsy

Histopathology Inventory*

	Control 1	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d	Control 2
Abdominal Cavity	X	X	X	X	X
Adrenals	X	X	X	X	X
Aorta					
Bone Marrow Smear	X	X	X	X	X
Bone	X	X	X	X	X
Brain	X	X	X	X	X
Cecum	X	X	X	X	X
Cervix					
Clitoral Gland	X	X	X	X	X
Colon	X	X	X	X	X
Connective Tissue	X	X	X	X	X
Cranial Cavity	X	X	X	X	X
Duodenum	X	X	X	X	X
Ear	X	X	X	X	X
Epididymis	X	X	X	X	X
Esophagus					
Eye	X	X	X	X	X
Fallopian Tube					
Foot/Leg	X	X	X	X	X
Gall Bladder					
Harderian Gland					
Heart	X	X	X	X	X
Hypophysis					
Ileum	X	X	X	X	X

Jejunum					
Kidneys	X	X	X	X	X
Lachrymal Gland	X	X	X	X	X
Larynx					
Liver	X	X	X	X	X
Lungs	X	X	X	X	X
Lymph Nodes, Cervical					
Lymph Nodes, Mandibular	X	X	X	X	X
Lymph Nodes, Mesenteric	X	X	X	X	X
Mammary Gland	X	X	X	X	X
Muscle	X	X	X	X	X
Nasal Cavity	X	X	X	X	X
Optic Nerves	X	X	X	X	X
Oral Cavity	X	X	X	X	X
Ovaries	X	X	X	X	X
Pancreas	X	X	X	X	X
Parathyroid	X	X	X	X	X
Penis	X	X	X	X	X
Peripheral Nerve					
Pharynx					
Pituitary	X	X	X	X	X
Prostate	X	X	X	X	X
Rectum					
Salivary Gland	X	X	X	X	X
Sciatic Nerve	X	X	X	X	X
Seminal Vesicles	X	X	X	X	X
Skin	X	X	X	X	X
Spinal Cord	X	X	X	X	X
Spleen	X	X	X	X	X
Sternum	X	X	X	X	X
Stomach	X	X	X	X	X
Tail	X	X	X	X	X
Testes	X	X	X	X	X
Thoracic Cavity	X	X	X	X	X
Thymus	X	X	X	X	X
Thyroid	X	X	X	X	X
Tongue					
Trachea	X	X	X	X	X
Ureter	X	X	X	X	X
Urinary Bladder	X	X	X	X	X
Uterus	X	X	X	X	X
Vagina	X	X	X	X	X
Zymbal Gland	X	X	X	X	X

*X: Organs examined in all rats that died and were sacrificed.

Toxicokinetics: Drug absorption was verified in blood samples taken in weeks 103 (males) and 104 (females).

Results:

Clinical signs: No treatment-related clinical signs.

Mortality:

End of Study Survival Rate

	Acamprosate Dose (mg/kg/d)				
	Control 1	**25**	**100**	**400**	**Control 2**
Males	64%	52%	44%	74%	54%
Females	44%	52%	50%	42%	56%

FIGURE 1

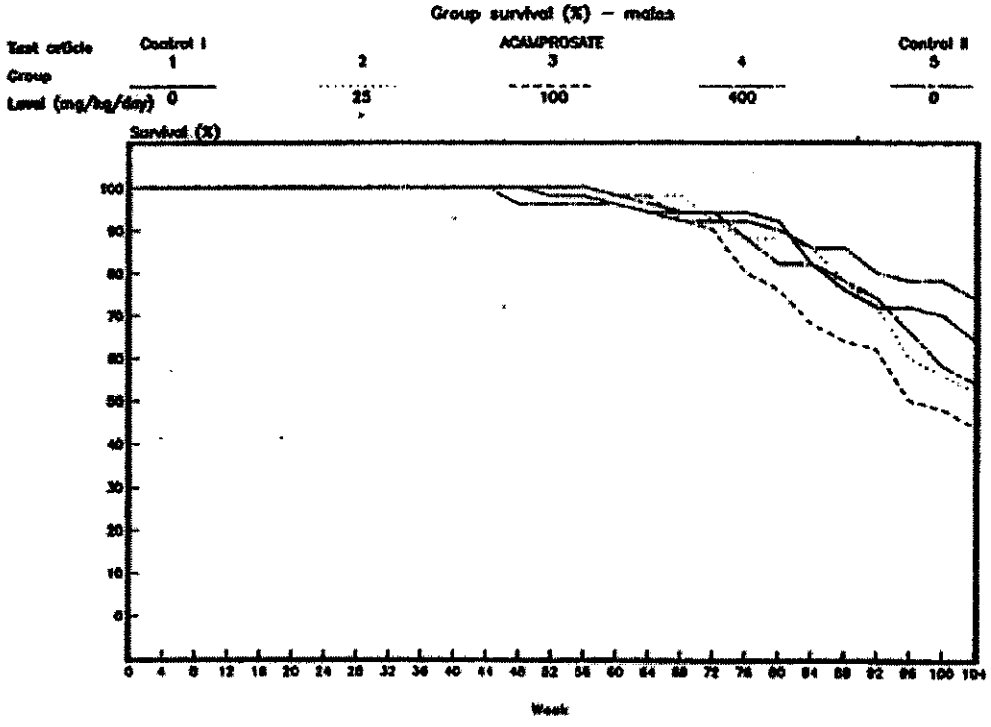
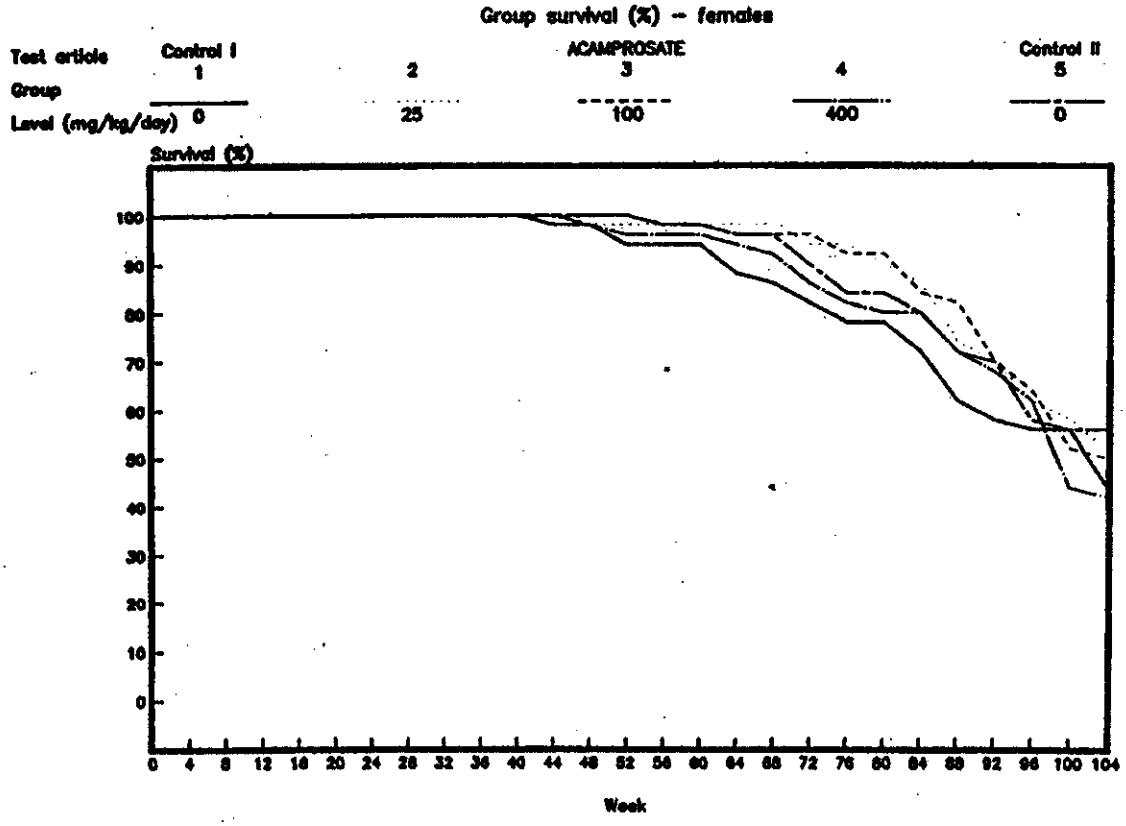


FIGURE 2



Cause of Death (# Animals, Bold for highlight only)

	Acamprosate Dose (mg/kg/d, Males)					Acamprosate Dose (mg/kg/d, Females)				
	C1	25	100	400	C2	C1	25	100	400	C2
Total deaths	**18**	**24**	**28**	**13**	**23**	**28**	**24**	**25**	**29**	**22**
Procedure/Trauma	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**
Neuropathy	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Vascular lesion	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Hemorrhagic lesion	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Liver lesion	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Urinary tract lesion	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
Skin subcutis lesion	**0**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Foot lesion	**1**	**1**	**1**	**2**	**0**	**0**	**1**	**0**	**0**	**1**
Oral cavity lesion	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Skin subcutis tumor	**0**	**3**	**3**	**0**	**4**	**0**	**0**	**1**	**0**	**2**
Brain tumor	**1**	**2**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**0**
Uterine tumor	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**
Mammary tumor	**0**	**0**	**0**	**0**	**0**	**7**	**6**	**4**	**7**	**5**
Pituitary tumor	**8**	**12**	**15**	**5**	**13**	**17**	**13**	**15**	**17**	**8**
Pituitary/mammary tumor	**0**	**0**	**0**	**0**	**0**	**1**	**2**	**2**	**1**	**1**
Hemolymorphoretic ular tumor	**0**	**0**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
Histiocytic sarcoma	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Multiple organ tumors	0	0	0	0	0	0	1	1	1	1
Other tumor	4	2	1	2	0	1	0	1	0	0
Undetermined	2	2	3	0	4	0	0	0	2	1

Body weights: Body weight 4-5% lower in high-dose male rats compared to control rats at the end of the 104 week study. Body weights were comparable to controls in remaining dose groups in males, and in all dose groups in females.

Mean Body Weight at One Year

MRC1: 676.0 g **MRC2**: 670.0 g **MRLD**: 682.3 g
MRMD: 676.8 g **MRHD**: 647.8 g

FRC1: 365.5 g **FRC2**: 378.7 g **FRLD**: 365.0 g
FRMD: 377.1 g **FRHD**: 372.9 g

FIGURE 3

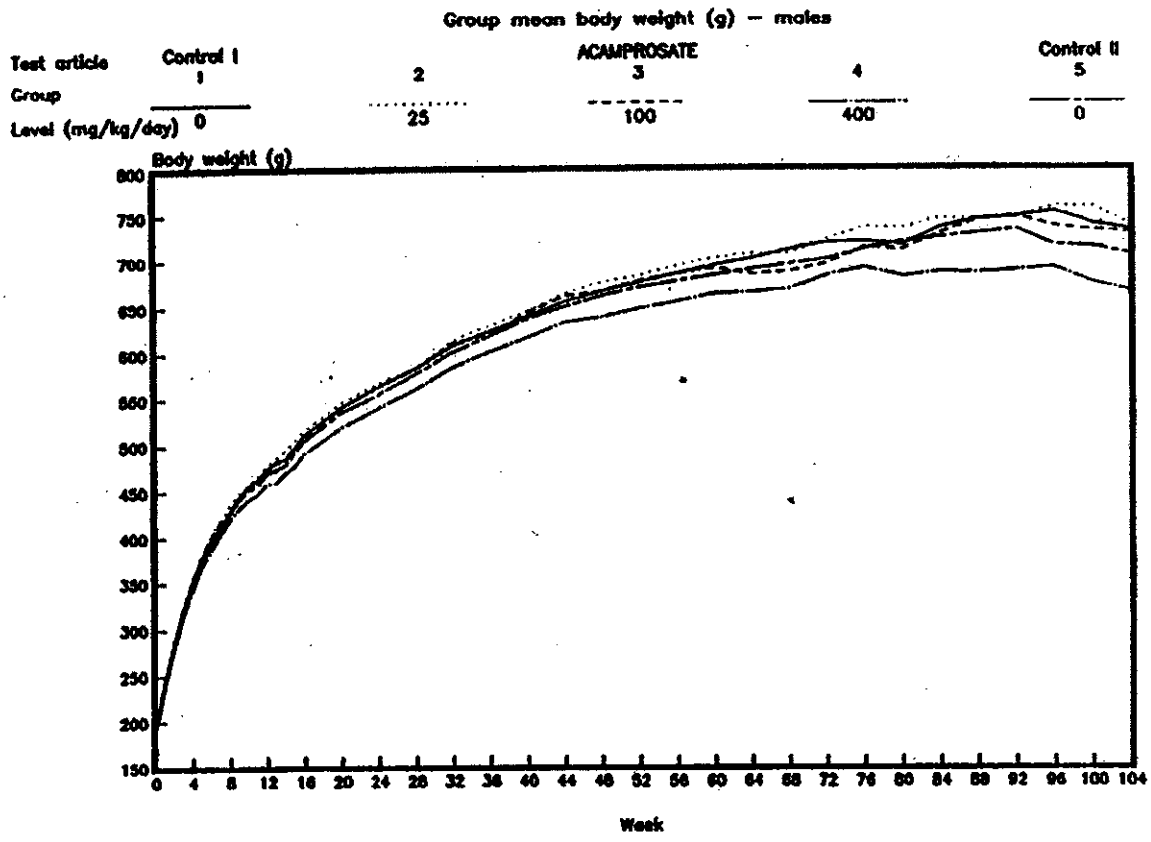
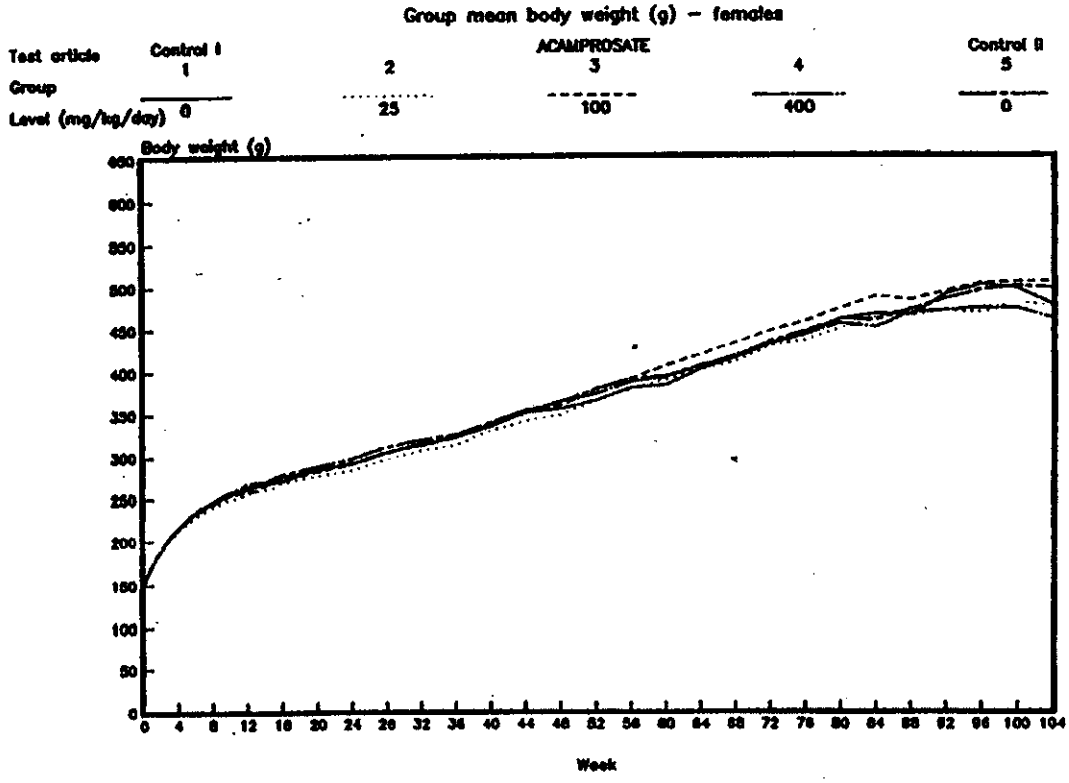


FIGURE 4



Food consumption: No treatment-related differences in food consumption.

Hematology: Higher mean total white blood cell count (36%-41% increase) in high dose males (mean 7500/cmm compared to 5500/cmm and 5300/cmm in the controls): due to increases in 4 males.

Clinical chemistry: No treatment-related effects.

Organ weights: NA

Gross pathology: Dark/enlarged adrenals, protruding eye, pale/mottled liver, dark liver area, hydronephrosis, enlarged pituitary, skin mass/sore/fur loss, prominent mammary, thickened stomach, dark/soft/small testis, uterus distension, abnormal teeth, tail sores, small seminal vesicle, foot/leg sores, to similar extent in all groups. Greater incidence of tail sores in high-dose males (26/50) than in control animals (14/50, 14/50).

