

increased water consumption at doses from 1000-2000 mg/kg/day, and increased urinary calcium and phosphorus at 2000 mg/kg/day. Brain, heart, liver, spleen and testes weights were slightly decreased in males at doses of 1000-2000 mg/kg/day. In the female mice, brain weights were decreased at 1000 and 2000 mg/kg/day and heart weights were decreased at 2000 mg/kg/day. *The MTD was determined to be > 2000 mg/kg.*

Subchronic toxicity of acamprosate was studied in Sprague Dawley rats treated by dietary administration at doses of 100 and 400 mg/kg/day (0.4x and 1.6x the MRHD on a BSA basis) for 3 and 13 weeks, and by oral gavage for 3 months. In the 3-week study, there was a dose-related increase in body weight gain in the females compared to controls, and a decrease in body weight gain in the males. Plasma acamprosate was not detected in the rats at 100 mg/kg/day; mean plasma concentrations in the males were 0.98-3.33 mg/l and in the females were 0.75-4.29 mg/l at 400 mg/kg/day; peak plasma levels occurred in the morning. A NOAEL was not established in this study. Dietary administration of acamprosate for 13 weeks at doses of 500, 1000, and 2000 mg/kg/day (2x, 4x, and 8x the MRHD on a BSA basis) resulted in loose feces primarily at the two upper doses, increased water consumption, decreased urinary volume in females, and increased urinary Ca. The necropsy showed decreased liver weights in the males, increased adrenal weights in males, and decreased ovarian and heart weights in females. Treatment-related firm contents in the ileum, watery distension and pale contents in the cecum, and soft, pale colon contents were observed in males at 500 mg/kg/day, and males and females at 1000 and 2000 mg/kg/day. *The MTD in this study was 1000 mg/kg/day based upon renal effects. A NOAEL was not identified in this study.*

Acamprosate administration by daily oral intubation, at doses of 320, 960, and 2400 mg/kg/day (1.3x, 4x, and 10x the MRHD on a BSA basis) for 90 days in rats, resulted in salivation and liquid diarrhea in 1 high-dose animal. Findings included reversible increases in gonad weight at 2400 mg/kg/day, and adrenal weight at 960 and 2400 mg/kg/day in the 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.* In a chronic (26-week) oral (gastric intubation) toxicity study (320, 960, and 2400 mg/kg/day; 1.3x, 4x, and 10x the MRHD on a BSA basis), drug-related deaths at 2400 mg/kg/d in male (17%) and female (57%) rats occurred between the weeks of 15 and 26 of dosing. Treatment-related findings included clinical signs (piloerection, subdued behavior, hypothermia, sudden weight loss, ptyalism and soft feces) and a dose-related increase in water consumption. Clinical chemistry changes included increases in alkaline phosphatase in the females and blood urea nitrogen, increased serum and urinary calcium, and acidified urine. The histopathology identified the liver, kidney, heart, lung, thymus, spleen, stomach, duodenum, cecum as target organs in the animals that died. Similar organs were identified in surviving animals in addition to the bladder, brain, and adrenal. Associated gross findings included decreases in heart and spleen weight, and increases in adrenal and kidney weight, and gastrointestinal system effects. 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 (4-week) toxicity in Beagle dogs was evaluated by the intravenous and oral routes. Intravenous treatment (20, 100 and 200 mg/kg/day) resulted in vomiting and injection site swelling and induration, with a dose-related increase in incidence. Treatment-related findings included clinical signs (salivation, tremors, chewing, and agitation), changes in clinical chemistry (increase in calcium and decrease in phosphorus levels), and macroscopic and microscopic changes (induration, swelling, hemorrhagic infiltration, periphlebitis and granulomatous inflammation at the injection site). *A NOAEL was not identified due to vomiting and injection site effects in a small number of low-dose dogs.* The plasma acamprosate measurements showed lower levels in females compared to males from 1 hour to 4 hours after dosing, suggesting higher clearance of acamprosate in the females. In a preliminary oral toxicity study, beagle dogs were given acamprosate at 1000 mg/kg/day (13.5x the MRHD on a BSA basis) by gastric intubation to determine the MTD for the 26-week study. A NOAEL was not identified due to liquid diarrhea in all animals after dosing and slight, reversible body weight reduction in the male dogs; no histologic assessment was performed.

In cynomolgus monkeys, 7-day oral acamprosate administration by gavage at 1 g/kg/day (8x the MRHD on a BSA basis) resulted in liquid diarrhea in all animals throughout the dosing period. A slight decrease in body weights was probably a result of the liquid diarrhea. *The NOAEL cannot be determined because clinical pathology and histopathologic examination were not performed.*

A chronic (26-week) oral (gavage) toxicity study in dogs given acamprosate at doses of 250, 500, and 1000 mg/kg/day (3.4X, 6.8X, and 13.5x the MRHD on a BSA basis) again showed diarrhea in all drug-treated dogs, with a dose-related increase in incidence and severity; the effect was reversible before each subsequent treatment. Cardiac rhythm and conduction abnormalities (1st and 2nd degree atrio-ventricular heart block, premature ventricular beat at lead II) that were possibly related to acamprosate administration were observed at the high dose after the first dose and at week 13. No findings in QT interval were noted and no findings were observed at week 26. 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 although no definitive target organs of toxicity were identified.* The doses studied (250-1000 mg/kg/day) represented 3.4X to 13.5X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis.

Despite the exposure margin achieved, the recommendation was made for a nonclinical study in a non-rodent species which would utilize higher dosing to adequately characterize the toxicity of acamprosate. An agreement was reached with the sponsor to provide a 1 month study in the dog in which the maximum feasible dose or a top dose high enough to characterize toxicity would be used. This study is reviewed below.

Study title: EMD 171 482 – 4 Week Oral Toxicity Study in Beagle Dogs**Key study findings:**

- Dogs were administered acamprosate by oral gavage for 28 days at a dose of 0, 750, 1500 or 3000 mg/kg to characterize the toxicity of acamprosate at higher doses than originally studied in 1- and 6-month repeated dose toxicity studies.
- *No target organs were identified in this study*
- One LD σ^7 was prematurely sacrificed on Day 1 of study. Gross pathology demonstrated massive edema and emphysema in the lungs and fluid in the trachea with foam up to the larynx, bloody contents in the jejunum and red discoloration in duodenal mucosa with bloody contents in jejunum. Pathologist diagnosis was lobular pneumonia and was probably due to aspiration of vomit.
- Clinical signs of vomiting were observed in all groups treated with acamprosate in a dose-dependent manner. MD and HD groups exhibited frequent vomiting throughout the treatment period, both before and after dosing. Soft stools and diarrhea noted in all treated animals throughout the study.
- Three MD ♀ demonstrated 2nd degree A-V block but 2/3 females also were observed with this characteristic during the pre-treatment period. No HD animals demonstrated this finding.
- No effects on cardiac function or conduction were detected.
- No treatment-related effects on hematology or clinical chemistry. Plasma Ca²⁺ and phosphorus concentrations were unaffected by treatment.
- No alterations in urinary parameters observed, however urinary calcium and potassium were not measured.
- No treatment-related changes observed with organ weights, gross or microscopic inspection of tissues of the animals sacrificed at the end of the 1-month study.
- TK assessment indicated that exposure was generally proportional to dose and not affected by gender or repeated dosing. Some degree of exposure plateau may be reached by 3000 mg/kg/day but the presence of significant vomiting in MD & HD dose groups combined with the poor oral absorption of acamprosate makes this assessment challenging to interpret. Peak plasma levels were reached within 1 hr of dosing.
- Doses utilized represent 10 – 42-fold the MRHD (BSA-adjusted based on a 50 kg individual); TK analysis of acamprosate concentrations in the dog appears to provide a 22 – 81-fold exposure margin above human exposure at steady state dosing (6884 ng•h/mL).
- *The NOAEL could not be defined due to vomiting and diarrhea at the lowest dose of 750 mg/kg/day (i.e. NOAEL < 750 mg/kg/day).*
- Although a maximum feasible dose was not utilized, *the toxicity of acamprosate was considered to be adequately characterized.*

Study no.: T 8838 (Document ID: 090006d180172678)

Volume 1 (Serial Submission #031 BP), Attachment 2

Conducting laboratory and location: Institute of Toxicology
Merck KGaA
64271 Darmstadt
Germany

Date of study initiation: 4/30/2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug: EMD 171 482 (3-Acetylaminopropanesulfonate, calcium salt (Acamprosate), Batch#: M284A, % Purity: Not specified

Methods

Doses: 0, 750, 1500 or 3000 mg/kg/day x 28 days

Species/strain: [] Beagle

Number/sex/group or time point (main study): 3 animals/sex/group

Route, formulation, volume, and infusion rate: Oral gavage of drug in demineralized water, 10 mL/kg body weight

Satellite groups used for toxicokinetics or recovery: None

Age: 9 – 10 months (both ♂ & ♀)

Weight (nonrodents only): ♂: 6.5 – 8.7 kg; ♀: 5.8 – 7.4 kg

Unique study design or methodology (if any):

Dose design: Dosing was selected based on a preliminary study using oral administration of acamprosate to two groups of beagle dogs. One group of dogs received acamprosate at a dose of 2500 mg/kg; the second group of dogs received acamprosate in escalating doses starting at 500 mg/kg and ascending up to 5000 mg/kg. Doses below 2000 mg/kg were judged to be “well tolerated” by the sponsor whereas dosing at 2500 and 5000 mg/kg led to more severe and dose-dependent clinical signs consisting of vomiting (in one case 15 times in one day with reddish material in the vomit) and an aqueous diarrhea which also included reddish material lasting up to 6 hr after drug treatment. No other clinical signs were described. Toxicokinetic analysis suggests that despite the significant vomiting and diarrhea that systemic absorption of drug still occurred with C_{max} and AUC values increasing supra-proportionally with dose.