Histopathology:

Non-neoplastic:

Increased incidence of pelvic mineralization in the kidney in high-dose male (grade 1, 54% in decedent and 57% in terminal kill, vs. control rate of 18% in C1 and 18% in C2 decedent, and 31% in C1 and 37% in C2 terminal kill) and female (grades 1 and 2, 93% in decedent and 90% in terminal kill, slight increases over control rates of 65% in C1 and 73% in C2 decedent and 82% in C1 and 86% in C2 terminal kill) rats. Pelvic mineralization was observed in the renal papilla-cortex junction, renal papilla and pelvic urothelium, and was associated with uroliths in the renal pelvic cavity.

Neoplastic:

A summary of neoplastic findings is provided in the table below. No significant treatment-related neoplastic findings were observed.

Rat Tumor Incidence

	Acamprosate Dose (mg/kg/d, male rats)					Acamprosate Dose (mg/kg/d, female rats)				
	C1	25	100	400	C2	C1	25	100	400	C2
Thyroid (Total # Examined)	49	24	27	48	50	46	48	48	48	48
C-cell adenoma (#)	10	3	3	5	10	5	8	7	12	6
C-cell carcinoma (#)	1	1	0	1	0	1	0	2	1	1
Combined Incidence (%)	22	17	11	13	20	13	17	19	27	15
Pancreas (Total #Examined)	50	50	49	48	50	49	24	25	50	49
Islet cell adenoma (#)	4	7	5	8	3	2	2	0	4	2
Islet cell carcinoma (#)	0	1	0	1	1	0	0	1	0	1
Combined Incidence (%)	8	16	10	19	8	4	8	4	8	6
Adrenal (Total # Examined)	50	49	49	49	50	49	33	34	50	50
Pheochromocytoma (#)	8	9	7	14	7	0	2	2	6	5
Malignant Pheochromocytoma (#)	1	0	1	3	0	0	0	0	0	0
Combined Incidence (%)	18	18	16	33	14	0	6	6	12	10

Neoplasm Summary (percent rats affected)

	Males					Females				
Dose (mg/kg/d)	C1	25	100	400	C2	C1	25	100	400	C2
Decedents										
Primary Neoplasms	89	100	93	85	96	100	100	100	90	100
Benign Neoplasms	72	92	79	77	87	100	100	100	86	100
Malignant Neoplasms	33	33	21	31	26	14	17	36	28	27
Metastatic Neoplasms	0	0	0	0	0	0	0	0	0	0
Locally Invasive neoplasms	0	0	0	0	0	0	0	0	0	0
Other Neoplasms	0	0	0	0	0	0	0	0	0	0
Terminal Kill										
Primary Neoplasms	91	85	100	92	96	95	81	96	100	93
Benign Neoplasms	91	81	100	92	93	95	81	92	100	89
Malignant Neoplasms	13	4	0	19	7	5	12	16	24	14
Metastatic Neoplasms	0	0	0	0	0	0	0	0	0	0
Locally Invasive neoplasms	0	0	0	0	0	0	0	0	0	0
Other Neoplasms	0	0	0	0	0	0	0	0	0	0

Toxicokinetics: Mean Acamprosate Plasma Levels (ng/ml)

	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d
Males	168.4	447.5	1339.2
Females	171.1	512.2	1737.5

AUC data not provided in this study. AUC data is taken from: 28-DAY ORAL (DIETARY ADMINISTRATION) TOXICOKINETIC STUDY IN THE RAT. .

Toxicokinetic Analysis in Rats Administered Dietary Acamprosate for 28 Days*

Dose (mg/kg/day)	Sex	Cmax (ng/ml)	Tmax (h)	AUC_{0-24}(h.ng/ml)
25	Male		9:00 am	1328.8
	Female		9:00 am	1429.4

	Male-Female	[redacted]	9:00 am	1379.1
100	Male		6:00 am	4300.6
	Female		9:00 am	5363.4
	Male-Female		9:00 am	4832.0
400	Male		3:00 am	17207.7
	Female		3:00 am	17317.2
	Male-Female		3:00 am	17292.7

*Study Number 537/059 (Submitted in NDA21-431 Amendment #003)

RAT HIGH DOSE/HUMAN MRD: (400 mg/kg/d)/(1998 mg/50kg/d) = 10X
RAT/HUMAN BSA RATIO:(400 mg/kg/d)(5.9)/(1998mg/50kg/d)(37) = 2360/1479 = 1.6X
RAT /HUMAN AUC RATIO: (17292ng.h/ml x 0.865*)/(6884ng.h/ml x 0.94*) = 2.3X

* Correction for protein binding:13.5% in rats and 6% in humans

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:

The carcinogenicity study in rats used an adequate number of animals, and showed adequate survival, parameters evaluated and duration of treatment. The sponsor demonstrated stability and homogeneity of acamprosate in the animal chow and drug absorption was verified in blood samples. The doses were acceptable though only marginally adequate. The high dose tested (400 mg/kg/day dietary, 2.3X the MRHD on an AUC basis) was at or near the MTD based on decreased body weights (-4% to -5%, high white blood cell count (-365 to-41%, increased incidence of tail sores, renal effects (pelvic mineralization at 54-86% in the controls, but no additional toxicity was observed. Furthermore, the high dose was considered to be below the MTD based on the results of the 13-week dietary toxicity study in rats. Nonetheless, the CAC committee concluded that the study in rats can be accepted based on overall toxicity and renal effects, particularly in the males.

Evaluation of tumor findings: No signifcant treatment-related neoplastic findings were noted..

Carcinogenicity summary: Carcinogenicity studies were conducted in mice and rats using dietary administration. The results of these studies were presented to the Executive CAC committee on March 19, 2002. In rats, acamprosate administered for 104 weeks at up to 400 mg/kg/d (1.6x the clinical high dose on a mg/m^2 basis, 2.3x the clinical high dose on an AUC basis) produced no clinical signs and no effects on the end-of-study survival rate or cause of death. Body weights were slightly lower, and total white blood cell count and incidence of tail sores were higher in high dose male rats throughout the study. In the histopathology examinations, pelvic mineralization in the kidney was increased in both decedent and terminal kill high-dose male and female rats. There was no evidence suggestive of increased carcinogenic potential, when the incidence of tumors were analyzed using Tests of Dose-Response Positive Linear Trend and Pairwise Tests (high dose vs. placebo). In the trend test for dose response, there were nonsignificant increases in benign adrenal pheochromocytoma, malignant mammary gland carcinoma, benign islet cell adenoma, thyroid C-cell adenoma and pituitary adenoma tumors in female rats; benign pancreatic Islet cell adenoma and adrenal pheochromocytoma, and

malignant adrenal pheochromocytoma and skin subcutis histiocytic sarcoma tumors in male rats. The Pairwise Tests showed non-significant increases in benign adrenal phaeochromocytoma and pancreatic islet cell adenomas in high dose male rats and benign thyroid C-cell adenomas and malignant mammary gland carcinomas in high dose female rats. Under the conditions of this study, acamprosate was not considered to be tumorigenic in rats. In the rat, the high dose (400 mg/kg/day) demonstrated an exposure level of 2.3x the clinical high dose (1998 mg/day in a 50 kg patient) on an AUC basis. Dosing in the rats was marginally adequate based on ICH guidelines.

A definitive assessment of the 91 week carcinogenicity study in the mouse could not be performed as the study was considered to be invalid by the Executive Carcinogenicity Assessment Committee due to inadequate dosing, nematode infestation and inadequate histologic assessment of the low- and mid-dose groups. The highest doses (400 mg/kg/day) used in the mouse and rat studies were 0.8x and 1.6x the highest proposed daily clinical dose in a 50 kg patient on a mg/m^2 basis, respectively. AUC data was not available in the mouse.

Carcinogenicity conclusions: Under the conditions of these studies, acamprosate was negative for carcinogenicity in rats. The study in mouse is considered to be inadequate to provide a definitive assessment of the carcinogenic potential. The results of the carcinogenicity studies in mice and rats were presented to the Executive CAC committee on March 19, 2002. The committee concluded that the doses used in the rat study were only marginally adequate based on ICH criteria, but the study can be accepted based on overall toxicity and renal effects, particularly in the male rats. The carcinogenicity study in mice is unacceptable because inadequate doses were used, based on lack of evidence for an MTD such as body weight effects. The mouse study results were confounded by nematode infestation, and histopathology evaluation was conducted on an inadequate number of mid- and high-dose animals. The committee recommended that the sponsor repeat the mouse carcinogenicity study.

Recommendations for Further Evaluation: A carcinogenicity study in mice should be repeated since dosing was inadequate to determine the full carcinogenic potential. The minutes of the Executive CAC meeting (see Addendum) were sent to the sponsor and the sponsor was informed in a teleconference of May 24, 2002 that a standard 2-year assay or an appropriate alternative mouse carcinogenicity assay could be performed. The sponsor was encouraged to submit the protocol and rationale for dose selection to obtain concurrence by the Executive Carcinogenicity Committee.

Labeling Recommendations: The results of the rat study should be reported in the appropriate section of the label. [

]

Addendum/appendix listing:

Executive CAC
Date of Meeting: March 19, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Joseph Sun, Ph.D., HFD-570, Alternate Member
Timothy McGovern, Ph.D., Team Leader
Kathleen Haberny, Ph.D., Presenting Reviewer

Author of Draft: Kathleen Haberny, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-431
Drug Name: Acamprosate
Sponsor: Lipha Pharmaceuticals, Inc.

Background: Acamprosate (Aotal, Campral) is being evaluated in the United States as a treatment for alcohol dependence in chronic ethanol abusers. Developed by Laboratoires Meram in 1987, acamprosate has been available commercially since 1989 for use in the treatment of alcohol abuse in over twelve European countries. The therapeutic dose in Europe and proposed dose in this submission is 2 x 333 mg tablets t.i.d. (1998 mg/day). [ ] patients have been treated in Europe with commercially available acamprosate (333 mg tablets) for maintenance of alcohol abstinence, since 1989.

The mechanism of action appears to involve alterations in gamma-aminobutyric acid (GABA) transmission and antagonism of excitatory amino acids, perhaps by restoring the inhibition/excitation balance that is possibly altered by chronic alcohol consumption. Acamprosate bioavailability is approximately 7%-15% in rats and 11% in humans. Distribution is predominantly to the GI tract, liver, kidney, lymph nodes and lungs. Acamprosate is approximately 13.5% protein bound in rats, but not bound to plasma proteins in humans. Information on protein binding in mice is not available. No evidence of acamprosate metabolism was found in rats, rabbits, or dogs. Acamprosate is excreted unchanged in urine and feces.

Acamprosate was not mutagenic in the Ames test, an *in vitro* assay with human lymphocytes and an *in vivo* mouse micronucleus test. Equivocal findings were observed in a Chinese hamster cell gene mutation test. Some of the assays were not conducted using currently accepted procedures.

The sponsor submitted the results of two 2-year carcinogenicity studies in rats and mice. The sponsor did not seek concurrence for dose selection by the Exec CAC prior to initiating the studies.

Rat Carcinogenicity Study: The doses tested were 25, 100, and 400 mg/kg/day by dietary

admixture. The study used an adequate number of animals, and showed adequate survival, parameters evaluated and duration of treatment. No significant treatment-related neoplastic findings were noted. Dosing in males is considered to be adequate, as the high dose was at or near the MTD based on decreased body weight, increased incidence of tail sores, and renal effects (pelvic mineralization) in the male rats. Dosing in female rats is marginally acceptable as it is considered to be approximately one-third of the MTD based on renal effects observed in a 13-week dietary administration study at 1000 mg/kg/day.

Mouse Carcinogenicity Study: The doses tested were 25, 100, and 400 mg/kg/day by dietary admixture. The study was terminated after 91 weeks due to animal mortality that was comparable among treatment groups. Although no significant treatment-related neoplastic findings were noted, the study is considered invalid due to dosing based on the lack of any dose-limiting toxicity at the highest dose, nematode infestation which could confound the study interpretation, and evaluation of animals for tumor incidence at the low and mid-doses that was inadequate for conducting a valid trend test.

Executive CAC Recommendations and Conclusions:

Rat: The rat carcinogenicity study is acceptable. Acamprosate is not considered to be tumorigenic in this model. Dosing approximated the MTD in males and was approximately one-third of the MTD in females.

Mouse: The Executive CAC Committee concluded that the carcinogenicity study in mice is unacceptable due to inadequate dosing, nematode infestation that confounded the study interpretation, and histopathology evaluation of low and mid-dose animals that was inadequate for conducting a trend test for tumor incidence.

The Committee recommended that the sponsor repeat the mouse carcinogenicity study. The sponsor is encouraged to submit the carcinogenicity study protocol, including the supporting data and rationale for dose selection for concurrence prior to starting the study.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
Division File, HFD 170
Timothy McGovern, Ph.D./Team leader, HFD-170
Kathleen Haberny, Ph.D./Reviewer, HFD-170
Lisa Basham/PM, HFD-170
ASeifried, OND

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Joe Contrera
4/25/02 02:33:31 PM

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: AOTA-Ca (ACAMPROSATE): FERTILITY IN THE MOUSE

Key study findings:

- Acamprosate at 320, 960, and 2400 mg/kg/day by oral gavage (0.65X, 2X, and 5X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis), given for 60 days before mating in male mice and for 14 days prior to mating through gestation in female mice, had no effect on male and female fertility and no effect on fertility of the F1 offspring; therefore the NOAEL for adverse effects on fertility ≥2400 mg/kg/day PO
- Although no toxicity was observed in the maternal mice at up to 2400 mg/kg/day PO, the maximum dose exceeded the ICH recommended maximum limit dose of 2 g/kg.
- The NOAEL for maternal toxicity is >2400 mg/kg/day PO

Study no.: 1578
Volume # 30, and page #: 1
Conducting laboratory and location: [redacted]

Date of study initiation: Report date given only: July 4, 1986
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug AOTA Ca (acamprosate), **lot #** not provided, **radiolabel** none, **and % purity:** not provided
Formulation/vehicle: Acamprosate dissolved in distilled water

Methods:

Species/strain: Swiss mouse, OF 1 – I.O.P.S. [redacted]
Ages: Not provided
Weights: 25 ± 1 g females, 30 ± 1 g males
Doses employed: 0, 320, 960, 2400 mg/kg given once daily (0.1 ml/10 g bodyweight)
Route of administration: Oral by intra-esophageal administration
Study design: Pregnant females given acamprosate daily for 14 days prior to mating and throughout gestation; males used for mating were treated for 60 days prior to mating; ½ females sacrificed on gestation day 19 and fetuses removed *in utero*, ½ females delivered litters normally on gestation day 20 or 21 and offspring observed until weaned
Number/sex/group: 12 F at 0 mg/kg, 13 F at 320 mg/kg, 12 F at 960 mg/kg, and 11 F at 2400 mg/kg; number of male mice not provided
Parameters and endpoints evaluated:
- For females sacrificed on day 19: Total number of fetuses in each uterine horn, fetus's sex, weights, macroscopic fetal examination (naked eye examination for cleft palate, number of digits on fore and hind legs), number of implantations and resorptions (Salewski's Method), skeletal modifications of fetuses (Alizarine test), malformations (Wilson's test)
- For females delivered on day 20-21: number of offspring, macroscopic examination of offspring (naked eye examination for cleft palate, number of digits on fore and hind legs), offspring sex, change in number of offspring on post-delivery days 0, 7, 14, 21

and 28, body weights on post-delivery day 0, 7, 14, 21 and 28, behavioral examination (De Visu test of outside cage behavior, Actimetry test of spontaneous mobility, Traction Test of equilibrium, sight test, and whistle blast hearing test) on day 28

- F1 General study: 12 female F1 offspring fertilized by same male and observed throughout gestation and delivery
- No gross or microscopic examination of male or female parental mice

Results:

In-life observations: Females appeared normal throughout gestation, no deaths

Females sacrificed on gestation day 19:

Total number of pregnancies 12, 13, 12 and 11 at 0, 320, 960 and 2400 mg/kg/day
No abortions observed
No treatment-related effects on numbers of implantations
No treatment-related effects on numbers of resorptions
No treatment-related effects on mean number of fetuses per mouse
No treatment-related effects on fetal weights, sex ratio, and fetal malformations

Females delivered on gestation day 20 or 21:

Total number of litters 16, 12, 14 and 14 at 0, 320, 960, and 2400 mg/kg/day
No treatment-related effects on number of offspring per female littering
No treatment-related effects on number of stillborn and dead offspring after littering
No treatment-related effects on number of malformed offspring

Offspring behavioral evaluation:

No treatment-related effects on motor activity, balance, sight, hearing

Terminal and necroscopic evaluations:

No treatment-related effects on malformations (hydronephrosis, exencephaly) of the F1 offspring

No treatment-related effects on F1 female pregnancy (gestation) and offspring

Summary of individual study findings: Acamprosate treatment at 320, 960, and 2400 mg/kg/day PO (0.65X, 2X, and 5X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis), daily for 60 days prior to mating in male mice and for 14 days prior to mating through gestation in female mice, had no effect on number of pregnancies, abortions, implantations, resorptions, mean number of fetuses per littering female, number of stillborn and dead offspring after littering compared to controls. There were no treatment-related effects on the number of malformed offspring, offspring behavior, equilibrium, motor activity, balance, sight and hearing, and F1 offspring fertility in mice. The NOAEL for fertility effects was ≥2400 mg/kg/day PO. No toxicity was observed in the maternal mice at up to 2400 mg/kg/day PO, a dose that exceeds the ICH recommended limit dose of 1 g/kg.

Study title: AOTA-Ca (ACAMPROSATE): ORAL (GAVAGE) FERTILITY STUDY IN THE RAT (SEGMENT I)

Key study findings:

- Acamprosate administration in CD(SD)BR rats at 0, 50, 225, 1000 mg/kg/day by oral gavage (0.2X, 0.9X, and 4X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis) resulted in hair coat staining in the anal region in the high dose Fo males, and renal hydronephrosis in the high dose F_1 females examined on day 21 (after delivery of the F_2 pups)
- Treatment-related increase in the incidence of skeletal variations and incomplete ossification in the F1 offspring
- No effects by acamprosate on male and female fertility in the rats or their offspring; NOAEL for adverse effects on fertility in rats 1000 mg/kg/day PO
- No maternal toxicity was observed up to 1000 mg/kg/day PO, a dose achieving the ICH recommended limit dose of 1 g/kg.

Study no.: 6688-537/22
Volume # 30, and page #: 97
Conducting laboratory and location: [redacted]
Date of study initiation: October 24, 1989
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** OTA 3011, **radiolabel** none, **and % purity:** [redacted]
Formulation/vehicle: Test article in distilled water

Methods:

Species/strain: CD(SD)BR [redacted]
Doses employed: 0, 50, 225, 1000 mg/kg/day
Route of administration: Oral by gavage
Study design: Acamprosate administered for 70 days prior to mating in the males and for 14 days prior to mating through mating, gestation and lactation to necropsy in the females; F_1 offspring were not treated; ½ F_0 females sacrificed on gestation day 20 and ½ sacrificed on after F_1 offspring weaning
Number/sex/group: 30/group
Parameters and endpoints evaluated:
Mortality: 2x daily
Clinical signs: daily
Body weights: weekly
Food consumption: Every 3 days
Fo generation sacrificed on gestation day 20 (1/2 Fo females): cesarean parameters, fetal examination
Cesarean and fetal parameters: examination of ovaries and uteri, pregnancy status, number of corpora lutea, number and intrauterine position of implantations (live and dead fetuses), early and late intrauterine deaths, fetal weights, external examination, sex, visceral and skeletal examination (Alizarin technique), internal abnormalities (Wilson

technique)
Macroscopic examination and histology (ovaries, uterus, cervix, vagina, lesions, testes, epididymides, seminal vesicles, prostate, coagulating gland) performed on all adult animals
Fo and F1 Litter Parameters (1/2 Fo females allowed to litter normally): gestation duration, abnormal behavior, F1 date of parturition and behavior, number of live and dead pups born, sex of live pups, clinical condition of pups from birth to weaning, development (pinna unfolding, tooth eruption, eye opening), functional tests (surface righting reflex, grip strength), pupillary reflex, auditory response, visual placing response, learning ability, swimming maze
Necropsy: macroscopic examination, histology, total implantation sites

Results:

Mortality: None
Clinical signs: Anal hair coat staining in the males at 1000 mg/kg/day (10% in week 1, > 20% from week 3 to termination in week 19);
Body weight: No treatment-related effects
Food consumption: No treatment-related effects
Toxicokinetics: Not done

In-life observations:

No treatment-related effects on nature and frequency of female estrous cycles, mating performance and pregnancy rate in the Fo females, mating index, fertility index (male and female), and fecundity index (male and female).

No treatment-related effects on uterine/implantation parameters (number of pregnant females with live fetuses, mean numbers of corpora lutea, mean numbers of implantations and fetuses per dam, pre- and post-implantations losses, number of dead fetuses) in the females sacrificed on day 20.

Increased proportion of male fetuses in treated groups

Sex Ratio of Fetuses in Rats given Oral Acamprosate Prior to and During Gestation (Observation on Gestation Day 20)

Parameter	0 mg/kg/d	50 mg/kg/d	225 mg/kg/d	1000 mg/kg/d
Number of male fetuses	83	99	92	110
Number of female fetuses	103	87	91	87
% male fetuses	44.6	53.2	50.3	55.8
% difference from control ratio	Reference	+19%	+13%	+25%

No treatment-related effects on number of females with live pups at day 21 post-partum, mean duration of gestation, number of implantation sites, number of pups born, number of pups alive on day 1, percent male pups day 1, number of pups alive on days 4, 7, 14 and 21 post-partum, gestation index, post-implantation survival index, liver birth index, viability index, mean fetal weight on days 1, 4, 7, 14 and 21, pups physical development, and pup functional tests

No treatment-related effects on F1 male induction of pregnancy, F1 female pregnancy rate, fertility index, number of females with live pups at day 21 post-partum, number of implantation sites, number of pups born, number of pups alive on days 1, 4, 7, 14, and 21, litter loss, gestation index, post-implantation survival index, liver birth index, viability index, F1 male

and female weight gain and food intake through week 22,

Performance of F1 females significantly decreased in the left but not the right channel in the swimming maze test in week 3

Terminal and necroscopic evaluations:

No treatment-related effects observed in the male necroscopic examinations

Female F1 necroscopic examination showed kidney hydronephrosis at the high dose (33% compared to 7% in the controls)

No treatment-related effects on fetal external malformations and variations

No treatment-related effects on fetal skeletal malformations

Significant increase in fetal skeletal variations

Skeletal Variations in Fetuses of Maternal Rats given Oral Acamprosate Prior to and During Gestations (Observation on Gestation Day 20)

Parameter	0 mg/kg/d	50 mg/kg/d	225 mg/kg/d	1000 mg/kg/d
Number of fetuses examined	97	96	95	101
Number of fetuses showing skeletal variations	94	94	95	101
% Fetuses examined with variations	96.9	97.9	100.0	100.0
Difference from Control Incidence	Reference	+1%	+3%	+3%

Increase in incomplete skeletal ossification of skull (up to +49% higher in high dose fetuses compared to controls), sternebrae (up to 104% and 46% higher in mid-dose and high dose fetuses, respectively, compared to controls), ribs (3% in high dose compared to 1% in controls), limbs (up to 65%-95% higher in high dose fetuses compared to controls)

No treatment-related effects on fetal external/visceral defects

Summary of individual study findings: Acamprosate administration in CD(SD)BR rats at 0, 50, 225, 1000 mg/kg/day by oral gavage (0.2X, 0.9X, and 4X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis) resulted in hair coat staining in the anal region in the high dose Fo males, and renal hydronephrosis in the high dose F1 females examined on day 21 (after delivery of the F2 pups). The NOAEL for maternal toxicity was > 1000 mg/kg/day PO. There was an increased ratio of male to female pups in the F1 generation in all acamprosate-treated groups. There was a treatment-related increase in the incidence of skeletal variations and incomplete ossification in the F1 offspring. However, acamprosate had no effect on male and female fertility in the rats or their offspring. The NOAEL for adverse effects on fertility in rats was 1000 mg/kg/day PO acamprosate.

Study title: AOTA-Ca (ACAMPROSATE): EMBRYOTOXICITY STUDY IN THE MOUSE

Key study findings:

- No embryo-fetal toxicity by acamprosate in mice at doses of 320, 960, and 2400 mg/kg/day PO (0.65X, 2X, and 5X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis), given on gestation days 6-14
- Slight dose-related increase in numbers of fetuses reaching maturity
- Slight dose-related decrease in number of females with malformed fetuses and number of

fetuses with malformations (exencephaly, hydronephrosis)

- Although maternal toxicity was not monitored in this study, the dosing exceeded the ICH recommended limit dose of 1 g/kg.

Study no.: 1578
Volume # 32, and page #: 1
Conducting laboratory and location: [redacted]
Date of study initiation: Not provided
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** not provided, **radiolabel** none, **and % purity:** not provided
Formulation/vehicle: Test article in distilled water

Methods:

Species/strain: Swiss mouse OF.1.IOPS (weights 25 ± 1 g)
Doses employed: 0, 320, 960, 2400 mg/kg/day (0.1 ml/10g body weight)
Route of administration: Oral by intra-esophageal intubation
Study design: Pregnant female mice were dosed once daily from day 6-14 of gestation, sacrificed on gestation day 19 and fetuses removed
Number/sex/group: 40 females/group
Parameters and endpoints evaluated: Number of pregnant females, number of fetuses, fetal weight, number of fetuses reaching maturity, partial and total resorptions per litter, morphological examination, number of implantations (Salewski's method), skeletal modifications (Alizarine test), fetal malformations (Wilson's test)

Results:

In-life observations:

No treatment-related effects on number of pregnant females, number of implantations per mated female, and number of fetuses per pregnant female

Slight increase in number of partial resorptions at 960 (27, 43.8%, +107% compared to control) and 2400 (29, 50.0%, +123% compared to control) mg/kg/day; incidence in controls was 13 (34.6%)

No treatment-related effects on number of abortions (percentage of females with resorptions only)

Increased number of fetuses reaching maturity (240, 341, 432, and 427 at 0, 320, 960, and 2400 mg/kg/day, respectively)

Terminal and necroscopic evaluations:

Dams:
Slightly decreased number of females with malformed fetuses at 960 (5, 15.6%) and 2400 (4, 11.1%) mg/kg/day compared to controls (6, 23.1%)

Offspring:
No treatment-related effects on fetal weights

Slightly lower numbers of fetuses with malformations (exencephaly and hydronephrosis) at low (9/324, 2.8%), mid- (5/405, 1.2%) and high (7/387, 1.8%) dose acamprosate compared to controls (9/219, 4.1%)

Summary of individual study findings:

Oral acamprosate at doses of 320, 960, and 2400 mg/kg/day (0.65X, 2X, and 5X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis), given by oral gavage on gestation days 6-14, was not embryo-fetal toxic in mice; the NOAEL for this effect is > 2400 mg/kg. There was a slight dose-related increase in numbers of fetuses reaching maturity, and decreased number of females with malformed fetuses and fetuses with malformations. Although maternal toxicity was not monitored, the high dose of 2400 mg/kg exceeds the ICH recommended limit dose of 1 g/kg.

Study title: AOTA-Ca (ACAMPROSATE): ORAL (GAVAGE) RANGE-FINDING STUDY IN THE PREGNANT RAT

Key study findings:

- Oral acamprosate at doses of 1000, 1500, and 2000 mg/kg/day given daily during gestation days 6-15 resulted in no mortality, and no effects on body weights and food consumption
- Treatment-related clinical signs were dose-related increases in incidence of fur staining and rough coat indicating a decline in general well-being
- Treatment-related increase in percentage of pregnant females, and decrease in percent pre-implantation loss
- Dosing in the definitive rat study could, theoretically, be greater since maternal toxicity was not observed at the high dose of 2000 mg/kg. However, dosing exceeds the ICH recommended limit dose of 1 g/kg

Study no.: 6158-537/20
Volume # 32, and page #: 47
Conducting laboratory and location: [redacted]
Date of study initiation: May 24, 1989
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate **lot # TOX 100, radiolabel** none, **and % purity:** [redacted]
Formulation/vehicle: Test article in distilled water

Methods:

Species/strain: CD(SD)BR [redacted] (ages 10-12 weeks, time mated)
Doses employed: 0, 1000, 1500, 2000 mg/kg/day (5 ml/kg body weight)
Route of administration: Oral by gavage
Study design: Female rats were dosed once daily from gestation days 6-15
Number/sex/group: 7 mated females/group
Parameters and endpoints evaluated: Mortality (2x daily), clinical signs (daily), body

weights (gestation days 1, 3, 6, 9, 12, 15, 18 and 20), food consumption (gestation days 1-3, 3-6, 6-9, 9-12, 13-15, 16-18, and 18-20), necropsy: macroscopic examination on gestation day 20, uterine/implantation data: pregnancy status, number of corpora lutea, number and intrauterine position of implantations (live fetuses, early and late fetal deaths, dead fetuses), fetal observations (weights, sex, external examination)

Results:

In-life observations:

Mortality: None

Clinical signs: The results of the clinical observations are presented in the following table:

Clinical Signs in Pregnant Rats Given Oral Acamprosate*

Observation	0 mg/kg/day	1000 mg/kg/day	1500 mg/kg/day	2000 mg/kg/day
Red vaginal discharge	0	0	1 (14%)	1 (14%)
Fur staining	3 (43%)	6 (86%)	5 (71%)	7 (100%)
% difference from control	Reference	+100%	(+65%)	(+133%)
Rough haircoat	2 (29%)	4 (57%)	7 (100%)	7 (100%)
% difference from control	Reference	+100%	+250%	+250%
Sores/lesions on head	0	0	2 (29%)	1 (14%)

*n=7/group; treated daily from gestation days 6-15; value represents number of rats; percent incidence in parentheses

Vaginal discharge was observed on day 14 only.

Body weight: No treatment-related effects

Food consumption: No treatment-related effects

Toxicokinetics: Not done

Terminal and necroscopic evaluations:

Increased percentage of pregnant females (71.4, 71.4, 85.7, and 100.0 at 0, 1000, 1500, and 2000 mg/kg/day, respectively).

Slight decrease in percent pre-implantation loss at low dose (12.3%) and high dose (14.8%) compared to controls (16.9%).

Post-implantation loss (1.1-3.8%) in acamprosate treated rats only, however within historical control range (1.9-4.3%)

No treatment-related effects on litter size, number of females with live fetuses, mean number of corpora lutea per female, mean number of implantations per female, mean number of fetuses per female, number of late intrauterine deaths, number of dead fetuses, % implantations, % male fetuses, mean litter weight and mean fetal weight

Summary of individual study findings:

In the dose range finding study in female rats, oral acamprosate at doses of 1000, 15000, and 2000 mg/kg/day given daily during gestation days 6-15 produced a dose-related increase in incidence of fur staining and rough coat. There was a treatment-related increase in percentage of pregnant females, and decrease in percent pre-implantation loss. The NOAEL for maternal toxicity is > 2000 mg/kg. Thus, the high dose for the definitive study in rats could, theoretically,

be greater. However, dosing exceeds the ICH recommended limit dose of 1 g/kg.

Study title: AOTA-Ca (ACAMPROSATE): AN ORAL (GAVAGE) TERATOLOGY STUDY IN THE RAT

Key study findings:

- Dose-limiting maternal toxicity was not observed although dosing exceeded the ICH recommended limit dose of 1 g/kg.
- Treatment-related increase in number of dams with malformed fetuses (1, 3, 4 and 4 at 0, 50, 300 and 2000 mg/kg/day, respectively, n=24/group)
- Treatment-related increase in number of fetuses with malformations (1, 3, 12 and 10 at 0, 50, 300 and 2000 mg/kg/day, respectively, n=24/group); malformations were predominantly hydronephrosis, malformed iris, retinal dysplasia, edema and microphthalmia/anophthalmia, in the absence of altered maternal weight change, and differences in litter and fetal weights compared to controls
- Incidence of hydronephrosis, malformed iris, retroesophageal subclavian artery, retinal dysplasia outside the range observed in the historical data, and considered to be treatment-related
- Acamprosate induced developmental toxicity in rats based on the results of this study, with the following target organs: kidneys, eyes and vascular system.
- The developmental effects were observed at 1.2x and 8x the MRHD of 1998 mg/d in a 50 kg patient on a BSA basis.
- NOAEL for developmental toxicity 50 mg/kg/day

Study no.: 6385-537/21
Volume # 32, and page #: 140
Conducting laboratory and location: [redacted]
Date of study initiation: July 12, 1989
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** 3002, **radiolabel** none, **and % purity:** [redacted]
Formulation/vehicle: Test article in distilled water

Methods:

Species/strain: CD(SD)BR rats [redacted] weights 223-282 g, ages 12-14 weeks at mating)

Doses employed: 0, 50, 300, 2000 mg/kg/day (0, 10, 60, 400 mg/ml, 5 ml/kg body weight)

Route of administration: Oral by gavage

Study design: Mated female rats were dosed daily on gestation days 6-15; the females were sacrificed on gestation day 20 and fetuses removed; females and fetuses were examined macroscopically

Number/sex/group: 24/dose

Parameters and endpoints evaluated: morbidity and mortality (2x daily), clinical signs

(daily), body weights (gestation days 0, 6, 9, 12, 15, 18, and 20), food consumption (gestation days 0, 3, 6, 9, 12, 15, 18, and 20), necropsy (macroscopic examination on gestation day 20), uterine/implantation data (pregnancy status, number of corpora lutea, number and intrauterine position of implantations, live fetuses, early and late intrauterine deaths, dead fetuses), fetal examination (weights, sex, and external, visceral and skeletal examination [Alizarin technique] for malformations and variations)

Results:

Mortality: None

Clinical signs: Skin sores and rough haircoat were observed to a greater extent in the mid-dose and high-dose dams compared to the controls. At 0, 50, 300, and 2000 mg/kg/day acamprosate, the numbers of dams with skin lesions were 1/24, 2/24, 1/24, and 4/24, respectively; and with rough haircoat were 10/24, 9/24, 14/24, and 14/24, respectively.