Toxicokinetics			
Dose	C_{max}	AUC	Exposure Margin over Clinical Therapeutic Concentrations in Humans (CL 1 mL and 5.9 $\mu\text{g}\cdot\text{h}/\text{mL}$)
2500	— $\mu\text{g}/\text{mL}$	507 $\mu\text{g}\cdot\text{h}/\text{mL}$	— $(C_{max}) / 86$ (AUC)
5000	— $\mu\text{g}/\text{mL}$	1450 $\mu\text{g}\cdot\text{h}/\text{mL}$	— $(C_{max}) / 246$ (AUC)

The sponsor chose 3000 mg/kg/day for the 1-month dog study due to the finding that at 5000 mg/kg/day clinical signs were worsened significantly from the 2500 mg/kg/day dose and that a 3000 mg/kg/day dose was likely to produce exposure levels which were highly in excess of the exposure observed in the clinical population.

Observation times and results

Mortality: Daily; along with evaluation of clinical signs. One male (#5591; 750 mg/kg/day) was sacrificed prematurely (Day 2 of study) for human reasons. Gross and histopathologic

assessment suggested animal condition deteriorated markedly due to aspiration of vomit and resulting lobar pneumonia and cardiac failure. Animal #3746 (male) was assigned to the 750 mg/kg/day dose on Day 2 and administered drug for 27 days (instead of 28). All other animals survived the study.

Comment: Based upon the presentation and lack of dose-dependency, the conclusion that animal 5591 aspirated vomit appears reasonable.

Clinical signs: Daily; with onset of recording of clinical signs 1 week prior to dosing initiation (week -1). Symptoms observed were soft stool and diarrhea in all treated animals throughout the treatment period. Isolated cases of vomiting in the LD group were observed as was one incidence of salivation on Day 14 in one LD♂. MD and HD groups exhibited frequent vomiting throughout the treatment period, occurring prior to dosing as well as extending in some cases beyond the 7 hour observation period. Reddish material was occasionally observed in the vomitus and feces of some MD and HD animals. No other observations were considered abnormal or treatment-related.

Animal #5591 (LD♂), which was sacrificed on Day 2 exhibited vomiting, salivation, cyanosis, labored respiration, reduced HR, lateral recumbency and hypothermia on Day 1 with symptoms occurring immediately after the first dose was administered.

Body weights: Weekly, at same day/time. Recording of body weight began 1 week prior to dosing initiation (week -1). Body weight was not observed to be affected by treatment with acamprosate at any of the doses administered.

Food consumption: Daily food intake was recorded starting 1 week prior to dosing initiation (week -1). Food was available to dogs for 2 hours daily (between 9:30 – 11:30 a.m.). Food consumption was not observed to be affected by treatment with acamprosate at any of the doses administered.

Ophthalmoscopy: Performed 4 weeks prior to dosing initiation (week -4) and at week 3. No treatment-related ophthalmologic abnormalities were observed in any group.

ECG: ECGs recorded using standard leads I, II and III and the Goldberger leads aVR, aVL and aVF were recorded. Lead II was evaluated. ECG recorded 3 weeks prior to dosing initiation (week -3) and at week 3 (2 hours before and 2 hours after treatment). Blood pressure was measured non-invasively by the oscillometric method at the coccygeal artery at the same time as ECG monitoring was performed. No treatment-related alterations in PQ, QRS or QT (corrected) intervals were detected. Three MD ♀ demonstrated 2nd degree AV heart block, two of which had a similar profile during the baseline (week -3). Only one of the three female dogs used demonstrated heart block which was confined to the treatment period and this was observed 2 hr after dosing. All other dogs were unremarkable. Heart rate appeared unaffected as well except for Animal #5591 (LD♂) which was sacrificed on Day 2 and had evidence of a markedly slowed HR in addition to severe clinical signs.

Hematology: Dogs were fasted ~ 20 hrs prior to blood sampling which was obtained from puncture of the jugular vein. Blood was obtained 2 weeks prior to dose initiation (week -2) and at week 4. Parameters evaluated are listed in the table below, from the sponsor's submission:

Parameter	Unit	Abbreviation	Instrument/Method
White blood cells (leukocytes)	/nL	WBC	(a)
Red blood cells (erythrocytes)	/pL	RBC	(a)
Hemoglobin	g/dL	HGB	(a)
Hematocrit	%	HCT	(a)
Mean cell volume	fL	MCV	(a)
Mean hemoglobin content	pg	MCH	(a)
Mean hemoglobin concentration	g/dL	MCHC	(a)
Platelets	/nL	PLT	(a)
Reticulocytes	%	RET	(b)
Erythrocyte sedimentation rate after 1 hour	mm/1h	ESR1	(a)
Erythrocyte sedimentation rate after 2 hours	mm/2h	ESR2	(a)
Lymphocytes	%	LYMPH	(a, c)
Absolute number of lymphocytes	/nL	LYMPH ABS	(a, c)
Neutrophilic granulocytes	%	NEU	(a, c)
Absolute number of neutrophilic granulocytes	/nL	NEU ABS	(a, c)
Eosinophilic granulocytes	%	EOS	(a, c)
Basophilic granulocytes	%	BASO	(a, c)
Monocytes	%	MONO	(a, c)
Prothrombin time	%, sec	PT %, PT sec	(d, R1)
Partial thromboplastin time	sec	PTT	(d, R2)
Thrombin time	sec	TT	(d, R3)
Instruments/Methods			
(a) _____			
(b) _____ lowcytometer			
(c) Visual differentiation by a microscope			
(d) : _____ R1 (Reagent 1): Neoplastin Plus R2 (Reagent 2): PTT-Reagenz. R3 (Reagent 3): Thrombin-Reagenz.			
(e) Westergren method			
The hematological examinations were recorded in the [1]			

No treatment-related effects were observed. Isolated alterations occurred but changes did not appear to be related to dose.

Animal #5591 showed evidence of altered hematologic status prior to premature sacrifice:

Parameter	RBC (RBC/pL)	Hemoglobin (g/dL)	Hematocrit (%)	WBC (WBC/nL)	Neutrophils (absolute) (Neu/nL)
Week -2	7.52	16.5	46.3	8.7	5.7
Day 1	9.78	21.5	62.5	20.9	19.6

Clinical chemistry: Dogs were fasted ~ 20 hrs prior to blood sampling which was obtained from puncture of the jugular vein. Blood was obtained 2 weeks prior to dose initiation (week -2) and at week 4. Measured parameters included the following:

Parameter in Serum	Unit	Abbreviation	Method	Instrument
Sodium	mmol/L	NA	/	(a)
Potassium	mmol/L	K		(a)
Calcium	mmol/L	CA		(a)
Chloride	mmol/L	CL		(b)
Inorganic phosphate	mmol/L	IP		(c)
Iron	µmol/L	FE		(c)
Glucose	mmol/L	GLUC		(c)
Urea	mmol/L	UREA		(c)
Creatinine	µmol/L	CREA		(c)
Total bilirubin	µmol/L	TBIL		(c)
Cholesterol	mmol/L	CHOL		(c)
Triglycerides	mmol/L	TRIG		(c)
Total protein	g/L	TP		(c)
Albumin	g/L	ALB		(c)
Alanine aminotransferase	U/L	ALAT	(c)	
Aspartate aminotransferase	U/L	ASAT	(c)	
Alkaline phosphatase	U/L	AP	(c)	

Instruments	
a) Automatic Electrolyte Analyzer	
b) Chloridometer	
c) Autoanalyzer	
d) Reflection Spectrophotometer	
e) Microscope	

No treatment-related alterations in clinical chemistry were observed. Plasma calcium and phosphorus concentrations were unchanged with treatment.