Body weight: No treatment-related effects

Food consumption: No treatment-related effects

Toxicokinetics: Not done

In-life observations: No treatment-related effects on numbers of pregnant females and on uterine/implantation values (number of corpora lutea, number and intrauterine position of implantations, live fetuses, early and late intrauterine deaths, dead fetuses).

No treatment-related effects on fetal parameters (percent male fetuses, fetal weights) except that the intra-litter difference in fetal weight was higher (>2g) in one litter each in the mid- and high dose groups compared to the other litters in all four groups (<1g). The two litters with high intra-litter differences in fetal weights also showed increased incidence of malformations (described below).

Terminal and necroscopic evaluations:

Dams: No treatment-related effects observed in macroscopic examination

Offspring: Dose-related increase in external fetal malformations: The following malformations were observed

Dose Acamprosate	Dam #	Fetus #	Malformation
0 mg/kg/day	23	L4	Encephalocoele Malformed interparietal Malformed occipital Microphthalmia Schistoglossia Bifid lower jaw
50 mg/kg/day	29	L2	Retroesophageal aortic arch
	31	R2	Microphthalmia
	40	R1	Microphthalmia
300 mg/kg/day	51	R9	Microphthalmia
	55	L1	Hydronephrosis
		L2	Hydronephrosis
		R6	Hydronephrosis

	60	L1	Malformed iris Retroesophageal subclavian artery Major fusion of sternebrae
		L4	Retinal dysplasia Hydronephrosis
		L5	Malformed iris Throat/neck edema Retroesophageal aortic arch Major fusion of sternebrae
		L8	Malformed iris Major fusion of sternebrae
		R1	Malformed iris Abnormal common carotid artery Major fusion of sternebrae
		R4	Malformed iris Retroesophageal subclavian artery Major fusion of sternebrae
		R6	Retinal dysplasia Hydronephrosis
	68	L5	Anophthalmia Eye socket reduced in size
2000 mg/kg/day	78	L3	Polydactyly
	85	R9	Hydronephrosis
	91	R3	Malformed frontals and parietals
	94	L1	Malformed iris Edema (thorax/neck) Retroesophageal subclavian artery
		L5	Malformed iris Abnormal common carotid artery
		L6	Retinal dysplasia
		L8	Malformed iris
		L10	Malformed iris Edema (throat/neck)
		R3	Anophthalmia Malformed iris Retroesophageal subclavian artery
		R4	Malformed iris

The incidences of the specific external malformations are presented in the following table:

Incidence of Malformations Observed in Fetuses of Female Rats Given Acamprosate*

Malformation	0 mg/kg/d (n=273)	50 mg/kg/d (n=284)	300 mg/kg/d (n=276)	2000 mg/kg/d (n=310)	Historical Incidence (%)
Encephalocoele	1				
Malformed interparietal, parietal, occipital or frontals	1			1	
Microphthalmia	1	2	1		
Schistoglossia	1				
Bifid lower jaw	1				
Retroesophageal aortic arch		1	1		
Hydronephrosis			5 (2%)	1 (0.3%)	0.02-0.12%
Malformed Iris			5 (2%)	6 (1.9%)	0%
Retroesophageal subclavian artery		1 (0.3%)	1 (0.4%)	2 (0.6%)	0.09%
Major fusion of sternebrae			5 (2%)		0.02%
Retinal dysplasia			2 (0.7%)	1 (0.3%)	0.02%

Edema (throat/neck)			1 (0.4%)	1 (0.3%)	0.02%
Abnormal common carotid artery			1 (0.4%)	1 (0.3%)	0.02%
Anophthalmia			1 (0.4%)	1 (0.3%)	0.02%
Eye socket reduced in size			1 (0.4%)		
Polydactyly				1 (0.3%)	-

*Dosing proceeded by oral gavage daily during gestation days 6-15; percent incidence in parentheses)

Summary of individual study findings:

There was a treatment-related increase in number of dams with malformed fetuses (1, 3, 4 and 4 at 0, 50, 300 and 2000 mg/kg/day, respectively), and in number of fetuses with malformations (1, 3, 12 and 10 at 0, 50, 300 and 2000 mg/kg/day, respectively). The malformations observed in the mid- and high dose groups were hydronephrosis, malformed iris, retroesophageal subclavian artery, retinal dysplasia, edema, abnormal common carotid artery, polydactlyly, and anophthalmia. The increased number of malformations at the mid- and high doses was predominantly due to a high number of malformations observed in one litter in each group. Hydronephrosis, retroesophageal subclavian artery, retinal dysplasia, throat/neck edema, abnormal common carotid artery, and anophthalmia are observed in the historical control data, and polydactyly is known to occur spontaneously in rats. The incidence of hydronephrosis, retroesophageal subclavian artery, retinal dysplasia and malformed iris are outside the range observed in the historical data, and can be considered to be treatment-related. These effects were observed in the absence of altered maternal weight change, and differences in litter and fetal weights compared to controls. Acamprosate induced developmental toxicity in rats based on the results of this study; the target organs are the kidneys, eyes and vascular system. The effects were observed at 1.2x and 8x the MRHD of 1998 mg/d in a 50 kg patient on a BSA basis. The NOAEL for embryo-fetal development in rats was 50 mg/kg/day PO in this study. Although dose-limiting maternal toxicity was not observed, the high-dose exceeded the ICH recommended limit dose of 1 g/kg.

Study title: AOTA-Ca (ACAMPROSATE): ORAL (GAVAGE) RANGE-FINDING STUDY IN THE PREGNANT RABBIT

Key study findings:

- Doses 800, 1200, and 1600 mg/kg/day by oral gavage were 6.5x, 10x, and 13x the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis
- Maternal toxicity at all doses included soft feces and fur staining, reduced body weight gain, gross lung discoloration and hair loss (two upper doses only)
- Food consumption was decreased 18% and 16% at 1200 and 1600 mg/kg/day, respectively (days 7-19)
- Percent post-implantation loss increased at high dose (5.3%) compared to control (2.7%)
- Mean litter weights decreased 6% at high dose, mean fetal weights decreased at all doses (10%, 12%, and 5% at 800, 1200, and 1600 mg/kg/day, respectively)
- NOAEL for embryo-fetal toxicity in rabbits not established due to decreased fetal weights at the low dose of 800 mg/kg/day PO
- Results indicate that the high dose in the definitive rabbit study should not exceed 800 mg/kg.

Study no.: 6172-537/24
Volume # 33, and page #:1
Conducting laboratory and location: [redacted]
Date of study initiation: May 17, 1989
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** OTA3002, **radiolabel none, and % purity:** [redacted]
Formulation/vehicle: Test article in distilled water

Methods:

Species/strain: Pregnant New Zealand White rabbits [redacted] weights 2.7-3.38 kg)

Doses employed: 0, 800, 1200, 1600 mg/kg/day (0, 200, 300, 400 mg/ml, 4 ml/kg body weight)

Route of administration: Oral by gavage

Study design: The rabbits were dosed daily from gestation days 7-19, and sacrificed on gestation day 29 for macroscopic examination and examination of the fetuses

Number/sex/group: 5 females/dose

Parameters and endpoints evaluated: Morbidity and mortality (2x daily), clinical signs (daily), body weight (gestation days 0, ,7, 12, 19, 24 and 29), food consumption (gestation days 0, 3, 7, 10, 12, 15, 17, 19, 21, 24, 27, and 29), terminal studies (necropsy and uterine/implantation data including pregnancy status, number of corpora lutea, number and intrauterine position of implantations, live fetuses, early and late intrauterine deaths, dead fetuses), and fetal data (weights, sex, external examination)

Results:

Mortality: None

Clinical signs: Soft feces and increased frequency of fur staining (suggesting decline in general well-being) at all active doses

Body weight: Treatment-related decrease in body weight gain at all doses (800-1600 mg/kg/day) during the treatment period on gestation days 7-19. Decreased body weight gains at the end of the study (gestation day 29) at the mid- (1200 mg/kg/day) and high (1600 mg/kg/day) doses. The results of the body weight observations in the female rabbits are presented in the following table:

% Body Weight Gain in Female Rabbits Administered Acamprosate*

Observation Period	0 mg/kg/day	800 mg/kg/day	1200 mg/kg/day	1600 mg/kg/day
Days 7-19	8.8	6.2 (-30%)	2.8 (-68%)	3.4 (-61%)
Days 0-29	22.9	23.5 (+3%)	21.2 (-7%)	17.9 (-22%)

*Acamprosate administered orally on gestation days 7-19; values in parentheses represent difference compared to control value

Food consumption: Treatment-related decrease in mean food consumption from gestation days 7-19 (161, 158, 132 and 136 g food/rabbit/day at 0, 800, 1200, and 1600 mg/kg/day acamprosate, respectively). The changes in food consumption were –2%, -18%, and –16% compared to the control daily food consumption.

Toxicokinetics: Not done

In-life observations: No treatment-related effects on the uterine/implantation parameters (pregnancy status, number of corpora lutea, number and intrauterine position of implantations, live fetuses, early and late intrauterine deaths, dead fetuses) except % post-implantation loss was increased at the high dose (2.7, 2.3, 2.3 and 5.3 at 0, 800, 1200, and 1600 mg/kg/day, respectively). The numbers of fetal deaths were 1 each in the control, low and mid-dose groups and 2 in the high-dose group. There was an increase in ratio of male to female fetuses in the acamprosate treated groups (44.4, 45.2, 46.5, and 52.8 at 0, 800, 1200, and 1600 mg/kg/day, respectively), but the ratios were within historical range. Mean litter weight was slightly decreased (6%) at the high dose, and mean fetal weights were decreased at all doses without a dose-effect (10%, 12%, and 5% at 800, 1200, and 1600 mg/kg/day, respectively) compared to the mean control fetal weight.

Terminal and necroscopic evaluations:

Dams: The results of the necropsy in the dams are presented in the following table:

Results of the Necropsy in Pregnant Rabbits Administered Acamprosate*

Observation	0 mg/kg/day (n=5)	800 mg/kg/day (n=5)	1200 mg/kg/day (n=5)	1600 mg/kg/day (n=5)
Kidney mottled	1			
Kidney hydronephrosis			1	
Liver mottled		2		
Lung dark or red focus		2	2	3
Skin subcutis – hair loss	1		4	4

*Acamprosate administered by oral gavage once daily from gestation days 7-19; values represent number of dams with the finding

Offspring: No external fetal abnormalities were observed.

Summary of individual study findings: The acamprosate doses of 800, 1200, and 1600 mg/kg/day by oral gavage were 6.5x, 10x, and 13x the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis. The clinical signs of soft feces and fur staining at all doses from 800-1600 mg/kg/day acamprosate suggested decreased well-being in the female rabbits. Body weight gains were decreased 30%, 68%, and 61% at 800, 1200, and 1600 mg/kg/day, respectively, during the treatment period from gestation days 7-19. Food consumption was decreased 18% and 16% at 1200 and 1600 mg/kg/day, respectively, during the treatment period from gestation days 7-19. Treatment related dark or red focus in the lung was observed in 2/5 dams at doses of 800 and 1200 mg/kg/day, and 3/5 at 1600 mg/kg/day. Hair loss was observed in the mid- (1200 mg/kg/day) and high (1600 mg/kg/day) groups. The percent post-implantation loss was increased at the high dose (5.3%) compared to control loss (2.7%). The mean litter weights was decreased 6% at the high dose and mean fetal weights were decreased at all doses (10%, 12%, and 5% at 800, 1200, and 1600 mg/kg/day, respectively). Based on maternal toxicity observed at the lowest dose, the maximum dose used in the definitive rabbit study should not exceed 800 mg/kg.

Study title: AOTA-Ca (ACAMPROSATE): ORAL (GAVAGE) TERATOLOGY STUDY IN THE RABBIT

Key study findings:

- Maternal toxicity included 1 death and one animal sacrificed *in extremis* at 1000 mg/kg/day, dose-related soft feces and stained fur, decreased body weight gains from 8%-39% at 100-1000 mg/kg/day from gestation days 7-19, and decreased food consumption at 1000 mg/kg/day. NOAEL for maternal toxicity = 300 mg/kg
- Decreased number and percent of fetuses with malformations and variations at 1000 mg/kg/day
- No treatment-related adverse effects on embryo-fetal development were observed in rabbits administered acamprosate at doses up to 1000 mg/kg/day (8x the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis)

Study no.: 6381-537/25
Volume # 33, and page #: 109
Conducting laboratory and location: [redacted]
Date of study initiation: July 13, 1989
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** 3002, **radiolabel** None, **and % purity:** [redacted]
Formulation/vehicle: Test article in distilled water

Methods:

Species/strain: New Zealand White rabbits [redacted] ages 20 weeks, weights 2.50-3.17 kg)

Doses employed: 0, 100, 300, 1000 mg/kg/day (0, 25, 75, and 250 mg/ml)

Route of administration: Oral by gavage

Study design: The pregnant females were dosed once daily from gestation days 7-19 (inclusive), and sacrificed on gestation day 29 for necroscopic and fetal examination

Number/sex/group: 16/group

Parameters and endpoints evaluated: Morbidity and mortality (2x daily), clinical signs (daily), body weights (gestation days 0, 7, 12, 19, 24, and 29), food consumption (gestation days 0, 3, 7, 10, 12, 15, 17, 19, 21, 24, 27, and 29), and terminal studies (gestation day 29). The terminal studies included necropsy (pregnancy status, cause of death [moribund females], macroscopic examination), uterine/implantation data (pregnancy status, number of corpora lutea, number and intrauterine position of implantations, live fetuses, early and late intrauterine deaths, dead fetuses), and fetal data (weights, external examination, sex, visceral examination, skeletal examination [Alizarin technique].

Results:

Mortality: There was one death on gestation day 19 and one dam was sacrificed *in*

extremis on gestation day 22 in the high dose group. The observations in the rabbit that died were polypnea, weight loss, poor general conditions, and dark discoloration of lung lobes. The observations in the rabbit that was sacrificed on day 22 were red vaginal discharge and uterine infection. Of note, no mortality was observed in the dose range-finding study at up to 1600 mg/kg.

Clinical signs: Soft feces in 13/14 surviving rabbits in the high dose group on gestation days 7-19 and occasionally in 2/16 rabbits in the low-dose group and 4/16 rabbits in the mid-dose group during the treatment period, fur staining in the high dose group,

Body weight: The results of the body weight observations are presented in the following table:

Mean Body Weights in Pregnant Rabbits Administered Oral Acamprosate

Gestation Day	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
0	2.74	2.76	2.80	2.77
7	3.15	3,16	3.18	3.18
12	3.35	3.30	3.36	3.29
19	3.56	3.48	3.56	3.44
24	3.67	3.60	3.67	3.58
29	3.78	3.67	3.75	3.71
% Body Weight Change (gd 0-29)	38.0	33.0 (-13%)	33.9 (-11)	33.9 (-11%)
% Body Weight Change (gd 7-19)	13.0	10.1 (-12%)	11.9 (-8%)	8.2* (-39%)

*p<0.01; gd: gestation day; values in parentheses represent difference from control

Food consumption: Consumption at the highest dose was decreased 23% between treatment days 7-12, 10% between treatment days 12-19, and 16% between treatment days 7-19 compared to controls

Toxicokinetics: Not done

Terminal and necroscopic evaluations:

Dams: No treatment-related effects in the macroscopic examination
No treatment-related effects on pregnancy status, number of corpora lutea, number and intrauterine position of implantations, live fetuses, early and late intrauterine deaths, and dead fetuses

Offspring: No treatment-related effects on number of male and female fetuses, % male fetuses, mean litter weight, and mean fetal weight
No treatment-related effects on number and percent of fetuses with external and visceral malformations and variations
Decreased number of fetuses with skeletal malformations (2 and 1 at 0 and 1000 mg/kg/day, respectively) and variations (107 and 100 at 0 and 1000 mg/kg/day, respectively)
Decreased percent fetuses examined with malformations (1.8% and 0.9% at 0 and 1000 mg/kg/day, respectively) and variations (96.4% and 93.5% at 0 and 1000 mg/kg/day, respectively)

Summary of individual study findings: There was 1 maternal death at 1000 mg/kg/day. Dose-related soft feces and stained fur, decreased body weight gains (from 11%-13% at 100-1000 mg/kg/day during the treatment period and from 8%-39% at 100-1000 mg/kg/day from gestation days 0-29), and decreased food consumption at 1000 mg/kg/day were observed in the dams. The number and percent of fetuses with malformations and variations was lower at the high dose of 1000 mg/kg/day than in the controls. There were no treatment-related adverse effects on embryo-fetal development in rabbits administered acamprosate at up to 1000 mg/kg/day (8x the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis). Based on the results of this study, acamprosate is not considered to be teratogenic in rabbits; NOAEL for developmental effects = 1000 mg/kg. The NOAEL for maternal toxicity is 300 mg/kg.

Study title: AOTA-Ca (ACAMPROSATE): EMBRYOTOXICITY STUDY IN THE RABBIT

Key study findings:

- Doses studied (200, 400, 800 mg/kg/day PO) were 1.5x, 3x and 6x the MRHD of 1998 mg/day in a 50 kg patients on a BSA basis.
- Decreased fetal weights at 400 and 800 mg/kg/day by oral gavage (-11% in both groups compared to controls)
- Non-dose-related increased number and percent of fetal malformations at 400 mg/kg/day (7/92 fetuses malformed, 7.6%) and 800 mg/kg/day (2/94 fetuses, 2.1%) compared to controls (1/94 fetuses, 1.1%); not dose-related
- Malformations included torsion of vertebrae (1 fetus) and hydronephrosis (4 fetuses in one litter and 2 fetuses in one litter) at the mid-dose and hydronephrosis in 1 fetus each in 2 litters at the high dose
- NOAEL for developmental effects = 200 mg/kg

Study no.: 1578
Volume # 33 and page #: 282
Conducting laboratory and location: [redacted]
Date of study initiation: Not provided, report date January 1989
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** 1395/11, **radiolabel** none, **and % purity:** Not provided
Formulation/vehicle: Test article dissolved in distilled water

Methods:

Species/strain: Standard female Burgundy Tawny rabbits (weights 3.2 ± 0.2 kg)
Doses employed: 0, 200, 400, 800 mg/kg/day (0.5-2 ml/kg)
Route of administration: Oral by intra-esophageal gavage
Study design: Pregnant rabbits were dosed once daily during gestation days 8-16 inclusive, and were sacrificed on gestation day 29; fetuses were removed for examination
Number/sex/group: 13, 13, 15 and 13 at 0, 200, 400, and 800 mg/kg/day
Parameters and endpoints evaluated: Number of females with only resorptions, mean

pregnancy rate, number of females with fetuses and resorptions, number of females with fetuses only, number of females with malformed fetuses, fetal number, number of fetuses reaching maturity, weight, sex, number of implantations and resorptions (Salewski's method), external morphological examination, visceral malformations (Wilson's test), skeletal malformations and variations (Alizarine test)

Results:

Mortality: No deaths
Clinical signs: Not done
Body weight: Not done
Food consumption: Not done
Toxicokinetics: Not done

In-life observations: No treatment-related effect on pregnancy rate
Terminal and necroscopic evaluations:

Dams: No treatment-related effects on number of fetuses per litter, number of implantations, number of partially resorbed litters, number of females without resorptions, number of females with fetuses and resorptions, numbers of partial and total resorptions per litter, number of females with fetuses only, abortions,
Offspring: Decreased mean fetal weight at 400 (28.8 g, -11%) and 800 (28.8g, -11%) mg/kg/day compared to control (32.5 g). No dose-related effects by acamprosate on the number of fetuses reaching maturity, number of sex ratio, macroscopic, visceral and skeletal malformations and variations. An increase in treatment-related fetal malformations was observed although the increase was not dose-related. The findings are presented in the following table:

Fetal Malformations in the Rabbit

	0 mg/kg/day	200 mg/kg/day	400 mg/kg/day	800 mg/kg/day
Number of females with malformed fetuses	1 (7.7%)	0 (0%)	3 (20%)	2 (15%)
Percent malformations per group	1.1% (1/94 fetuses)	0%	7.6% (7/92 fetuses)	2.1% (2/94 fetuses)
Total Malformations per group	"1 Malformed fetus"		1 Torsion of vertebrae 4 Hydronephroses 2 Hydronephroses	1 Hydronephrosis 1 Hydronephrosis

Summary of individual study findings: Acamprosate slightly decreased fetal weights at 400 and 800 mg/kg/day by oral gavage (-11% in both groups compared to controls). There was an increase in the number of females with malformed fetuses at 400 mg/kg/day (3 females) and 800 mg/kg/day (2 females) acamprosate compared to controls (1 female with malformed fetus). The malformations at the mid and high doses were torsion of vertebrae (1 MD fetus) and

hydronephrosis (4 fetuses in one female and 2 fetuses in one female at 400 mg/kg/day, and 1 fetus each in 2 females at 800 mg/kg/day). The doses studied (200, 400 and 800 mg/kg/day PO) were 1.5x, 3x and 6x the MRHD of 1998 mg/day in a 50 kg patients on a BSA basis. The NOAEL for embryo-fetal toxicity in rabbits was 200 mg/kg/day PO in this study. Maternal toxicity was not indicated although the high dose is not significantly below the ICH recommended limit dose of 1 g/kg and maternal toxicity was observed in New Zealand white rabbits at 800 mg/kg in a dose-range finding study.

Study title: ORAL STUDY OF THE EFFECTS OF AOTA-Ca (ACAMPROSATE) ON SEGMENT II OF REPRODUCTION IN THE RABBIT

Key study findings:

- Study conducted with New Zealand white rabbits to further investigate potential for embryo-fetal toxicity following observations of hydronephrosis at 400 and 800 mg/kg/day PO acamprosate in a previous study (study #1578) with female Burgundy Tawny rabbits.
- The NOAEL for embryo-fetal toxicity was 800 mg/kg/day (6x the MRHD of 1998 mg/d in a 50 kg patients on a BSA basis) by oral gavage given daily during gestation days 8-16 in rabbits; consistent with another study in New Zealand White rabbits (NOAEL of 1000 mg/kg)

Study no.: 2273
Volume # 34 and page #: 1
Conducting laboratory and location: [

]
Date of study initiation: October 7, 1987
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate**, lot #** 1395/11**, radiolabel** none**, and % purity:** [ ]
Formulation/vehicle: Test article dissolved in distilled water

This study was conducted to re-examine the potential embryo-fetal toxicity of acamprosate in rabbits after a visceral malformation (hydronephrosis) was observed at the rate of 7.6% (7/92 fetuses) in the mid-dose group (400 mg/kg/day) in a previous study (#1578 above; doses of 200-800 mg/kg/day PO). Of note, the previous study assessed female Burgundy Tawny rabbits while the current study uses New Zealand white rabbits.

Methods:

Species/strain: Standard female New Zealand rabbits (weights 3.25-3.44 kg)
Doses employed: 0, 200, 400, 800 mg/kg/day (0.5-2 ml/kg)
Route of administration: Oral by intra-esophageal gavage
Study design: Pregnant rabbits were dosed once daily during gestation days 8-16 inclusive, and were sacrificed on gestation day 29; fetuses were removed for examination
Number/sex/group: 13, 13, 12, and 13 at 0, 200, 400, and 800 mg/kg/day
Parameters and endpoints evaluated: Mortality, body weights (days 8, 16, 29), mean pregnancy rate, fetal number, weight, sex, and morphological examination, total number

of implantations and resorptions (Salewski's method), fetal malformations (Wilson's test)

Results:

Mortality: No deaths
Clinical signs: Not done
Body weight: No treatment-related effects
Food consumption: Not done
Toxicokinetics: Not done

In-life observations: No treatment-related effect on pregnancy rate
Terminal and necroscopic evaluations:

Dams: No treatment-related effects on number of fetuses per litter, number of implantations, resorptions,
Offspring: No treatment-related effects on the number of fetuses reaching maturity, mean fetal weight, sex ratio, macroscopic and visceral malformations and variations. The internal malformations are presented in the following table:

	0 mg/kg/day (n=87)	200 mg/kg/day (n=85)	400 mg/kg/day (n=81)	800 mg/kg/day (n=91)
Number of malformed fetuses	3	2	0	3
Percent malformed fetuses	3.4	2.3	0	3.3
Malformations	Exencephalia/coelosoma/ modified hind leg Unilateral retinal detachment Hydrocephalia	Unilateral agenesis Single kidney	None	Abdominal fistula Anencephalia/sundactylia/ absent digits Unilateral retinal detachment

Summary of individual study findings:

This study was conducted to re-examine the potential embryo-fetal toxicity of acamprosate in rabbits after a visceral malformation (hydronephrosis) was observed at the rate of 7.6% (7/92 fetuses) in the mid-dose group (400 mg/kg/day) in a previous study (#1578 above) at the dose of 200-800 mg/kg/day PO. Abdominal fistula was observed in one high dose (800 mg/kg/day, 6X MRHD) fetus in the absence of other toxicity, and is not considered to be treatment-related. Hydronephrosis was not observed in this study. The NOAEL for embryo-fetal toxicity by acamprosate in this study was 800 mg/kg/day PO, consistent with the results of another study in New Zealand rabbits (NOAEL of 1000 mg/kg). Maternal toxicity was not indicated although the high dose is not significantly below the ICH recommended limit dose of 1 g/kg and maternal toxicity was observed in New Zealand white rabbits at 800 mg/kg in a dose-range finding study.

Study title: AOTA-Ca (ACAMPROSATE): PERI-NATAL STUDIES IN THE MOUSE

Key study findings:

- Doses studied (320, 960, and 2400 mg/kg/day by oral gavage) were approximately 0.5x, 2x, and 5x the MRHD of 1998 mg/d in a 50 kg patient on a BSA basis.
- Increased incidence of still-born offspring and increased number and percent females with offspring dying after birth
- No treatment-related effects on maternal performance of mice (F_0 generation) and their offspring (F_1 generation) during gestation and lactation
- No treatment-related effects on behavioral function (activity, balance, behavior, sight and hearing) of the F_1 and F_2 generation offspring
- NOAEL for adverse effects on prenatal and postnatal development by acamprosate in mice was 320 mg/kg/day PO under the conditions of this study

Study no.: 1578
Volume # 34, and page #: 125
Conducting laboratory and location: [ ]
Date of study initiation: Not provided, report date January 1989
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** 1395/11, **radiolabel** none, **and % purity:** [ ]
Formulation/vehicle: Test article dissolved in distilled water

Methods:

Species/strain: Swiss Mouse, OF.1.-IOPS
Doses employed: 0, 320, 960, 2400 mg/kg/day
Route of administration: Oral by gavage
Study design: The pregnant mice were dosed once daily from gestation day 15 through day 28 of lactation; the mice littered on gestation days 20 or 21
Number/sex/group: 24 females/group (weights 25 ± 1 g)
Parameters and endpoints evaluated: Examination of still-born offspring (F_1), number of offspring, number of offspring deaths and disappearances, offspring macroscopic examination and weights (days 0 [birth], 7, 14, 21, and 28), sex, behavioral tests (day 28) including De Visu (activity outside cage), actimetry (spontaneous mobility), traction test (recovery and equilibrium by grasping horizontal metallic thread), sight (ophthalmoscopy) and hearing (reaction to whistle blast), offspring reproductive function (number of F_2 offspring, number of malformed and still-born F_2 offspring)

Results:

Mortality: No deaths
Clinical signs: Not done
Body weight: Not done
Food consumption: Not done
Toxicokinetics: Not done

In-life observations:

Dams: Treatment-related increase in number and percent females delivering still-born

offspring (1 [5.3%], 1 [5.0%], 4 [18.2%] and 3 [13.0%] at 0, 320, 960, and 2400 mg/kg/day, respectively)
Slight increase in number and percent females with offspring dying after birth in the low and mid doses (14 [74%], 18 [90%], 21 [95%], and 17 [74%] at 0, 320, 960, 2400 mg/kg/day, respectively)
No treatment-related effects on number of malformed offspring per female, number of fetuses at birth and at the end of the observation period (postnatal day 28)
Offspring: Increase in number of still-born offspring (1 [0.4%], 2 [0.9%], 4 [1.5%], and 4 [1.4%] at 0, 320, 960, and 2400 mg/kg/day, respectively)
No treatment-related effects on male and female weights and growth rate, and on behavioral parameters of activity level, balance, sight and hearing
No treatment-related effects on delivery of F2 offspring
No treatment-related effects on behavior, motor activity, balance, sight and hearing of the F2 offspring

Terminal and necroscopic evaluations:

Dams: Not done
Offspring: No malformations in the mid and high dose F1 offspring; twisted tails in 2 offspring at 320 mg/kg/day not considered to be treatment related.
No treatment-related effects on macroscopic observations in the F2 offspring

Summary of individual study findings: The doses studied (320, 960, and 2400 mg/kg/day by oral gavage) were approximately 0.5x, 2x, and 5x the MRHD of 1998 mg/d in a 50 kg patient on a BSA basis. Acamprosate, administered during gestation days 15 through birth (gestation day 20 or 21), and throughout lactation (through postnatal day 28), produced an increased incidence of still-born offspring and increased number and percent females with offspring dying after birth at 960 and 2400 mg/kg/day. There were no treatment-related effects on maternal performance of mice (F_0 generation) and their offspring (F_1 generation) during gestation and lactation, and no treatment-related effects on behavioral function (activity, balance, behavior, sight and hearing) of the F_1 and F_2 generation offspring. The NOAEL for adverse effects on prenatal and postnatal development by acamprosate in mice was 320 mg/kg/day PO under the conditions of this study.

Study title: AOTA-Ca (ACAMPROSATE): ORAL (GAVAGE) PERI AND POST-NATAL STUDY IN THE RAT

Key study findings:

- Acamprosate given at doses of 50, 300 and 2000 mg/kg/day (up to 8x the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis) by oral gavage in maternal rats, daily from gestation day 14 through lactation day 20
- Slightly higher pup weights measured on post-partum days 4 and 7 in the mid-dose and high dose groups compared to controls
- NOAEL for prenatal and postnatal development in rats 2000 mg/kg/day PO
- NOAEL for maternal toxicity > 2000 mg/kg; high dose exceeds the ICH recommended limit dose of 1 g/kg.

Study no.: 6494-537/23

Volume # 35, and page #: 1
Conducting laboratory and location: [redacted]
Date of study initiation: Not provided, Report date October 1991
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** OTA3011, **radiolabel** none, **and % purity:** [redacted]
Formulation/vehicle: Test article dissolved in distilled water

Methods:

Species/strain: CD(SD)BR[redacted] (ages 11-13 wks, weights 200-286 g)
Doses employed: 0, 50, 300, 2000 mg/kg/day
Route of administration: Oral by gavage
Study design: The rats were dosed daily from gestation day 14 through lactation day 20, and were sacrificed on lactation day 21
Number/sex/group: 24 mated females/group
Parameters and endpoints evaluated: Morbidity and mortality (2x daily), clinical signs (daily), body weights (gestation days 0, 6, 10, 14, 17, and 20, and lactation days 1, 7, 14 and 21), food consumption (gestation days 0, 3, 6, 8, 10, 14, 17, and 20, and lactation days 1, 4, 7, 10, 14, 17, 19, and 21), date of mating and parturition, gestation duration, abnormal behavior, number of pups born live and dead, number and sex of live pups (post-partum days 1, 4, 7, 14 and 21), clinical signs of pups (daily), litter weights (post-partum days 1, 4, and 21), developmental parameters in the pups (pinna unfolding, tooth eruption, eye opening), functional tests in the pups (righting reflex, grip reflex, pupillary reflex, auditory response, visual placing response), necropsy (day 21, females and pups, macroscopic examination for structural or pathological changes), number of implantations per uterus.