Animal #5591, sacrificed prematurely, had the following alterations in clinical chemistry:

Parameter	K (mmol/L)	Glucose (nmol/L)	Urea (mmol/L)	Creatinine (µmol/L)	ALT (U/L)	AST (U/L)
Week -2	4.9	4	5.1	51	23	17
Day 1	7.35	19.9	10.8	103	59	77

Urinalysis:

Measured parameters included the following:

Parameter in Urine	Abbreviation	Method	Instrument
Glucose	GLU	Reflectometry	(d)
Bilirubin	BIL	Reflectometry	(d)
pH value	PH	Reflectometry	(d)
Protein	PROT	Reflectometry	(d)
Urobilinogen	URO	Reflectometry	(d)
Blood	BLO	Reflectometry	(d)
Sediment	SED	visual	(e)

Intestine, large				
Cecum	F	H.E.	all	all
Colon	F	H.E.	all	all
Rectum	F	H.E.	all	all
Intestine, small				
Duodenum	F	H.E.	all	all
Jejunum	F	H.E.	all	all
Ileum	F	H.E.	all	all
Kidney	F	H.E.	all	all
Liver	F	H.E.	all	all
Lung (with mainstem bronchi)	F	H.E.	all	all
Lymph nodes				
mandibular	F	H.E.	all	all
mesenteric	F	H.E.	all	all
Mammary gland (thoracic, inguinal)	F	H.E.	all	all
Mesenteric vessel	F	H.E.	all	all
Muscle, skeletal (thigh)	F	H.E.	all	all
Nerve, optic	S	H.E.	all	all
Nerve, sciatic	F	H.E.	all	all
Pancreas (endocrine, exocrine)	F	H.E.	all	all
Parathyroid	F	H.E.	all	all
Pituitary	F	H.E.	all	all
Reproductive organs, female				
Ovary	F	H.E.	all	all
Oviduct	F	H.E.	all	all
Uterus (cornu/corpus/cervix)	F	H.E.	all	all
Vagina	F	H.E.	all	all
Reproductive organs, male				
Epididymis	B	H.E.	all	all
Prostate	F	H.E.	all	all
Testis	B	H.E.	all	all
Salivary gland (mandibular, parotid)	F	H.E.	all	all
Skin	F	H.E.	all	all
Spinal cord (cervical, thoracic, lumbar)	F	H.E.	all	all
Spleen	F	H.E.	all	all
Stomach (cardiac, fundic, pyloric)	F	H.E.	all	all
Tattoo	F			
Thymus	F	H.E.	all	all
Thyroid	F	H.E.	all	all
Tongue	F			
Trachea	F	H.E.	all	all
Urinary bladder	F	H.E.	all	all
All tissues showing abnormality	F	H.E.	all	all

No treatment-related effects were observed at the end of the 4 week study.

Comment: No treatment-related effects were observed in the 26-week study either.

Toxicokinetics: Blood samples taken from the jugular vein on Days 1 and 25 prior to dosing and 0.5, 1, 2, 4, 6 and 24 hr after treatment. Blood samples were additionally taken on days 8, 15, and 22 and 24 hr post treatment. All samples were stored at -18°C until analysis.

Toxicokinetic analysis conducted by [J

Dose Day	Male		Female		All Animals			
	C _{max} (µg/mL)	AUC ₀₋₂₄ (µg·h/mL)	C _{max} (µg/mL)	AUC ₀₋₂₄ (µg·h/mL)	T _{max} (hr)	C _{max} (µg/mL)	AUC ₀₋₂₄ (µg·h/mL)	AUC/Dose
750 mg/kg/day								
Day 1	48	149	59	179	0.8	55	168	0.22
Day 25	52	131	39	163	1	44.8	151	0.20
1500 mg/kg/day								
Day 1	121	352	128	396	0.5	125	394	0.25
Day 25	115	295	112	374	0.5	114	335	0.22
3000 mg/kg/day								
Day 1	226	474	230	461	0.5	229	477	0.16
Day 25	328	627	257	488	0.5	293	558	0.19

As can be seen in the above table, exposure appeared to be generally proportional to dose between 750 and 3000 mg/kg/day though there may be some plateau of exposure developing at the 3000 mg/kg/day dose as can be seen in the dose-normalized AUC (AUC/dose) column, though not when analysis is confined to C_{max}. Peak plasma levels were generally reached within 1 hr of oral administration. No gender differences were noted and acamprosate did not appear to accumulate with repeated dosing over the one month period of the study.

Other:

Analysis of Test Material: Suspensions were analyzed at week 1 and week 4 for appropriate concentrations and the following results observed:

Group	Acamprosate Required (mg/10 mL)	Acamprosate Week 1 (mg/10 mL)/% nominal	Acamprosate Week 4 (mg/10 mL)/% nominal
1	0	0	0
2	750	724/97%	732/98%
3	1500	1435/96%	1481/98%
4	3000	2831/94%	2896/97%

Blood Pressure: Blood pressure was measured non-invasively by the oscillometric method at the coccygeal artery at the same time as ECG monitoring was performed. Blood pressure was not affected by treatment.

Body Temperature: Temperature was measured at the same time as the ECG and blood pressure readings were taken. With the exception of animal #5591 which was sacrificed on Day 2, dogs did not develop significant change in body temperature with administration of acamprosate as measured during week 3.

Reflexes: Pupillary light reflex, corneal and lid reflexes, flexor reflex and anal reflex were tested 3 weeks prior to dose initiation and on week 3 of study. Reflexes were assessed as being normal in all dogs and unaffected by treatment.

Individual study conclusions

Results from this 1-month repeat-dose toxicity oral toxicity study in beagle dogs with acamprosate at a dose up to 3000 mg/kg/day reveals little toxicity other than the clinical signs of vomiting and diarrhea which appear to be dose-dependent in its severity. No alterations in clinical chemistry were observed, even in the plasma Ca^{2+} and phosphorous levels which might have been expected to have been elevated based on the previous 6 month study reviewed by Dr. Haberny. Notably, these electrolytes were not found to be elevated in the 6 month study at 1 month and 3 month interim evaluations, only at the end of the study. Urinary Ca^{2+} and inorganic phosphate levels were not evaluated in this study in contrast to the 6-month study previously conducted in which these parameters were observed to be significantly elevated.

Toxicology conclusions: As noted by Dr. Haberny in the original NDA review, the toxicity following single dose administration is considered low in mice and rats following intravenous, intraperitoneal and oral administration. The oral median lethal doses are approximately 10x higher than the IV LD₅₀ values suggesting poor oral bioavailability in rodents. Oral and IV LD₅₀ values in mice were 7700-8370 and 720-771 mg/kg respectively, and in rats were 6160-9340 and 730 mg/kg respectively. Chronic dosing in rats produced death at 2400 mg/kg and identified the liver, kidney, heart, lung, thymus, spleen, stomach, duodenum, cecum, bladder, brain, and adrenal as target organs of toxicity. Chronic dosing in dogs up to 1000 mg/kg did not identify target organs of toxicity although potential drug-related changes in cardiac rhythm and conduction abnormalities were noted.

Included with this resubmission, a 1-month repeated-dose oral toxicity study conducted in dogs with dosing up to 3000 mg/kg/day (42-fold the human therapeutic dose; producing 81-fold the systemic exposure in humans associated with therapeutic dosing at steady state) did not reveal alterations in cardiac rhythm nor identify target organs of toxicity though toxicity was considered to have been adequately characterized due to the presence of dose-dependent clinical signs of vomiting and diarrhea which were seen at all dose levels but were observed to increase in frequency and severity at the higher doses tested. Additionally, a preliminary study using doses up to 5000 mg/kg did not reveal overt toxicities other than those described which became extremely severe at the highest dose tested, though this study was used only to assess oral tolerance and was not used to assess changes in clinical pathology parameters or histopathologic changes. Similar to effects observed in dogs, 7-day administration of acamprosate to rhesus monkeys at a dose of 1000 mg/kg/day produced clinical signs of diarrhea without evidence of alterations in clinical pathology, gross or microscopic observations.