Results:

Mortality: No maternal deaths
Clinical signs: No treatment-related effects
Body weight: No treatment-related effects
Food consumption: No treatment-related effects
Toxicokinetics: Not done

In-life observations:

Dams:
No treatment-related effects on pregnancy incidence and duration of gestation
No treatment-related effects on mean litter size

Offspring:
No treatment-related effects on survival to lactation day 21
No treatment-related effects on percent male offspring
Increased mean pup weight on post-partum days 4 and 7 compared to controls in the mid-dose and high dose pups. The mean pup weights are presented in the following table:

Mean Pup Weights (g)*

Post-partum Day	0 mg/kg/day	50 mg/kg/day	300 mg/kg/day	2000 mg/kg/day
1	6.1	6.1 (+0%)	6.2 (+2%)	6.5 (+7%)
4	8.6	8.8 (+2%)	9.3 (+8%)*	9.6 (+12%)*
7	14.8	15.0 (+1%)	15.5 (+5%)	15.8 (+7%)
14	31.7	32.4 (+2%)	33.3 (+5%)	33.0 (+4%)
21	50.2	51.0 (+2%)	52.8 (+5%)	52.3 (+4%)

*p<0.05; Values in parentheses represent percent difference from controls

No treatment-related clinical signs
No treatment-related effects on tooth eruption
Earlier attainment of pinna unfolding and eye opening at the high dose. The results of the treatment-related effects on developmental parameters in the pups are presented in the following table:

Physical Development in Pre-weaned Pups

Pinna Unfolding (% Litters on Day Post-Partum)					
Acamprosate Dose (mg/kg/d)	Pups Attaining Criterion (%)	Day 2	Day 3	Day 4	Day 5
0 mg/kg/d	50	10	62	100	
	75	0	38	100	
	100	0	0	90	100
50 mg/kg/d	50	4	63	100	
	75	4	33	96	100
	100	0	4	92	100
300 mg/kg/d	50	25	67	100	
	75	8	38	100	
	100	0	8	100	
2000 mg/kg/d	50	22	65	100	
	75	4	52	100	
	100**	4	35	100	
Eye Opening (% Litters on Day Post-Partum)					
	Pups Attaining Criterion (%)	Day 13	Day 14	Day 15	Day 16
0 mg/kg/d	50	0	43	100	100
	75	0	14	90	100
	100	0	10	81	100
50 mg/kg/d	50	0	25	96	100
	75	0	17	92	100
	100	0	8	71	100
300 mg/kg/d	50	0	29	96	100
	75	0	8	88	100
	100	0	4	79	100
2000 mg/kg/d	50*	22	70	100	
	75**	13	57	100	
	100*	4	43	87	100

*p<0.05; **0<0.01

No treatment-related effects on mean scores for surface righting, grip reflex, auditory startle response, pupillary reflex, and visual placing

Terminal and necroscopic evaluations:

Dams: No treatment related effects observed in the necropsy on day 21
Offspring: No treatment-related effects observed in the necropsy on day 21

Summary of individual study findings: Acamprosate given at doses of 50, 300 and 2000 mg/kg/day (up to 8x the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis) by oral gavage in maternal rats, daily from gestation day 14 through lactation day 20, resulted in slightly higher pup weights measured on post-partum days 4 and 7 in the mid-dose and high dose groups compared to controls. Also, pinna unfolding and onset of eye opening, two criteria of post-partum development, were attained slightly earlier at the mid and high doses. These effects are not considered to be adverse developmental effects by acamprosate. NOAEL for prenatal and postnatal development in rats 2000 mg/kg/day PO. The NOAEL for maternal toxicity is > 2000 mg/kg. Dosing exceeded the ICH recommended limit dose of 1 g/kg.

Reproductive and developmental toxicology summary:

The results of the oral fertility studies in mice and rats are summarized in the following table.

Species/Strain	Oral Acamprosate Dose (mg/kg/d)	Duration	Maternal Toxicity	Fertility/Other Effects	Reference
Mouse (Swiss OF.1-IOPS) n=25-28/grp)	0,320,960, 2400	M :60 d before mating, F: 14 d before mating thru gestation	None	None	Study 1578 Vol. 30
Rat CD(SD)BR) n=30/sex)	0,50,225, 1000	M:70 d before mating thru mating to sacrifice, F: 14 d before mating thru lactation to sacrifice	None	No effects on fertility. At 1000: hydronephrosis in females. All grps: slight skeletal ossification.	Study 6688-537/22 Vol. 30

In mice administered acamprosate at 320, 960, and 2400 mg/kg/day (0.65X, 2X, and 5X the MRHD on a BSA basis) the NOAEL for fertility and for general toxicity in male and female mice was ≥2400 mg/kg/day PO.

Acamprosate administration in CD(SD)BR rats at 0, 50, 225, 1000 mg/kg/day (0.2X, 0.9X, and 4X the MRHD on a BSA basis) resulted in hair coat staining in the anal region in the high dose Fo males, and renal hydronephrosis in the high dose F1 females. There was a treatment-related increase in the incidence of skeletal variations and incomplete ossification in the F1 offspring. However, acamprosate had no effect on male and female fertility in the rats or their offspring. The MTD for maternal toxicity was 1000 mg/kg/day and the NOAEL for fertility effects was 1000 mg/kg/day.

The results of the embryo-fetal developmental studies on oral acamprosate in mice, rats and rabbits are summarized below.

Species/Strain	Oral Acamprosate Dose (mg/kg/d)	Duration	Maternal Toxicity	Embryo/Feto-toxicity	Teratogenicity	Ref.

Mouse (Swiss) n=40 /dose	0,320,960, 2400	Gestation days 6-14	None	960 & 2400: increased partial resorptions & fetuses	Not significant	Study 1578 Vol. 32
Rat CD(SD)BR) n=7 /dose	Dose-range-finding: 0,100,1500, 2000	Gestation days 6-15	None	None	Not significant	Study 6158-537/20 Vol. 32
Rat CD(SD)BR) n=24 /dose	0,50,300,2000	Gestation days 6-15	None	skeletal variations, hydro-nephrosis, malformed iris, retroesopha-geal subclavian artery, retinal dysplasia	Not significant	Study 6385-537/21 Vol. 32
Rabbit (New Zealand white) n=5 /dose	Dose-range-finding: 0,800,1200, 1600	Gestation days 7-19	All treated grps: soft feces, food consumption , body wt gain , dark lung lobe foci, mottled liver lobes	1600: % post-implantation loss , non-dose-related in fetal wt	Not significant	Study 6172-537/24 Vol. 33
Rabbit (New Zealand white) n=16 /dose	0,100,300, 1000	Gestation days 7-19	1000: soft feces, food consumption , body wt gain	None	Not significant	Study 6381-537/25 Vol. 33
Rabbit (Burgundy tawny) n=13-15 /dose	0,200,400,800	Gestation days 8-16	None	400,800: slight in fetal wt, 400: hydronephrosis incidence	Not significant	Study 1578 Vol. 33
Rabbit (New Zealand white) n=13 /dose	0,200,400,800	Gestation days 8-16	None	None	Not significant	Study 2273 Vol. 34

Acamprosate, administered by oral gavage at doses of 320, 960, and 2400 mg/kg/day (0.65X, 2X, and 5X the MRHD on a BSA basis) on gestation days 6-14 in mice resulted in a slight dose-related increase in the numbers of fetuses reaching maturity, and a slight decrease in the number of females with malformed fetuses and fetuses with malformations. Maternal toxicity was not monitored.

A dose range finding study in female rats at doses of 1000, 15000, and 2000 mg/kg/day produced no significant maternal toxicity although the dose exceeded the ICH recommended limit dose. There was a treatment-related increase in percentage of pregnant females, and decrease in percent pre-implantation loss. In the definitive study in rats, there was a treatment-related increase in the number of dams with malformed fetuses (1, 3, 4 and 4 at 0, 50, 300 and 2000 mg/kg/day, respectively), and in number of fetuses with malformations (1, 3, 12 and 10 at 0, 50, 300 and 2000 mg/kg/day, respectively). The malformations observed in the mid- and high dose groups were hydronephrosis, malformed iris, retroesophageal subclavian artery, retinal dysplasia, edema,

abnormal common carotid artery, polydactlyly, and anophthalmia. The increased number of malformations was due to a high number of malformations observed in one litter in each group. The incidence of hydronephrosis, malformed iris, retroesophageal subclavian artery, retinal dysplasia was outside the range observed in the historical data, and can be considered treatment-related. These effects were observed in the absence of altered maternal weight change, and differences in litter and fetal weights compared to controls. Acamprosate is considered to be embryotoxic in rats based on the results of this study, and the target organs are the kidneys, eyes and vascular system. The embryotoxicity was observed at 1.2x and 8x the MRHD on a BSA basis. The NOAEL for embryo-fetal development in rats was 50 mg/kg/day PO in this study.

In the dose range-finding study in pregnant New Zealand White rabbits, acamprosate was administered at doses of 800, 1200, and 1600 mg/kg/day by oral gavage. The clinical signs of soft feces and fur staining at all doses from 800-1600 mg/kg/day acamprosate suggested decreased well-being in the female rabbits. Body weight gains were decreased (30%, 68%, and 61% at 800, 1200, and 1600 mg/kg/day, respectively) during the treatment period from gestation days 7-19; food consumption was decreased (18% and 16% at 1200 and 1600 mg/kg/day, respectively). Treatment related dark or red focus in the lung was observed in 2/5 dams at doses of 800 and 1200 mg/kg/day, and 3/5 at 1600 mg/kg/day. Hair loss was observed in the mid- (1200 mg/kg/day) and high (1600 mg/kg/day) groups. The percent post-implantation loss was increased at the high dose (5.3%) compared to control loss (2.7%). The mean litter weights was decreased 6% at the high dose and mean fetal weights were decreased at all doses (10%, 12%, and 5% at 800, 1200, and 1600 mg/kg/day, respectively). Based on the results of this study the maximum dose in the definitive rabbit study should not exceed 800 mg/kg/day. In the definitive study, pregnant rabbits were administered acamprosate by oral gavage at doses of 100, 300 and 1000 mg/kg/day from gestation days 7-19. There was 1 maternal death and one animal sacrificed *in extremis* at 1000 mg/kg/day. Dose-related soft feces and stained fur, decreased body weight gains (11%-13% at 100-1000 mg/kg/day during the treatment period, and 8%-39% at 100-1000 mg/kg/day from gestation days 0-29), and decreased food consumption at 1000 mg/kg/day were observed in the dams. The number and percent of fetuses with malformations and variations was lower at the high dose of 1000 mg/kg/day than in the controls. There were no treatment-related adverse effects on embryo-fetal development in rabbits administered acamprosate; NOAEL for developmental effects = 1000 mg/kg/day (8x the MRHD on a BSA basis).

Two earlier developmental studies were performed in Burgundy Tawny and New Zealand White rabbits. In Burgundy Tawny rabbits, acamprosate administration at 200, 400, and 800 mg/kg/day (1.5x, 3x and 6x the MRHD on a BSA basis) by oral gavage on gestation days 8-16 resulted in slightly decreased fetal weights at 400 and 800 mg/kg/day (-11% in both groups compared to controls). There was an increase in the number of females with malformed fetuses at 400 mg/kg/day (3 females) and 800 mg/kg/day (2 females) acamprosate compared to controls (1 female with malformed fetus). The malformations were torsion of vertebrae (1 fetus) and hydronephrosis (4 fetuses in one female and 2 fetuses in one female at 400 mg/kg/day, and 1 fetus each in 2 females at 800 mg/kg/day). The NOAEL for embryo-fetal toxicity in rabbits was 200 mg/kg/day PO in this study. Maternal toxicity was not monitored.

The effects in the Burgundy tawny rabbits were re-examined in New Zealand white rabbits. No treatment-related embryo-fetal toxicity was observed by acamprosate given at up to 800 mg/kg/day (3x the MRHD on a BSA basis) during gestation days 6-18 in rabbits, under the

conditions of this study. Therefore the NOAEL for embryo-fetal toxicity by acamprosate in this study was 800 mg/kg/day PO.

The results of the Segment III oral (gavage) studies on perinatal and postnatal development in mice and rats are presented in the following table.

Species/Strain	Oral Acamprosate Dose (mg/kg/d)	Duration	Maternal Toxicity	Perinatal/Postnatal Toxicity	Reference
Mouse (Swiss OF.1.IOPS) n=24 /dose	0,320,960, 2400	gestation d 15 thru parturition	None	Increased incidence of still-born offspring at MD and HD	Study 1578 Vol. 34
Rat CD(SD)BR) n=24 /dose	0,50,300, 2000	gestation d 14 thru lactation d 21	None	Not significant	Study 6494-537/23 Vol. 35

In mice administered doses of 320, 960, and 2400 mg/kg/day (0.5x, 2x, and 5x the MRHD on a BSA basis) during gestation days 15 through birth and throughout lactation (through postnatal day 28), the observations were increased incidence of still-born offspring and increased number and percent females with offspring dying after birth at 960 and 2400 mg/kg/day. There were no treatment-related effects on maternal performance of mice (Fo generation) and their offspring (F1 generation) during gestation and lactation, and no treatment-related effects on behavioral function (activity, balance, behavior, sight and hearing) of the F1 and F2 generation offspring. The NOAEL for adverse effects on prenatal and postnatal development by acamprosate in mice was 320 mg/kg/day PO under the conditions of this study.

Acamprosate given at doses of 50, 300 and 2000 mg/kg/day (up to 8x the MRHD on a BSA basis) in maternal rats, daily from gestation day 14 through lactation day 20, resulted in slightly higher pup weights measured on post-partum days 4 and 7 in the mid-dose and high dose groups compared to controls. Also, pinna unfolding and onset of eye opening were attained slightly earlier at the mid and high doses; effects are not considered to be adverse developmental effects. The NOAEL for pre- and postnatal development in rats is considered to be ≥2000 mg/kg/day PO.

Reproductive and developmental toxicology conclusions: Acamprosate produced no adverse effects on fertility in oral studies in rats and mice. In the rats, the highest dose was associated with fetal toxicity (slight decreases in mean fetal weights and skeletal ossification and hydronephrosis). Effects on embryo-fetal development were observed in rats and included hydronephrosis, malformed iris, retroesophageal subclavian artery, and retinal dysplasia. No effects were noted in mice or New Zealand White rabbits. However, an increased incidence of renal hydronephrosis was observed in Burgundy Tawny rabbits. No effects on perinatal or postnatal development were observed in mice or rats. However, an increased incidence of still-born offspring was noted in the mouse study.

The nonclinical developmental findings associated with acamprosate should be considered in relation to the known reproductive effects of ethyl alcohol, which include the characteristics of

fetal alcohol syndrome (craniofacial dysmorphism, intrauterine and postnatal growth retardation, retarded psychomotor and intellectual development) and milder forms of neurological and behavioral disorders in humans.

Labeling recommendations: Based on embryo-fetal development effects observed in rats and rabbits and increase in still-born mice, the Pregnancy Category for this drug should be Category C. This recommendation and associated findings will be reflected in the label.

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: ACAMPROSATE: AND MK-801 NEUROTOXICITY STUDY BY A SINGLE ADMINISTRATION TO CD RATS

Key study findings:

- No neurotoxic effects by acamprosate in the retrosplenial and posterior cingulate (RS/PC) cortices in rats at 2000 mg/kg by oral gavage, measured at 4, 12, and 24 hours after dosing
- Study validity demonstrated by necrosis and microglia in the RS/PC by the positive control, MK-801

Study no: 203/000127
Volume # 35, and page #: 213
Conducting laboratory and location: [redacted]

Date of study initiation: 22-31 August, 2000
GLP compliance: Yes
QA reports: yes (x) no ( ):
Drug Acamprosate, **lot #** M202C, **radiolabel** none, **and % purity:** [redacted]
Formulation/vehicle: Test article dissolved in distilled water

Methods (unique aspects):
Dosing:

Species/strain: CD®BR rats[redacted]
#/sex/group or time point (main study): 4/sex/treatment/timepoint
Satellite groups used for toxicokinetics or recovery: None
Age: 40-44 days
Weight: 97.2-133.5 g males and 131.7-152.5 g females
Doses in administered units: 2000 mg/kg single dose
Route, form, volume, and infusion rate: Oral gavage, 200 mg/ml, 10 ml/kg
Negative Control Article: Isotonic saline 0.9% (5 ml/kg SC)
Positive Control Article: MK-801 [redacted] Batch 5A/18526, 5 mg/kg SC)

Observations and times:

Clinical signs: Once daily
Body weights: Day of treatment and prior to necropsy
Food consumption: Once daily

Histopathology: Perfused (heparinized phosphate buffer) brains were isolated at 4, 12 and 24 hours after dosing, immersed in 10% neutral buffered formalin containing 1% zinc sulfate (24 h), and trimmed (2mm slices) through the posterior cingulate and retrosplenial cortices, dehydrated, embedded in paraffin, and sectioned at 5 mcm; sections were stained with either hematoxylin and eosin, or for Glial Fibrillary Acidic Protein, or Griffonia simplificifolia B4 lectin (GSL 1-B4) according to the method of Fix *et al.* (1996); sections were examined by light microscopy.

Toxicokinetics: Not done

Results:

Mortality: No deaths

Clinical signs: No treatment-related effects by acamprosate; MK-801 resulted in reduced muscle tone, muscle spasms, reduced awareness

Body weights: No treatment-related effects by acamprosate; mean 33g loss in females and 5g loss in males given positive control article, MK-801

Food consumption: No treatment-related effects in the rats that received acamprosate; 43% decrease in males and 90% decrease in females given positive control article, MK-801

Histopathology: No treatment-related effects in the rats given acamprosate; positive control (MK-801) treated rats (75% males and 100% females) showed minimal to moderate neuronal necrosis in the retrosplenial cortex (layers 3 and 4) at 24 hours after dosing, and microglia in layers 3 and 4 of the retrosplenial cortex (50% females) at 24 hours after dosing.

Summary of individual study findings: Acamprosate, administered to rats at the dose of 2000 mg/kg by oral gavage, produced no evidence of neuronal vacuolation, necrosis, or microglia in the retrosplenial and posterior cingulate cortices, measured at 4, 12, and 24 hours after dosing. The study was conducted according to the method of Fix *et al.* (1996), to demonstrate the potential for non-competitive NMDA receptor antagonists to induce the neurotoxic effect known as the "Olney Lesion". The validity of the study was demonstrated by the observation of neuronal necrosis and microglia in the retrosplenial cortex by the positive control article, MK-801, at 24 hours after dosing.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Overall Summary and Evaluation: Acamprosate is being studied in the United States as a treatment for alcohol dependence in chronic ethanol abusers and has been available commercially since 1989 in over twelve European countries. The therapeutic dose in Europe and proposed dose in this submission is 2 x 333 mg tablets t.i.d. (1998 mg/day). Clinical trials (8 pharmacodynamic, 17 pharmacokinetic, several small efficacy studies early in development, 12 large efficacy phase III studies and one phase IV open label study) were conducted in 11 European countries. The clinical safety data indicate that acamprosate, at therapeutic doses (1332-1998 mg/d PO for up to 1 yr) is well tolerated and produces minor dose-related adverse effects, including gastrointestinal effects (nausea and diarrhea) and pruritus. [ ] patients have been treated in Europe with commercially available acamprosate (333 mg tablets).

In the Pharmacodynamics and Mechanism of Action studies in rats, oral and intraperitoneal acamprosate reduced voluntary ethanol consumption in ethanol dependent, but not non-dependent rats, and reversed some effects of acute ethanol and acetaldehyde toxicity and alcohol withdrawal without altering ethanol kinetics in rodents. Alcohol-induced hyperactivity in mice was inhibited by oral acamprosate. Continuous administration of 1% acamprosate in drinking water or a single acute IP administration reduced ethanol consumption in ethanol dependent, but not in non-dependent rats. The onset of action of acamprosate at 1% in drinking water, in reducing alcohol consumption was approximately 15 days. The mechanism of action appears to involve alterations in gamma-aminobutyric acid (GABA) transmission and antagonism of excitatory amino acids, perhaps by restoring the inhibition/excitation balance that may be upset by alcohol consumption. The exact mechanism of action remains unknown.

Acamprosate produced minor and variable behavioral effects in rodents. In mice, sustained hypothermia was observed at extremely high IP doses only, and antagonized amphetamine, chlordiazepoxide and morphine-induced hyperactivity were noted after oral dosing. In rats, acamprosate antagonized harmaline-induced hyperactivity at 450 mg/kg PO. Acamprosate had no anxiolytic effects, no effects on hypnotic activity or pentobarbitone-induced narcoses, and no effects on food and water consumption in mice. Acamprosate had no sedative or muscle relaxant effects at doses, no effects on fighting behavior after electroshock and no effects on mouse-killing behavior in rats. Tests in rodents demonstrated no evidence of anti-depressant, neuroleptic, anticonvulsant, and analgesic activity. In the serotonergic system, acamprosate was inhibitory during high serotonergic activity, and excitatory during low serotonergic activity.

Acamprosate had no anti-inflammatory or spasmolytic activity, and was slightly anti-allergic in ovalbumin-induced generalized edema in rats. No cardiovascular effects were observed in normotensive rats, but acamprosate decreased blood pressure and heart rate in spontaneously hypertensive rats. Acamprosate inhibited sodium nicotinate-induced peripheral vasodilation in guinea pigs, and prevented hemolysis in isolated rabbit erythrocyte membrane. At the dose of 100 mg/kg IV, acamprosate had little effect on respiratory rate, diuresis, choleresis, duodenal motility and rectal temperature in dogs. In cardiovascular studies in mongrel dogs, slight dose-related decreases in heart rate (up to -16%), increased respiratory rate and slight increases in the PR interval and QRS interval were observed at all doses from 30-1000 mg/kg IV; no effect on QT interval was noted. There were several observations of 2nd degree auriculo-ventricular heart

block and ventricular premature beat at lead II in the ECG evaluations in the 26-week study in dogs. *In vitro*, acamprosate had no effects on barium chloride and acetylcholine-induced contractions, and only slightly antagonized histamine dihydrochloride-induced contractions in isolated rat duodenum.

Drug interaction studies with drugs likely to be co-administered in the treatment of alcohol abuse showed no interactive effects with the anticonvulsants phenobarbitone, sodium valproate and diazepam, the antidepressants imipramine and fluvoxamine, and the anxiolytic drug meprobamate. Acamprosate slightly antagonized the anxiolytic effect of chlorazepate dipotassium, diazepam and potentiated atrium effects. There were no interactive effects with the neuroleptics haloperidol, sulpiride and chlorpromazine, but acamprosate slightly antagonized tiapride in a test of inhibition of apomorphine-induced climbing. Also, slight attenuation of sleep delay and sleep duration effects by butobarbitone and blood pressure effects by the hepatic ethanol metabolism inhibitor, disulfiram, were observed.

Acamprosate bioavailability by the oral route was variable in animal studies, with gastrointestinal absorption of approximately 7-16% in rats, 13-61% in dogs, and 55% in rabbits. Distribution of acamprosate by the oral route was predominantly to the gastrointestinal tract, kidneys, liver, lungs, and bone marrow in rats. In addition to these tissues, acamprosate was found in the adrenal glands and lacrymal glands in beagle dogs. Acamprosate crossed the blood brain barrier with highest brain concentrations appearing at 30 minutes after dosing. The brain:plasma AUC ratio was 0.17. Acamprosate also crossed the placenta. There was no evidence of acamprosate metabolism in rats, rabbits, dogs, and *in vitro* in human microsomes and hepatocytes. Excretion studies in rats, rabbits and dogs showed that intravenous acamprosate is primarily excreted renally and the oral form is generally excreted in feces, suggesting limited absorption from the gastrointestinal tract. In humans, a single oral dose was recovered in urine at 11% and in feces at 88% over five days. Acamprosate was excreted into milk in Wistar rats, resulting in a peak milk:plasma ratio of 1.34 at 4 hours after dosing. Comparative analysis showed most of the radioactivity by oral ^{35}S acamprosate appeared in the feces while most of the radioactivity after dosing with oral ^{45}Ca acamprosate was measured in the carcass, suggesting incorporation of the calcium moiety into bone. Protein binding was low in rat, dog, and human plasma, with higher percent binding in rat (approximately 13.5%) than in dog (2.4%) and human (6%) samples.

Acamprosate pharmacokinetics were studied in mice, rats, rabbits and dogs. Dietary administration of acamprosate at 100 mg/kg/d resulted in Cmax levels below the limit of detection in most mice. Oral acamprosate at 100 mg/kg/d produced peak plasma levels of [ ] mg/l in rats and [ ] ng/l in dogs. In comparison, in clinical studies, the proposed 1998 mg/d dose for 8 days resulted in a steady state Cmax of [ ] mg/l. The increases in Cmax and AUC values were slightly less than dose proportional in the 28-day oral study in rats. The Tmax was ~ 0.5 hours in rats and 2 hours in dogs. After 1000 mg/kg/d PO in rabbits, the Cmax of [ ] mcg eq/ml was detected at 2 hours. The Tmax decreased by approximately 50% with repeated dosing in humans. The single dose (400 mg/kg) AUC was 44 mg.h/l in rats and 240 mg.h/l in dogs. After 1000 mg/kg/d PO in rabbits, total exposure (AUC) over 24 hours was 522.1 mcg eq.h/ml. In humans the AUC measured from 0-24 hours at steady state after 1998 mg/d for 18 days was 6884 ng.h/l. The half-life of acamprosate after administration of single oral doses of 400 mg/kg was 31 hours in rats and 2.4 hours in beagle dogs. In humans, the half-life at steady state after oral treatment at the dose of 666 mg was 17 hours.

Single dose toxicology was studied in mice, rats, and rabbits. The LD50 values in mice were 1.5 g/kg IP, 0.72-0.77 g/kg IV, and 7.70-8.4 g/kg PO. Intravenous acamprosate resulted in treatment-related convulsions, apathy, ataxia and injection site wounds, with deaths at doses of ≥500 mg/kg. The deaths were attributed to cardiac arrest and were associated with gross observations of hard, contracted heart, retention of blood in the auricles and aorta, and intestinal congestion and liquid contents. Acamprosate produced apathy, and decreased bodyweight gain and food consumption at 7 g/kg or greater by the oral route. In Swiss mice, acamprosate administration by the intraperitoneal (IP, 1-3.0 g/kg), intravenous (IV, 0.25-1.0 g/kg) and oral (PO, gavage, 6-10 g/kg) routes produced treatment-related reduction in motor activity, muscle spasms, generalized hypotonia and bradycardia by the IP route, reduced motor activity, ptosis, and paralysis of the lower limbs by the oral route, and agitation with convulsions and death by the IV route.

In Sprague-Dawley rats, the LD50 values were 1.25 g/kg IP and 9.34 g/kg PO. The clinical signs were reduced motor activity, muscular hypotonia, generalized paralysis, diarrhea, salivation. Deaths were correlated with GI tract congestion. In CD Sprague Dawley rats administered IV acamprosate at doses of 0.125-1.000 g/kg and observed for 14 days, the NOAEL was 0.125 g/kg. Injection site wounds were observed at 0.500-0.750 g/kg and convulsions followed by deaths at 1 g/kg. The macroscopic examination showed splenomegaly and discolored liver at 0.75 g/kg with no histopathologic changes. Spleen weights were increased at doses ≥0.5 g/kg corresponding with hypertrophy of the white pulp and/or the red plug in the microscopic examination. The LD50 in that study was 0.71 g/kg in the males and 0.73 g/kg in the females. Oral (gavage) acamprosate, given to CD Sprague-Dawley rats at doses of 5-8.75 g/kg PO, resulted in transient apathy and diarrhea in all treated animals, and deaths at the higher doses. The oral LD50 was 6.45 g/kg in the male rats, 5.93 g/kg in the female rats (25x the MRHD on a BSA basis).

In the Burgundy tawny rabbit, the minimum lethal dose was 2.23 g/kg IV. An infusion of 15% solution resulted in decreased blood pressure, cardiac arrest and death. Oral acamprosate administration at single doses of 600 mg/kg in rabbits resulted in wet stools and no deaths.

Subchronic toxicology studies were performed in mice, rats, dogs and monkeys. CD-1 VAF mice administered dietary doses of 125-137 and 534-564 mg/kg/day for 2 weeks induced wounds and hair loss in the high dose group, and increased food consumption and body weight gains in both dose groups. Plasma sampling demonstrated good absorption by the dietary route indicated that sampling can be conducted at any time of day. In a13-week dietary study in CD-1 mice, acamprosate intake (500, 1000, 1500 and 2000 mg/kg/day) induced increased water consumption and urinary calcium and phosphorus. Brain, heart, liver, spleen and testes weights were slightly decreased in males at doses of 1000-2000 mg/kg/day; brain weights were decreased at 1000 and 2000 mg/kg/day and heart weights were decreased at 2000 mg/kg/day in females. The MTD was not established in this study.

Sprague Dawley rats were treated by dietary administration at doses of 100 and 400 mg/kg/day for 3 and 13 weeks. A dose related increase in body weight gain in the females, and a decrease in body weight gain in the males were at 3 weeks. Plasma acamprosate was not detected at 100 mg/kg/day; mean plasma concentrations in the males were 0.75-4.29 mg/l. Dietary administration for 13 weeks (500, 1000, and 2000 mg/kg/day) resulted in loose feces and increased water

consumption. Urinalysis showed decreased urinary volume and increased urinary Ca. Treatment-related firm contents in the ileum, watery distension and pale contents in the cecum, and soft, pale colon contents were observed. The MTD in this study was 1000 mg/kg/day; a NOAEL was not identified.

Acamprosate administration by daily oral intubation (320, 960, and 2400 mg/kg/day) for 90 days in rats resulted in salivation and liquid diarrhea in 1 high-dose animal. Reversible increased adrenal weight was observed in male rats. The histopathology examination showed distended kidney tubule sections from coagulum accumulations, attributed to early senile nephrosis in 3 high dose recovery rats. A NOAEL could not be identified because a full histopathology assessment was not conducted. A chronic (26-wk) oral toxicity study (320, 960 and 2400 mg/kg/day) produced drug related deaths at 2400 mg/kg/d between weeks 15 and 26 and also identified kidney toxicity. Treatment-related clinical signs in animals that died were piloerection, subdued behavior, hypothermia, and sudden weight loss and ptyalism and soft feces in the rats that survived. Increased in water consumption in all treated groups, increases in alkaline phosphatase, blood urea nitrogen, serum and urinary calcium, and acidified urine were observed. Dose-related decreases in heart and spleen weight and increases in adrenal and kidney weights were noted. Gross findings were predominantly in the gastrointestinal system (distension, liquid contents, gas, hypertrophy). Microscopic findings in the animals that died were noted in the liver, kidney, heart, abdominal aorta, lung, thymus, spleen, stomach, duodenum, and cecum. Findings in surviving animals were similar with additional findings noted in the brain. The NOAEL was not identified in this study due to an increased incidence of dyskeratosis and inflammation of the stomach and vacuolation of the cerebellum at the mid-dose and the lack of histopathology evaluation at the low dose.

Subchronic toxicity in Beagle dogs was evaluated by the intravenous and oral routes. Intravenous treatment at doses of 20, 100 and 200 mg/kg/day resulted in vomiting, clinical signs, and injection site induration, swelling, hemorrhagic infiltration, periphlebitis and granulomatous inflammation at the injection site. Plasma measurements were lower in females suggesting higher clearance in the females. A NOAEL was not observed due to vomiting and injection site effects in a small number of low-dose dogs. Beagle dogs were given acamprosate at 1000 mg/kg/day (13.5x the MRHD on a BSA basis) by gastric intubation in a preliminary oral toxicity study. The MTD by the oral route is considered to be 1000 mg/kg/day due to liquid diarrhea in all animals after dosing and slight, reversible body weight reduction in males. The results of a 26-week oral (gavage) study in dogs (250, 500, and 1000 mg/kg/day) showed diarrhea in all treated dogs, with a dose-related increase in incidence and severity. Although several cardiac rhythm and conduction abnormalities were observed that were possibly related to acamprosate administration, no QT effects were observed. There was a dose-related increase in urinary calcium in all acamprosate-treated animals. The NOAEL was not established due to diarrhea and increased urinary calcium at the low dose.

In cynomolgus monkeys, 7-day oral acamprosate administration by gavage at 1 g/kg/day resulted in liquid diarrhea and a slight decrease in body weights. A NOAEL could not be determined.

Acamprosate was negative in two Ames tests and negative for clastogenicity in the *in vitro* chromosome aberration assay in human lymphocytes and in two *in vivo* mouse micronucleus tests. Dosing in the chromosome aberration assay without metabolic activation and incubation

times with activation did not meet currently accepted criteria. Equivocal results were produced when tested for possible mutagenic activity in the point mutation test at the HPRT locus in Chinese hamster V79 cells in the absence of metabolic activation. A significant increase in the number of mutants was observed at a concentration of 300 μg/ml in the absence of metabolic activation in the first experiment and at concentrations of 100, 1000, and 3000 μg/ml in the second experiment. In a third test, conducted without metabolic activation, there was no significant increase in the number of mutants at any concentration. Thus, the genotoxic potential of acamprosate cannot be ruled out. Also, dosing with meatbolic activation does not appear to be adequate. Thus, the point mutation test and the in vitro chromosome aberration test should be repeated.

Studies to evaluate the carcinogenic potential of acamprosate were conducted in mice and rats. The results of the carcinogenicity studies were presented to the Executive CAC committee on March 19, 2002. In rats, no significant drug-related neoplastic findings were noted at doses up to 400 mg/kg/d by dietary admixture (up to 2.3x the MRHD on an AUC basis). The Executive CAC committee concluded that the rat carcinogenicity study is acceptable, although dosing in the female rats is marginally adequate. Although no significant treatment-related neoplastic findings were noted in the 91-week mouse carcinogenicity study, the study is considered invalid due to dosing based on the lack of any dose-limiting toxicity at the highest dose, nematode infestation which could confound the study interpretation, and evaluation of animals for tumor incidence at the low and mid-doses that was inadequate for conducting a valid trend test. The committee recommended that the sponsor repeat the mouse carcinogenicity study.

Reproductive toxicology studies on fertility were conducted in mice and rats. In mice, the NOAEL for fertility effects and for general toxicity in male and female mice was ≥2400 mg/kg/day PO following oral treatment with 320, 960, and 2400 mg/kg/day by oral gavage. There were no treatment-related effects on the number of malformed offspring, offspring behavior, equilibrium, motor activity, balance, sight and hearing, and F1 offspring fertility in mice. In CD(SD)BR rats, oral acamprosate (0, 50, 225, 1000 mg/kg/day) had no effect on male and female fertility in dams or their offspring; the NOAEL for adverse effects on fertility in rats was 1000 mg/kg/day. There was a treatment-related increase in the incidence of skeletal variations and incomplete ossification in the F1 offspring. No maternal toxicity was noted.