6.6.6.4 Genetic toxicology

Study title: EMD 171 482 – *In vitro* mammalian chromosome aberration test (human lymphocytes)

Key findings:

- According to ICH guidance S2A “Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals”, the highest concentration of test article should reach 5000 µg/mL or that which produces 80-90% cytotoxicity, or in the absence of cytotoxicity the lowest precipitating concentration should be used. The highest concentration of test article used in the main studies was 1580 µg/mL, a concentration of test article which resulted in the formation of precipitate.
- EMD 171 482 (acamprosate) did not produce polyploidy or produce mitotic inhibition at any dose tested up to 5000 µg/mL though the test article was noted to precipitate at ≥ 1580 µg/mL and led to the choice of this concentration as the top dose in the main studies.
- EMD 171 482 did not produce a biologically significant increase in chromosomal aberrations nor produce polyploidy at concentrations tested up to 1580 µg/mL in the presence or absence of metabolic activation. Positive controls produced the expected increase in chromosomal aberrations in all study conditions.
- *In agreement with the conclusions of the sponsor, EMD 171 482 (acamprosate) did not induce a clastogenic effect with exposure to human peripheral blood lymphocytes in the absence or presence of metabolic activation under the conditions of this assay.*

Study no.: T15446

Volume #, and page #: N 000 (Amendment #039) Vol. 2, Attachment #3

Conducting laboratory and location: Merck KGaA, Institute of Toxicology, 64271 Darmstadt, GERMANY

Date of study initiation: 11/15/2002

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: EMD 171 482 (Acamprosate), Batch M284A, 100% purity

Methods

Strains/species/cell line:

Human peripheral blood lymphocytes obtained from a healthy male donor. Whole blood cultures established after addition of phytohemagglutinin in chromosome medium B, fetal calf serum, heparin, penicillin G, streptomycin.

Doses used in definitive study:

158 µg/mL, 500 µg/mL and 1580 µg/mL EMD 171 482 . 1t (H₂O)

Basis of dose selection:

No reduction in mitotic index noted with incubation of lymphocytes in a preliminary experiment with doses of EMD 171 482 up to 5000 µg/mL with or without metabolic activation. Precipitate observed at ≥ 1580 µg/mL so this was chosen as the top dose.

Negative controls:

Solvent, H₂O

Positive controls:

Without metabolic activation:

Mitomycin C (MMC) 0.15 µg/mL in H₂O, []

With metabolic activation

Cyclophosphamide (CPA)/Endoxan 4 µg/mL in H₂O. []

Incubation and sampling times:

Whole blood cells were cultured for 48 hr prior to addition of test agent, positive control or negative (solvent) control in the presence or absence of S9 liver fractions (aroclor 1254 1% final concentration). The following incubation durations were tested in the two main studies:

	- S9	+ S9
1 st Series	5 hr	5 hr
2 nd Series	29 hr or 48 hr	5 hr

Three hours prior to harvesting, cells colchicine was added to a final concentration of 0.1 µg/mL to arrest cell division in metaphase. Cells were fixed, placed on slides and stained. Total culture times were therefore 77 hr in the first series and 77 or 96 hr in the second series from the start of culturing.

Therefore the schedule of the study was as follows:

Series	Culture Start	Culture Time	Treatment Time	Extra Culture Time		Total Culture Time
1 st (-S9/+S9)	0 hr	48 hr	5 hr	24 hr	<i>Preparation</i>	77 hr
2 nd (-S9)			29 hr	0 hr		77 hr
			48 hr	0 hr		96 hr
2 nd (+S9)			5 hr	24 hr		77 hr
			5 hr	43 hr		96 hr

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Two slides were prepared for each culture (concentration) of test article, positive control and 4 slides were prepared for the negative control. 100 well-spread metaphases were counted per slide giving a total of 200 well-spread metaphases counted per treatment condition (400 per solvent control) which is adequate. Counting was conducted by microscopic assessment at 1250x

magnification. Structural aberrations were scored as: gaps, breaks (chromatid, isobreak = chromosomal break), exchanges (chromosome or chromatid), multiple aberrations (cells with > 5 aberrations excluding gaps), specific aberrations (atypical chromosomes, pulverized metaphases). Scoring for polyploidy and determination of mitotic index was based on scoring 1000 mitoses per slide and 1000 cells per slide, respectively.

Descriptive statistics:

Group means were calculated for the following parameters:

Percentage of observed aberrant metaphases/culture (gaps excluded)

Percentage of observed aberrant metaphases/culture (gaps included)

Number of mitoses per 1000 cells/culture

Number of polyploid cells per 1000 mitotic cells/culture

Statistical tests:

Percentage of observed aberrant metaphases/culture with gaps excluded were compared pairwise with negative (solvent) control. The Fisher's Exact Test was performed against one-sided observations.

The critical parameter for assessment is the number of aberrant metaphases (excluding gaps) per 100 cells. Criteria for a positive result primarily rested on biologically and statistically significant increases in chromosomal aberrations above negative (solvent) controls in a concentration-dependent fashion or at the same concentration in two independent experiments. In order for the test article to be considered a clastogen, the number of aberrant metaphases must additionally be above the range of the historical negative controls. Criteria for a valid study result also included the findings of a statistically significant increase in chromosomal aberrations to be found in the positive control group and a level of chromosomal aberrations in the negative (solvent) control group that is within historical background for exposure to the solvent.

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ON ORIGINAL

Study outcome:

No test article effects on polyploidy or mitotic index were observed under any condition in this study as can be seen in the table below compiled from several tables provided by Sponsor:

Test Material and Condition Studied	Concentration (µg/mL)	Polyploid Metaphases per Culture		Mitotic Index (%)	
		Metaphases scored	Mean (%)	Mean	Relative M.I. (% neg. control)
Series #1 - S9 / 5 hr treatment					
Solvent	-	4000	0.03	7.5	100
EMD 171 482	158	2000	0.10	6.1	81
	500	2000	0.10	5.8	77
	1580	2000	0.00	5.9	79
MMC	0.15	2000	0.05	6.9	92
Series #1 - S9 / 8 hr treatment					
Solvent	-	4000	0.18	8.2	100
EMD 171 482	158	2000	0.10	9.8	120
	500	2000	0.00	11.4	139
	1580	2000	0.15	8.7	106
CPA	4.0	2000	0.1	9.4	115
Series #2 - S9 / 29 hr or 48 hr treatment					
Solvent	-	4000	0.10	4.9	100
	- #	4000	0.13	8.3	100
EMD 171 482	158	2000	0.00	3.7	76
	500	2000	0.15	4.5	92
	1580	2000	0.20	5.3	108
	1580 [#]	2000	0.20	5.0	60
MMC	0.15	2000	0.10	2.1	43
Series #2 - S9 / 8 hr treatment					
Solvent	-	4000	0.05	5.5	100
	- #	4000	0.10	11.8	100
EMD 171 482	158	2000	0.00	4.2	76
	500	2000	0.00	6.0	109
	1580	2000	0.00	5.5	100
	1580 [#]	2000	0.10	10.0	85
CPA	4.0	2000	0.15	3.6	66

Preparation time is 96 hr; all other cultures are 77 hr.

Assessment of Structural Chromosomal Aberrations by Treatment Group

Treatment/ Dose	Cells Scored	% Aberrant Cells			Chromatid type							Chromosome type					Other	
		Incl. Gaps	Excl. Gaps	w/ex- change	G	IG	B	M	F	S	EX	IB	IM	IF	ID	EX	MULT	Sp
Series #1-59 Treatment 65 hr																		
Solvent	400	3.25	1.75	0	6	0	5	0	0	0	0	1	0	0	0	0	0	1*
EMD 158 µg/mL	200	1.00	0.50	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
EMD 500 µg/mL	200	3.50	3.50	0.5	0	0	2	0	2	0	1	3	0	0	0	0	0	1
EMD 1580 µg/mL	200	3.00	2.50	0.50	2	0	4	0	0	0	0	0	0	0	0	0	0	1*
MMC 0.15 µg/mL	200	8.00	6.00**	1.50	4	0	2	0	0	0	3	6	0	1	0	0	0	0
Series #1-59 Treatment 5 hr																		
Solvent	400	0.25	0.25	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
EMD 158 µg/mL	200	2.00	1.00	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0
EMD 500 µg/mL	200	2.50	2.00*	0	3	0	3	0	0	0	0	1	0	0	0	0	0	0
EMD 1580 µg/mL	200	1.50	1.00	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0
CPA 4 µg/mL	200	11.00	10.00**	4.50	2	1	8	0	0	0	8	2	0	1	1	1	0	0

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ON ORIGINAL

Series (2) / S9 / Treatment (6) hr																	
Solvent	400	1.00	0.50	0	2	0	1	0	0	0	0	0	0	1	0	0	0
Solvent #	400	2.00	1.25	0	2	1	5	0	0	0	0	0	0	0	0	0	0
EMD 158 µg/mL	200	2.00	1.00	0	2	0	2	0	0	0	0	0	0	0	0	0	0
EMD 500 µg/mL	200	1.50	1.50	0	0	0	3	0	0	0	0	0	0	0	0	0	0
EMD 1580 µg/mL	200	1.50	1.00	0	1	0	2	0	0	0	0	0	0	0	0	0	0
EMD 1580 µg/mL [#]	200	1.50	1.00	0	1	0	2	0	0	0	0	0	0	0	0	0	0
MMC 0.15 µg/mL	200	16.50	16.50 ^{**}	10.50	0	0	11	0	0	0	21	7	0	0	0	0	0
Series (2) / S9 / Treatment (5) hr																	
Solvent	400	1.75	1.25	0	2	0	3	0	0	0	0	2	0	0	0	0	0
Solvent #	400	1.75	0.75	0	4	0	3	0	0	0	0	0	0	0	0	0	0
EMD 158 µg/mL	200	0.50	0.50	0	0	0	1	0	0	0	0	0	0	0	0	0	0
EMD 500 µg/mL	200	1.00	0.5	0	1	0	1	0	0	0	0	0	0	0	0	0	0
EMD 1580 µg/mL	200	1.50	1.50	0.50	0	0	1	0	0	0	1	1	0	0	0	0	0
EMD 1580 µg/mL [#]	200	0.50	0.50	0	0	0	1	0	0	0	0	0	0	0	0	0	0
CPA 4 µg/mL	200	13.50	12.00 ^{**}	5.00	5	1	6	0	0	0	10	14	0	0	0	0	1 [*]

* Atypical chromosome

Preparation time 96 hr (all other preparation times 77 hr)

* p ≤ 0.05 (= 0.045)

** p ≤ 0.01

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, m = minute; ib = iso-break, im = iso-minute; f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, sp = specific aberrations (a = atypical chromosome)

EZ = Internal study numbers, CPA = Cyclophosphamide, MMC = Mitomycin C, * including cells carrying exchanges,

Historical Control Data

Study number	Mean aberrant metaphases (excl. gaps) [%] ^a															
	Solvent controls ^b				Positive controls ^c											
	- S9 mix		+ S9 mix		- S9 mix		+ S9 mix									
T13382	1.7	1.0	3.3	1.5	4.5	13.6	13.0	9.3	17.0	7.0						
T13384	2.5	2.0	2.0	1.5	4.0	3.3	2.5	2.0	17.5	11.6	14.0	16.0	22.7	32.0	18.0	18.0
T13421	1.0	2.5	2.5		5.8	3.0	1.0		7.2	16.5	19.0		6.5	13.0	7.5	12.4
T13675	4.0	2.0			5.5	5.0			13.4	12.3			22.0	18.0		
T13966	2.8	0.0			nd				8.0	14.5			nd			
T14196	2.5	2.5	0.5		5.8	2.5	1.3		7.5	20.0			18.5	13.0		
T14235	0.5	2.5	2.5		1.3	1.4	5.0		4.5	14.7			13.0	20.6		
T14264	2.3	0.5	3.0		3.0	1.8	2.0		8.7	30.0			17.0	17.5		
T14401	nd				3.5	1.5	1.5	2.3	4.5	nd			10.5	15.0	11.0	
T14463	1.0	1.8			1.5	0.3			10.5	7.0			9.5	14.0		

Historical Controls:				
Mean ± SD:	1.9 ± 1.0	2.9 ± 1.6	13.3 ± 5.7	15.1 ± 5.9
Range:	0.0 - 5.8		4.5 - 30.0	6.5 - 32.0

a: Mean values from 2 to 4 individual cultures, for each culture 100 metaphases evaluated, of studies performed in the laboratory using the described protocol

b: All solvent controls from different preparation times are pooled for each study

c: MMC (0.15 or 0.25 µg/ml) in the absence and CPA (4, 14 or 20 µg/ml) in the presence of S9 mix

nd: Not determined

EMD 171 482 was judged to be non-clastogenic with exposure to human peripheral blood lymphocytes in the absence or presence of metabolic activation. Study validity was confirmed by the significant increase in chromosomal aberrations in cells exposed to positive control substances (MMC or CPA) and the lack of chromosomal aberrations outside the background historical range for negative control in the solvent (negative) control groups. The single statistically significant finding observed at EMD 171 482 500 µg/mL in Series #1 was considered not biologically significant as 1) higher concentrations of test article did not produce statistically significant elevations of chromosomal aberrations; 2) no increased incidence of chromosomal aberrations were observed at this concentration or higher in any other experimental condition; and, 3) chromosomal aberrations observed in solvent control cultures were unusually low (0.25% vs. 2.9 ± 1.6 historically) but within the normal historical background range (0.0 – 5.8%).

Study title: EMD 171 482 – *In vitro* mammalian cell gene mutation test (HPRT/V79)**Key findings:**

- Incubation of HRPT-deficient V79 cells with acamprosate in the presence or absence of metabolic activation did not lead to the production of mutants using concentrations up to 5000 µg/mL (-S9) and 2810 µg/mL (+S9) and exposures of 3 (-S9 and +S9 conditions) and 24 hr duration (-S9 condition only).
- According to ICH guidance S2A “Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals”, the highest concentration of test article should reach 5000 µg/mL or that which produces 80-90% cytotoxicity, or in the absence of cytotoxicity the lowest precipitating concentration should be used. The highest concentration of test article used in the main studies was 1580 µg/mL, a concentration of test article which resulted in the formation of precipitate. Dose selection for this study was adequate with exposure to acamprosate in the absence of S9 reached the limit concentration of 5000 µg/mL without evidence of cytotoxicity or precipitate formation. The concentrations selected were considered adequate with exposure to acamprosate in the presence of S9 metabolic activation due to the observation of precipitate formation at concentrations \geq 1580 µg/mL and lack of clear concentration-related cytotoxicity with the exception of excessive toxicity at 5000 µg/mL necessitating a top dose of 2810 µg/mL.
- Positive and negative control substances generated the expected results which support study validity.
- *In agreement with the conclusions of the sponsor, acamprosate is considered non-mutagenic under the conditions of this assay.*

Study no.: T15444

Volume #, and page #: N 000 (Serial Submission #039), Vol. 2, Attachment #4

Conducting laboratory and location: Merck KGaA, Institute of Toxicology, D-64271 Darnstadt, GERMANY

Date of study initiation: 10/24/2002

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: EMD 171 482 (Acamprosate), Batch M284A, Purity
[] %

Methods**Strains/species/cell line:**

The V79 cell line established from the Chinese hamster lung were used in this study. This line has a mutational defect in the HPRT gene which renders the enzyme it encodes nonfunctional and sensitive to the selection compound 6-thioguanine (6-TG). Cells from low passages were used for this study and appropriate cell culture conditions were used.

Doses used in definitive study:

First series (with and without S9 mix): 158, 500 and 5000 µg/mL EMD 171 482 in H₂O

Second series (without S9 mix): 158, 500 and 5000 µg/mL EMD 171 482 in H₂O

Second series (with S9 mix): 158, 500 and 2810 µg/mL EMD 171 482

Basis of dose selection:

Doses were selected such that the top concentration would be:

1. either 5000 µg/mL (or 10 mM, whichever was lower) which would be the testing limit, or
2. excessive (>80-90%) cytotoxicity or
3. insolubility of test article, change the pH or osmolality of the culture medium.

If the compound demonstrated dose-dependent cytotoxicity, the top concentration of test article would be the one which produced only ~ 10 – 20% cell viability or if this was not reached the limit of 5000 µg/mL would be used. Four analyzable concentrations would be chosen unless cytotoxicity was the reason for top concentration selection in which five concentrations would be used. Concentration levels are usually separated by $\sqrt{10}$. The Range-Finding test for cytotoxicity involved incubation of V79 cells in the presence or absence of S9 mix at varying concentrations up to the limit of 5000 µg/mL with subsequent reduction of the vital stain MTT (which is broken down by mitochondrial dehydrogenases of viable cells to a formazane derivative which can be assessed photometrically) serving as an endpoint for cytotoxicity.

Negative controls:

Sterile H₂O.

Positive controls:

Treatment Condition	Chemical	Solvent	Final Concentration (culture medium)
- S9 mix	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	Acetone	1 µg/mL
+ S9 mix	7,12-Dimethylbenz[a]anthracene (DMBA)	DMSO	10 µg/mL

Incubation and sampling times:

Experimental Series	Treatment	- S9	+ S9
1 st Series	TA, positive control or negative (solvent) control	24 hr	3 hr (10% S9 mix)
2 nd Series	TA, positive control or negative (solvent) control	3 hr	3 hr (5% S9 mix)

	Wash/Culture Duration	Media Culture	Determination
Both Series	8 days (nonselective)	8 days (selective media)	Mutation Frequency
		8 days (non-selective)	Cloning Efficiency

S9 preparation:

From the livers of male Harlan Wistar rats treated with Aroclor 1254 500 mg/kg i.p.