Teratogenicity studies on oral acamprosate were conducted in mice, rats and rabbits. In mice, camprosate (320, 960, and 2400 mg/kg/day, PO) resulted in a slight dose-related increase in the numbers of fetuses reaching maturity, and a slight decrease in the number of females with malformed fetuses and fetuses with malformations. The NOAEL for developmental effects is 2400 mg/kg. In rats, a treatment-related increase in the number of dams with malformed fetuses and in number of fetuses with malformations was observed; the increases were due to a high number of malformations in one litter per group. The malformations included hydronephrosis, malformed iris, retroesophageal subclavian artery, retinal dysplasia which were outside the range observed in the historical data and occurred in the absence of maternal toxicity or differences in litter and fetal weights. Acamprosate is considered to be embryotoxic in rats based on the results of this study, and the target organs are the kidneys, eyes and vascular system. The NOAEL for embryo-fetal development in rats was 50 mg/kg/day PO in this study. In New Zealand White rabbits, no embryo-fetal effects were at doses up to 1000 mg/kg. In Burgundy Tawny rabbits, acamprosate administration (200, 400, and 800 mg/kg/day, PO) resulted in slightly decreased

fetal weights at 400 and 800 mg/kg/day (-11% in both groups compared to controls). There was an increase in the number of females with malformed fetuses at 400 mg/kg/day or greater; the malformations were torsion of vertebrae and hydronephrosis. The NOAEL for embryo-fetal toxicity was 200 mg/kg/day in this study.

Studies on peri- and postnatal development were conducted in mice (320, 960, and 2400 mg/kg/day, PO) and rats (50, 300 and 2000 mg/kg/day, PO). In mice, an increased incidence of still-born offspring and increased number and percent females with offspring dying after birth at 960 and 2400 mg/kg/day. There were no treatment-related effects on maternal performance of mice (Fo generation) and their offspring (F1 generation) during gestation and lactation, and no treatment-related effects on behavioral function (activity, balance, behavior, sight and hearing) of the F1 and F2 generation offspring. The NOAEL for adverse effects on prenatal and postnatal development by acamprosate in mice was 320 mg/kg/day PO under the conditions of this study. In rats, no significant effects were noted; the NOAEL for prenatal and postnatal development in rats is considered to be ≥2000 mg/kg/day. No maternal toxicity was observed in this study.

Acamprosate was evaluated for the potential to induce neurotoxicity in rat retrosplenial and posterior cingulate cortices ("Olney Lesion") according to the method of Fix *et al.* (1996), based on inhibitory effects on NMDA receptor activity. No evidence of neuronal vacuolation, necrosis, or microglia was found in the retrosplenial and posterior cingulate cortices, when measured at 4, 12, and 24 hours after dosing at 2000 mg/kg by oral gavage. In another special toxicology study, acamprosate produced no nitrosamines in the WHO nitrosation assay procedure (NAP). In comparison, the positive control diethanolamine demonstrated nitrosatability.

Conclusions: Acamprosate showed efficacy in reduction of voluntary ethanol consumption in rodents with a delayed onset of action of approximately 15 days. This effect was observed in ethanol-dependent, but not in non-dependent rats. Acamprosate decreased some effects of ethanol, such as analgesia, hyperactivity or hypoactivity, and staggering, decreased ethanol absorption and elimination in rats, and decreased many of the signs of ethanol withdrawal in mice. The mechanism of action appears to involve alterations in gamma-aminobutyric acid (GABA) transmission and antagonism of excitatory amino acids, and may restore an altered inhibition/excitation balance induced by chronic alcohol consumption. Acamprosate bioavailability by the oral route is variable in animals but is generally low. Distribution is primarily to the gastrointestinal tract, kidney and liver, and acamprosate crosses the blood-brain barrier and placenta. There is no evidence of acamprosate metabolism in animals and humans. Oral acamprosate is excreted in feces with a minor fraction excreted in the urine. Protein binding is also low in animals and humans.

The toxicity following single dose intravenous, intraperitoneal and oral administration is considered low in rodents. The oral median lethal doses (6-9 g/kg) are approximately 10x higher than the IV LD_{50} values suggesting poor oral bioavailability in rodents. Clinical signs of reduced motor activity, hypotonia, apathy, gastrointestinal and cardiovascular effects were observed.

Repeated dosing in the sub-chronic and chronic studies at doses of up to 2 g/kg/day resulted in toxicity in rats, including loose feces, increases in slight urinary calcium, and histologic changes in kidney, liver, heart, brain, thymus, spleen, and GI system. Dogs treated with up to 1 g/kg/day

oral acamprosate showed dose related diarrhea, and increased urinary calcium after 26 weeks of treatment. Acamprosate produced no adverse effects, except for diarrhea, in monkeys at doses up to 1 g/kg/day for seven days. Target organ toxicities appear to be related to the calcium moiety; these include kidney, heart, thymus, GI system and brain. In mice, dogs or monkeys, no specific target organs of toxicity were identified, though increased plasma and urinary Ca++ in mice and dogs were observed.

Acamprosate was negative for mutagenicity in the Ames test, and for clastogenicity in the chromosome aberrations assay in human lymphocytes and in the Mouse Micronucleus test. Equivocal results were observed in a point mutation assay in Chinese hamster V79 cells The test should be repeated to further assess the mutagenic potential of acamprosate. The *in vitro* chromosome aberration assay in human lymphocytes should be evaluated at higher doses without metabolic activation.

Acamprosate was negative for carcinogenicity in a two-year rat study. The carcinogenicity study in mice is unacceptable because inadequate doses were used, based on lack of evidence for an MTD. The mouse study results were confounded by nematode infestation, and histopathology evaluation was conducted on an inadequate number of mid- and high-dose animals. The committee recommended that the sponsor repeat the mouse carcinogenicity study.

No adverse effects on male and female fertility were observed in mice and rats. Embryo-fetal developmental effects were observed in mice, rats and rabbits. The treatment-related malformations in rats included hydronephrosis, malformed iris, retroesophageal subclavian artery, and retinal dysplasia. In Burgundy Tawny rabbits, an increased number of females with malformed fetuses (torsion of vertebrae and hydronephrosis). In addition, an increased incidence of still-born offspring occurred in a perinatal or postnatal toxicity in mice. However, the findings in animals should be considered in relation to known adverse developmental effects of ethyl alcohol, which include the characteristics of fetal alcohol syndrome (craniofacial dysmorphism, intrauterine and postnatal growth retardation, retarded psychomotor and intellectual development) and milder forms of neurological and behavioral disorders in humans.

Acamprosate, administered to rats at the dose of 2 g/kg by oral gavage, produced no evidence of neuronal vacuolation, necrosis, or microglia in the retrosplenial and posterior cingulate cortices, measured at 4, 12, and 24 hours after dosing.

General Toxicology Issues:

The results of the studies in animals indicate a slight potential for adverse cardiovascular effects (AV heart block, ventricular premature beat) and a higher possibility of adverse gastrointestinal effects, particularly diarrhea. Renal effects were noted in the animal studies, including increased urinary calcium, increased kidney weights, changes in urine volume, degenerative renal tubulopathy and renal pelvis mineralization. Additional toxicities by acamprosate were tissue calcifications, hyperkeratosis, cardiac myolysis, and vacuolation and thrombus in the cerebellum. No definitive target organs of toxicity were identified in a non-rodent species. The genotoxic potential of acamprosate was not ruled out due to equivocal results in Chinese hamster V79 cells, and inadequate dosing in the gene mutation assay in Chinese hamster V79 cells and in the chromosome aberration assay. Although acamprosate was negative for carcinogenicity in a 2-

year rat study, the study in mice was not considered to be valid. Acamprosate induced various embryo-fetal developmental effects while no effects of fertility parameters were observed.

Recommendations:

1. This NDA is not considered to be approvable from a non-clinical perspective.

2. The following deficiencies in the nonclinical program were identified:
   a) The toxicity profile of acamprosate has not been adequately characterized in a non-rodent species.
   b) The gene mutation study (No. 85065) produced equivocal results in the absence of metabolic activation and dosing was not adequate due to the lack of dose-limiting effects
   c) In the in vitro chromosome aberration study (No. 86002) in human lymphocytes, dosing and incubation time with metabolic activation was inadequate according to current standards
   d) Only one valid carcinogenicity study has been completed with acamprosate as the mouse carcinogenicity study is deemed to be invalid by the CDER Executive Carcinogencicity Assessment Committee.

3. The above noted deficiencies in the non-clinical program should be addressed in the following way:
   a) Perform a one-month oral toxicity study in dogs using adequate doses to either characterize the toxicity profile or achieve the maximum feasible dose.
   b) The gene mutation assay in Chinese hamster V79 cells and the chromosome aberration assay should be repeated using adequate dosing and procedures according to current standards.
   c) A carcinogenicity study in mice should be repeated; a standard 2-year assay or an appropriate alternative model may be performed. The sponsor is encouraged to submit a study protocol with supporting data for concurrence of dose selection by the Executive Carcinogenicity Committee prior to initiating the carcinogenicity study.

Labeling:

The proposed Carcinogenicity, Mutagenicity and Impairment of Fertility, Pregnancy, Labor and Delivery, and Nursing Mothers sections of the Label are reproduced below:

Carcinogenicity, Mutagenicity and Impairment of Fertility

[ ]

3 page(s)
of draft labeling
redacted from the
approval package

X. APPENDIX/ATTACHMENTS:

Drug: Acamprosate

	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Pediatric	[redacted]			[redacted]				[redacted]
Adult	>12	[redacted]	[redacted]	1998	50	39.96	37	1478.52

	route	mg/kg/d	conv. factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
rat	[redacted]		6	2400	1.6	---	######	---
mouse			3	0	---	---	---	---
extra			---	---	---	---	---	---
extra			---	---	---	---	---	---
extra			---	---	---	---	---	---
Reproduction and Fertility:								
rat	[redacted]		6	6000	4.1	N/A	######	N/A
rat			6	0	---	N/A	---	N/A
mouse			3	7200	4.9	N/A	######	N/A
dog			20	0	---	N/A	---	N/A
Teratogenicity:								
mouse	[redacted]		3	2880	1.9	N/A	######	N/A
rat			6	1800	1.2	N/A	######	N/A
rabbit			12	4800	3.2	N/A	######	N/A
rat			6	300	0.2	N/A	1/5	N/A
rabbit			12	2400	1.6	N/A	######	N/A
Overdosage:								
mouse	[redacted]		3	0	---	---	---	---
rat			6	0	---	---	---	---
dog			20	0	---	---	---	---
rabbit			12	0	---	---	---	---
Other: Reproduction								
rat	[redacted]		6	0	---	---	---	---
guinea pig			8	0	---	---	---	---
monkey			12	0	---	---	---	---
rabbit			12	12000	8.1	---	######	---
mouse			3	960	0.6	---	1/2	---

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Kathy Haberny
6/10/02 08:42:20 AM
PHARMACOLOGIST

Timothy McGovern
6/10/02 08:47:30 AM
PHARMACOLOGIST
I concur.

45 DAY MEETING CHECKLIST

FILEABILITY:

On initial overview of the NDA application: **YES** **NO**

PHARMACOLOGY:

(1) On its face, is the pharmacology section of the NDA organized in a manner to allow substantive review to begin? Yes

(2) Is the pharmacology section of the NDA indexed and paginated in a manner to allow substantive review to begin? Yes

(3) On its face, is the pharmacology section of the NDA legible so that substantive review can being? Yes

(4) Are all required (*) and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity, teratogenicity*, effects on fertility*, juvenile studies, acute adult studies*, chronic adult studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetics studies, etc)? No 9/12day tox in dog, 1mo tox in dog requested to characterize to profile in dogs (Tim) No

(5) If the formulation to be marketed is different from the formulation used in the toxicology studies, has the sponsor made an appropriate effort to either repeat the studies using the marketed product or to explain why such repetition should be required? NA

(6) Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57? Yes

(7) Has the sponsor submitted all special studies/data requested by the Division during Pre-submission discussions with the sponsor? Yes

(8) On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted rationale to justify the alternative route? Yes

(9) Has the sponsor submitted a statement(s) that all the pivotal Pharm/Tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? Yes

(10) Has the sponsor submitted a statement(s) that the Pharm/Tox studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns? Yes

(11) From a pharmacology perspective, is this NDA fileable? If "no", please state below why it is not. /S/ Yes

Reviewing Pharmacology Officer Date

/S/

Supervisory Pharmacology Officer Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Kathy Haberny
2/15/02 09:30:32 AM
PHARMACOLOGIST

Timothy McGovern
2/19/02 04:23:26 PM
PHARMACOLOGIST
I concur.

Executive CAC
Date of Meeting: March 19, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Joseph Sun, Ph.D., HFD-570, Alternate Member
Timothy McGovern, Ph.D., Team Leader
Kathleen Haberny, Ph.D., Presenting Reviewer

Author of Draft: Kathleen Haberny, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-431
Drug Name: Acamprosate
Sponsor: Lipha Pharmaceuticals, Inc.

Background: Acamprosate (Aotal, Campral) is being evaluated in the United States as a treatment for alcohol dependence in chronic ethanol abusers. Developed by Laboratoires Meram in 1987, acamprosate has been available commercially since 1989 for use in the treatment of alcohol abuse in over twelve European countries. The therapeutic dose in Europe and proposed dose in this submission is 2 x 333 mg tablets t.i.d. (1998 mg/day). [ ] patients have been treated in Europe with commercially available acamprosate (333 mg tablets) for maintenance of alcohol abstinence, since 1989.

The mechanism of action appears to involve alterations in gamma-aminobutyric acid (GABA) transmission and antagonism of excitatory amino acids, perhaps by restoring the inhibition/excitation balance that is possibly altered by chronic alcohol consumption. Acamprosate bioavailability is approximately 7%-15% in rats and 11% in humans. Distribution is predominantly to the GI tract, liver, kidney, lymph nodes and lungs. Acamprosate is approximately 13.5% protein bound in rats, but not bound to plasma proteins in humans. Information on protein binding in mice is not available. No evidence of acamprosate metabolism was found in rats, rabbits, or dogs. Acamprosate is excreted unchanged in urine and feces.

Acamprosate was not mutagenic in the Ames test, an *in vitro* assay with human lymphocytes and an *in vivo* mouse micronucleus test. Equivocal findings were observed in a Chinese hamster cell gene mutation test. Some of the assays were not conducted using currently accepted procedures.

The sponsor submitted the results of two 2-year carcinogenicity studies in rats and mice. The sponsor did not seek concurrence for dose selection by the Exec CAC prior to initiating the studies.

Rat Carcinogenicity Study: The doses tested were 25, 100, and 400 mg/kg/day by dietary admixture. The study used an adequate number of animals, and showed adequate survival, parameters evaluated and duration of treatment. No significant treatment-related neoplastic findings were noted. Dosing in males is considered to be adequate, as the high dose was at or near the MTD based on decreased body weight, increased incidence of tail sores, and renal effects (pelvic mineralization) in the male rats. Dosing in female rats is marginally acceptable as it is considered to be approximately one-third of the MTD based on renal effects observed in a 13-week dietary administration study at 1000 mg/kg/day.

Mouse Carcinogenicity Study: The doses tested were 25, 100, and 400 mg/kg/day by dietary admixture. The study was terminated after 91 weeks due to animal mortality that was comparable among treatment groups. Although no significant treatment-related neoplastic findings were noted, the study is considered invalid due to dosing based on the lack of any dose-limiting toxicity at the highest dose, nematode infestation which could confound the study interpretation, and evaluation of animals for tumor incidence at the low and mid-doses that was inadequate for conducting a valid trend test.

Executive CAC Recommendations and Conclusions:

Rat: The rat carcinogenicity study is acceptable. Acamprosate is not considered to be tumorigenic in this model. Dosing approximated the MTD in males and was approximately one-third of the MTD in females.

Mouse: The Executive CAC Committee concluded that the carcinogenicity study in mice is unacceptable due to inadequate dosing, nematode infestation that confounded the study interpretation, and histopathology evaluation of low and mid-dose animals that was inadequate for conducting a trend test for tumor incidence.

The Committee recommended that the sponsor repeat the mouse carcinogenicity study. The sponsor is encouraged to submit the carcinogenicity study protocol, including the supporting data and rationale for dose selection for concurrence prior to starting the study.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
Division File, HFD 170
Timothy McGovern, Ph.D./Team leader, HFD-170
Kathleen Haberny, Ph.D./Reviewer, HFD-170
Lisa Basham/PM, HFD-170
ASeifried, OND

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Joe Contrera
4/25/02 02:33:31 PM