S9/Cofactor solution (i.e. S9 mix):

3 volumes S9 to 1 volume cofactor solution containing:

197 mM glucose-6-phosphate

28 mM NADP

26 mM NADH

11 mM NAPDH in PBS-HEPES

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): 1.5×10^6 cells were placed in Petri dishes (adequate), two cultures were maintained per concentration of the test article and positive control and three cultures for the negative control. An appropriate duration of exposure to test material and negative and positive controls was provided. Controls were themselves appropriate and no untreated controls were necessary as historical control data were provided. Subsequent cultures began on Day 5 with 6 replicates for the mutation frequency determination via selective (6-TG) media and 3 replicates for the cloning efficiency assay in nonselective media. V79 cells were cultured for 6 days prior to placement into selective or nonselective media in order to give time for optimal expression of the mutant (i.e. normal) gene.

The study was judged to be valid if positive controls (MNNG and DMBA) produced clear increases in mutation frequency (≥ 5 -fold mean mutation frequency of negative controls as well as negative historical controls) and if negative control cultures demonstrated a mean mutation frequency of < 20 cells per 1×10^6 cells.

The test article would be judged to be *non-mutagenic* if:

- 1) No increase in mutation frequency (< 2 -fold above the mean of the actual negative controls or the mean mutation frequency is < 20 cells per 1×10^6 cells) occurs in the two independent experimental series performed, or
- 2) Weak increase (< 5 -fold increase above negative controls) in one study series and no increase in the other series are observed.

The test article would be judged to be *mutagenic* if:

- 1) A clear increase (> 5 -fold increase above negative controls and negative control historical data) at similar concentrations in the two experimental series tested, or
- 2) Clear increase in one experimental series and a weak increase in the other series, or
- 3) Weak effects occur in a dose dependent fashion over ≥ 2 doses and occur over the same concentrations in both experimental series.

Comment: The Agency does not recognize the distinction of "weak" effects in genetic toxicology data. The above criteria were not used to determine study results.

Study outcome:

Test Material (µg/mL)	Number of Parallel Cultures	Solvent (µg/plate)	Mean 6-TG Resistant Colonies	Mean Mutation Frequency per 1×10^8 cells
Without S9				
1st Series				
Negative control	3	300 H ₂ O	13	6.7
EMD 150 µg/mL	2	300 H ₂ O	5	1.5
EMD 500 µg/mL	2	300 H ₂ O	13	4.8
EMD 1580 µg/mL	2	300 H ₂ O	39	8.2
EMD 5000 µg/mL	2	300 H ₂ O	6	1.2
Positive control: MNNG 1 µg/mL	2	60 acetone	2554	996
2nd Series				
Negative control	3	300 H ₂ O	15	3.3
EMD 150 µg/mL	2	300 H ₂ O	25	6.2
EMD 500 µg/mL	2	300 H ₂ O	14	3.6
EMD 1580 µg/mL	2	300 H ₂ O	10	2.9
EMD 5000 µg/mL	2	300 H ₂ O	23	5.9
Positive control: MNNG 1 µg/mL	2	60 acetone	2554	4350
With S9				
1st Series				
Negative control	3	180 H ₂ O	8	2.0
EMD 150 µg/mL	2	180 H ₂ O	6	1.5
EMD 500 µg/mL	2	180 H ₂ O	10	3.5
EMD 1580 µg/mL PE	2	180 H ₂ O	13	4.6
EMD 5000 µg/mL PE*	2	180 H ₂ O	0.5	0
Positive Control: DMBA 10 µg/mL	2	18 DMSO	1059	3220
2nd Series				
Negative control	3	180 H ₂ O	14	2.8
EMD 150 µg/mL	2	180 H ₂ O	10	2.3
EMD 500 µg/mL	2	180 H ₂ O	14	3.2
EMD 1580 µg/mL PE	2	180 H ₂ O	13	3.0
EMD 2810 µg/mL PE	2	180 H ₂ O	9	3.0
Positive Control: DMBA 10 µg/mL	2	18 DMSO	527	249

*, Lost due to toxicity of the test material; ^{PE}, Precipitate noted

The results of this study indicate that, under the conditions of the experiment, EMD 171 482 was non-mutagenic in the *in vitro* mammalian cell gene mutation assay. EMD 171 482 exposure at 1580 µg/mL in the 1st series in the presence of S9 mix did produce a mutation frequency which was more than double that seen in the negative controls (4.6 vs. 2.0 cells per 1 x 10⁶ cells). But this effect was well within the historical negative control data for this condition in this laboratory (range: 1.5 - 7.5 mutants per 1 x 10⁶ cells; mean mutation frequency: 5.6 ± 4.7 mutants) and did not demonstrate evidence of a concentration-dependent response. Both positive and negative controls returned the appropriate results, though DMBA in the 1st series produced a mean mutation frequency (3220 per 1 x 10⁶ cells) that was above the range of historical control values (1.5 - 7.5, mean: 614 ± 561) determined in this laboratory. Negative (solvent) controls were well within the range defined by historical control data in all series. Although there was a trend toward an increase in the mutation frequency in the first series +S9, this effect was within historical control range and therefore not considered to be positive. Although the criteria for a positive or negative response are not acceptable to the Agency, the study appears valid and I agree with the conclusions of the sponsor, i.e., under the conditions of the assay, acamprosate tested negative (not mutagenic) in the *in vitro* mammalian cell gene mutation test (HPRT/V79).

2.6.6.5 Carcinogenicity

No new carcinogenicity studies were submitted for the 2nd cycle review of this NDA though a repeat of the mouse carcinogenicity bioassay was recommended by the Executive Carcinogenicity Assessment Committee (Exec CAC) in a meeting of 3/19/2002, the minutes of which were immediately faxed to the sponsor. The Exec CAC considered the mouse study 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 sponsor provided an expert review of the mouse study, conducted by Dr. L. J.

which was in concurrence with the sponsor's opinion that the mouse carcinogenicity study was adequate to allow for a risk assessment and safety profile of acamprosate for clinical use. A brief summary of the argument made and this reviewer's opinion is presented below (a more detailed discussion can be found in Appendix A which contains the review sent to members of the Exec CAC for their input):

Dr. L. J. presented the following supports for his conclusion that the mouse carcinogenicity study should be considered adequate for assessing human risk:

1. Negative results from all *in vitro* and *in vivo* genetic toxicology tests
2. No data indicating nematode infestation in the mouse carcinogenicity study had any experimental impact
3. No significant treatment-related neoplastic findings in the rat carcinogenicity study
4. No reported association with an increased incidence of neoplasia in human patients using acamprosate clinically in Europe (1 million patients exposed for variable durations over the last decade)

5. No significant treatment-related neoplastic findings in the mouse carcinogenicity study
6. Mouse carcinogenicity study relevance supported by a ~4.6-fold exposure ratio over the recommended human dose, therefore providing an adequate safety factor.

This reviewer is in general agreement with the arguments made that the nematode infestation was unlikely to have affected the results of the mouse bioassay and that it is unlikely that the microscopic assessment of tissues from the LD & MD groups would have detected an increased tumor incidence in these groups since as no evidence of tumors were detected in the HD group. Additionally, the negative carcinogenicity study in the rat, the negative genetic toxicology studies submitted and the absence of evidence reporting a link between acamprosate usage in Europe adds to the general comfort surrounding use of this compound; however, this was known at the time of the initial evaluation of the submission and does not directly augment or enhance the mouse carcinogenicity assay which was the primary concern to which the Exec-CAC responded. Ultimately, however, the lack of the use of either a MTD dose or an exposure profile that approaches the 25-fold margin advocated in ICH guidelines fails to support the claim that the negative tumorigenicity found in the mouse bioassay is credible or would have relevance to the human as a means of risk assessment.

2.6.6.6 Reproductive and developmental toxicology

No additional reproductive toxicology studies were submitted nor required for the 2nd cycle review of this NDA.

2.6.6.7 Local tolerance

No local tolerance studies were submitted in support of this NDA.

2.6.6.8 Special toxicology studies

No additional special toxicology studies were submitted nor required for the 2nd cycle review of this NDA.

2.6.6.9 Discussion and Conclusions

Studies submitted for the 2nd cycle review of this NDA included a 1-month repeated-dose oral toxicity study in the dog designed to characterize the toxicity of acamprosate at dosing higher than that used in previously reviewed chronic dog studies as well as a repeat of two genetic toxicology studies and a 28-day repeat-dose toxicokinetic study in the mouse to support the previously submitted carcinogenicity study in this species. An additional component of this submission was an expert review of the mouse carcinogenicity study and related information which argued for the adequacy of the mouse bioassay in support of clinical risk assessment.

Increasing the oral dose of acamprosate up to 3000 mg/kg/day (42-fold the clinically therapeutic dose in humans) in a 1-month repeated-dose toxicity study in the dog was ineffective in eliciting new toxicities or target organs when compared with the 6-month repeat-dose toxicity study in the dog (with dosing up to 1000 mg/kg/day) reviewed in the 1st cycle of this NDA. Adverse effects observed were limited to clinical signs of dose-dependent increase in severity and frequency of vomiting and diarrhea which was not linked to test article administration (i.e. vomiting was not confined to the immediate post-dosing period) but instead occurred throughout the day.

The sponsor has adequately addressed the concerns regarding the adequacy of two genetic toxicology studies in the first submission. Specifically, a repeat of the chromosomal aberration assay in human lymphocytes and the mammalian cell gene mutation assay in V79/HPRT cells, indicated that under the conditions of the assay, acamprosate tested negative for mutagenicity at doses which were adequate.

The expert review of the mouse carcinogenicity study presented several lines of argument that the mouse carcinogenicity study was adequate to allow for a risk assessment and safety profile of acamprosate for clinical use. Review of these arguments were not persuasive and the summary of this reviewer's assessment was submitted along with the expert review to the Exec CAC for concurrence or disagreement. Members of the original and current Exec CAC concurred with the conclusions of the Division. These comments are summarized in Appendix A.

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

The sponsor did not submit a tabulated summary of toxicology studies for this NDA.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The sponsor provided additional studies which: 1) characterized the toxicity in a non-clinical species (dog) at a dose higher than used previously, 2) repeated the two *in vitro* genetic toxicology tests found inadequate in the first cycle of the NDA submission, and 3) examined acamprosate toxicokinetics in the mouse with repeated-dosing at the dose level administered in the mouse carcinogenicity study to provide an understanding of the likely systemic exposure achieved in this mouse study which could then be compared with the known therapeutic exposure in humans to aid in risk assessment and determination of study adequacy. No new toxicities or target organs were identified in the repeat-dose toxicity study in the dog. Repeat of the genetic toxicology studies demonstrated an absence of clastogenicity and mutagenicity. The sponsor also provided an expert review of the mouse carcinogenicity study which argued for adequacy of the mouse carcinogenicity bioassay but this was not persuasive as the maximum tolerated dose was not used in the study and information from the submitted mouse toxicokinetic study suggests that systemic exposure attained in the mouse was less than 5-fold the exposure known to exist in humans with therapeutic dosing of acamprosate. As this is

significantly less than the alternative dosing strategy which may be used for carcinogenicity studies, which suggests at least a 25-fold exposure margin, the mouse carcinogenicity study is still considered inadequate.

Unresolved toxicology issues (if any): The carcinogenic potential of acamprosate in the mouse has not been resolved at this time. This information should be obtained by the Agency as part of a Phase 4 commitment by the sponsor.

Recommendations: From the nonclinical pharmacology and toxicology perspective, this NDA is approvable, pending a solid commitment from the sponsor to initiate carcinogenicity assessment in a second species within a reasonable time frame following an approval action. The sponsor is strongly encouraged to submit their carcinogenicity protocol to the Division for Exec CAC concurrence prior to study initiation.

Suggested labeling: NOTE: Labeling subject to change.

DESCRIPTION

[

]

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

| []
[]
Nursing Mothers
[]

Signatures (optional):

Reviewer Signature Adam M. Wasserman, Ph.D.

Supervisor Signature R. Daniel Mellon, Ph.D. Concurrence Yes X No

APPENDIX/ATTACHMENTS

APPENDIX A

**Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals**

Application (IND/NDA) number:	21-431
Submission date and number:	Original submission 12/27/2001 Currently 2 nd cycle received 2/4/04
Division:	HFD-170
Project Manager:	Lisa Basham-Cruz
CAS#:	77337-76-9 (acid)
Drug Name:	Acamprosate (Calcium Acetylhomotaurinate)
Pharmacological Classification:	Alcohol dependence
Sponsor/Applicant	Lipha Pharmaceuticals, Inc.
Sponsor/Applicant contact name:	Anita M. Goodman, M.D. Executive Vice-President and COO
Sponsor telephone and fax #:	212-398-4602; fax 212 398-5026
Date submitted (stamp date):	Complete (Class 2) response to Action Letter 2/4/04
45-day date (from submission stamp date):	N/A
P/T Reviewer(s):	Adam M. Wasserman, Ph.D. Previously: Kathleen Haberny, Ph.D.
Date Review Completed:	
Date of CAC review:	
CAC members:	David Jacobson-Kram, Ph.D. (Chair) Joe Contrera, Ph.D. Bob Osterberg, Ph.D. Abby Jacobs, Ph.D. R. Daniel Mellon, Ph.D. (Supervisor)

Background:

This is the review of the Class 2 complete response to Lipha Pharmaceuticals, Inc. acamprosate HFD-170 Action Letter as it relates to the deficiencies of the initial carcinogenicity studies which were originally described by Dr. Kathleen Haberny in her review of the original NDA submission. The Executive CAC supported Dr. Haberny's conclusions in the meeting of 3/19/2002 (DFS date 4/25/02) that the mouse bioassay was unacceptable and recommended that the carcinogenicity study in mice be repeated "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 2-year rat carcinogenicity study was found to be acceptable based on overall toxicity especially in male rats, though only "marginally" acceptable in female rats.

The Sponsor had not sought Exec CAC concurrence for their carcinogenicity protocols prior to initiating or during the course of their studies (conducted over the years 1989 – 1991) and review of these studies revealed the lack of the use of a MTD dose in mice while in rats the top dose used was considered to be at or near MTD in males due to end-of-study observations of decreased body weight, increased incidence of tail sores and renal pelvic mineralization. Female rat dosing was considered marginally acceptable as the top dose used (400 mg/kg/day) was only 1/3 of the MTD (1000 mg/kg/day) based on renal effects observed in a 13-week dietary administration study.

As part of their Complete Response to the Division's Action Letter, the Sponsor did not repeat the mouse carcinogenicity study as recommended, instead submitting an expert report (Amendment #031) from [redacted] in which he discussed several additional studies submitted to the NDA as part of the Complete Response as well as the original data, and concluded that "the mouse carcinogenicity study provides adequate data to support human risk assessment for acamprosate". The

Sponsor hopes that if this conclusion is not supported by the Executive CAC and Division that they would be allowed to conduct the study as a Phase IV commitment.

The arguments made by Dr. [] center around 6 main points:

1) **Negative findings in all *in vitro* and *in vivo* genetic toxicology studies**

- a. This includes two new studies submitted along with the most recent submission which include EMD 171 482 – *In vitro* Mammalian Chromosome Aberration Test (Human Lymphocytes) and EMD 171 482 – *In vitro* Mammalian Cell Gene Mutation Test (HPRT/V79).

- 2) **No data indicating that the nematode infestation in the mouse carcinogenicity study had any clinical or pathogenic effect.** Dr. [] next argued against the importance of the finding of nematode infestation in all treatment groups and believes it likely they were non-pathogenic and not clinically relevant to the study. Published literature was submitted as an attachment to support his position (Appendix A). Dr. [] commented that the most common nematode infestation in laboratory mice is the mouse pinworm *Syphacia obvelata* which is considered relatively non-pathogenic and is found to occur primarily in the lower GI tract including colon and cecum. Incidence within a colony over time is usually 100% but at specific times may vary depending on the diagnostic method and the age of the mouse (older mice are more resistant). Supporting literature was provided (Flynn, 1973). Clinical signs are relatively rare except in mice with heavy pinworm burdens in which case the most common signs are rectal prolapse, intussusception, fecal impaction and diarrhea (Percy and Barthold, 1993). Dr. [] pointed to the lack of clinical signs observed in the mouse carcinogenicity study that were considered possibly treatment-related. At necropsy, impaction was reported in the colon and/or rectum of only 2/510 mice. Intussusception also was reported to occur only in 2/510 mice. Only one of these mice, a group 5 (Control) mouse with intussusception was reported to have nematode infestation by the reviewing pathologist. Dr. [] concludes that little evidence exists that significant clinical effects resulted from nematode infestation in any particular group and thus it should not be considered a confounding variable with regards to carcinogenicity assessment and data interpretation.

Reviewer's comment: It is not known for certain that *Syphacia obvelata* was the offending nematode as no microscopic typing was conducted, but it is the most likely parasite according to published literature; however, the other common nematode found in mice (*Aspicularis tetraoptera*) is not considered to be particularly pathogenic either. However, helminthic infection (which includes the oxyurids [pinworm nematodes]) has been suggested to modulate humoral responses to non-parasitic antigens, especially in athymic nude mice (Capron et al., 2004; Wilson & Maizels, 2004). Sato and colleagues (1995) demonstrated that infection of AKR/J mice with *Syphacia obvelata* increased the antibody production to sheep red blood cells when compared with uninfected mice. It seems likely, however, that these infestations are common enough to be considered as a contributor to the historical background data.

3) **No significant treatment-related neoplastic findings in the rat carcinogenicity study**

Reviewer's comment: Though the rat carcinogenicity study was ultimately deemed acceptable by the Executive CAC, it should be stated that a MTD of 1000 mg/kg/day was established in a 13-week dose range-finding study (due to renal effects at 2000 mg/kg/day) and that the high dose used in the rat carcinogenicity study (400 mg/kg/day) was only ~ 1/3 of this dose and only judged to be an adequate estimation of the MTD in male rats based on mild decreases in body weight (<5%), elevated WBCs, tail sores and evidence of renal pelvic mineralization encountered in the 2-year rat bioassay in the HD group. This dose was found also found only marginally acceptable for female rats in the same assay as no effects on weight or clinical chemistry were observed, though a similar renal toxicity was noted. Based on a 28-Day oral (dietary administration) toxicokinetic study in the rat, the exposure margin achieved when dosing the rat at 400 mg/kg/day (17,293 ng·h/mL) provides a 2.9-fold safety factor over the average human exposure to acamprosate at steady-state therapeutic dosing (5095 ng·h/mL). Although it is possible that during the course of a 2-year bioassay the exposure might differ from that seen in the 28-day study, the low level of acamprosate metabolism observed across species and the absence of evidence of impaired renal function, which is nearly exclusive route of elimination of the unchanged drug, mitigates concern.

- 4) No reported association with an increased incidence of neoplasia in human patients using acamprosate clinically in Europe (with more than one million patients exposed for variable periods of time during the past decade).
- 5) No significant treatment-related neoplastic findings in the mouse carcinogenicity study. Dr. [] conceded that the histopathologic evaluation of low- and mid-dose animals were inadequate for conducting a trend test for tumor incidence but argued that the study was conducted according to the standards of the time in which histopathology was to be assessed in:
1. All animals in the HD and Control groups
 2. All animals found dead or sacrificed moribund
 3. All low- and mid-dose animals demonstrating evidence of gross pathology

Reviewer's Comment: As the numbers of examined LD and MD animals was inconsistent between tissues, I assume point #3 meant that any tissue from LD and MD animals that had evidence of gross pathology was examined microscopically but a complete histologic work-up was not necessarily done.

Raw data from the acamprosate mouse carcinogenicity study including the archival slides were transferred from [] to Merck (Germany) archives but the original materials were found to be desiccated and therefore uninterpretable. Thus, the data as originally submitted was determined to be the sole source for study assessment. Dr. [] asserted that a trend test on tumor incidence across groups can be made under the assumption that all animals necropsied in the low- and mid-dose groups which had grossly normal organs/tissues – and which would not have been subject to histology – did not have a hidden neoplasm which would have been revealed by microscopic assessment. With such an assumption, the total number of examined animals would be 51 across all groups. Submitted with this report are two papers (Appendix B) – one of which was co-authored by Dr. [] – which describe the correlation observed between findings of microscopic neoplasms and the presence of gross tissue abnormality in the rat to be high with the exception of small endocrine tissues (thyroid, adrenal and pituitary) and large organs such as liver and lung which have extensive parenchymal surfaces which reduces the likelihood of surface detection of concealed lesions. Overall, in rats Dr. [] and colleagues found that 70 – 76% of neoplasms discovered microscopically were from tissues considered to have gross abnormalities (Kulwich et al., 1980). In another reference paper in which mice were the subjects of assessment, Frith and colleagues (1979) conclude that:

“If only gross lesions are examined microscopically, a significant number of microscopic neoplasms will be missed. This is true especially for the thymus, lungs, adrenal glands, Harderian gland and urinary bladder where 50% or more of the neoplasms would have been missed by failing to examine at least one histological section from each organ. Conversely, for some organs such as liver and mammary gland or for tissues having tumors consisting of large mass, a single tissue section does not greatly increase ones ability to detect neoplastic lesions beyond that offered by a thorough gross examination at necropsy.”

In addition, these authors comment that compounds that produce tumors with long latency for induction or a slow progression would be less likely to generate positive findings on gross inspection and without the benefit of microscopic analysis be judged incorrectly as being non-tumorogenic in nature.

Dr. [] suggests that the absence of an increase in tumors in endocrine tissues of the high dose group when compared with the control groups in the mouse carcinogenicity study strongly suggests that the low- and mid-dose groups would be unlikely to demonstrate evidence of elevated tumor incidence compared with controls as well.

Reviewer's comments: The absence of increased tumor incidence in tissues of the HD mice vs. controls does lend strength to the argument that it is unlikely that statistically significant tumor increases would be found in low- or mid-dose groups. The generally high correlation observed between findings of neoplasm with tissues

having grossly abnormal features with the exception of non-endocrine (or lung or liver) tissues enhances the presumption of study accuracy, though not necessarily study adequacy (i.e. adequate dosing).

- 6) Adequate “safety” margins between the likely acamprosate exposures in the 2-year mouse carcinogenicity study and known human steady-state plasma levels of acamprosate with therapeutic dosing for risk assessment analysis. Derived from the newly submitted mouse toxicokinetic study entitled “Acamprosate: 28 Day Oral (Dietary Administration) Toxicokinetic Study in the Mouse”, [Study Number 0537/060 [See review in the Pharmacokinetic/Toxicokinetic Section 2.6.4.8 “Other Pharmacokinetic Studies” contained in this NDA review]. This study, completed in September 2002, was designed to address Agency’s concerns about the adequacy of dosing during the mouse carcinogenicity study. The study was designed to administer approximately 400 mg/kg/day of acamprosate (calcium acetylhomotaurinate) in the diet to both male and female mice for 28 days. Subsequent analysis of the food consumption revealed that males ingested 407.7 mg/kg/day and females 433.2 mg/kg/day of acamprosate. Thus, dosing in this study approximated that seen in the submitted carcinogenicity study. As before, no overt toxicity was observed and the drug was well tolerated. The AUC₀₋₂₄ exposures of male and female mice were 21,372 ng*h/mL and 33,512 ng*h/mL, respectively. The clinically therapeutic dose of acamprosate, used outside the United States, is 2 x 333 mg tablets t.i.d. for a total dose of 1998 mg/day. Exposure to this dose in humans has been reported to be 5905 ng*h/mL (NDA 21-431, Vol. 36 p.70). Thus, the acamprosate exposure in mouse at the highest dose of 400 mg/kg/day is approximately 3.6 – 5.6 greater than that reported in humans at steady state at clinically therapeutic levels.

Reviewer’s comments: According to ICH S1C “Dose Selection for Carcinogenicity Studies of Pharmaceuticals”, the recommendation to use a 25-fold (AUC) exposure rodent:human plasma ratio as an alternative to administering the compound of interest at the MTD was based on a retrospective analysis of 35 carcinogenicity studies in animals of compounds given at the MTD for which adequate rodent and human pharmacokinetic data was available to compare exposure. Approximately 2/3 of the studies analyzed demonstrated that the MTD in rodent produced an exposure ≤ 10-fold that observed in humans. For IARC 1 and 2A drugs with positive rodent (rat) carcinogenicity studies, it was determined that a body surface area-adjusted dose (substituting for the true AUC exposure) ratio ≥ 10 would detect the carcinogenic potential of these compounds. The targeting of a 25-fold systemic exposure ratio was therefore proposed to be an acceptable alternative to the determination and use of a MTD as the high dose in 2-year rodent carcinogenicity assays. A mean AUC ratio of ~ 4.6 between mouse and human as determined by the 28-day mouse toxicokinetic study is only approximately 1/5th the exposure ratio which is recommended by ICH guidelines. A 13 week study conducted in CD-1 mice (study # 138/88827) with dietary dosing up to 2000 mg/kg/day failed to reveal a MTD dose (MTD ≥ 2000 mg/kg/day) with only increases in water consumption and increased calcium excretion was observed without evidence of renal changes at the HD. Slight decreases in the weights of certain organs (brain, liver, heart, spleen and testes, ♂; brain and heart, ♀) were not considered toxicologically significant by the Sponsor or by the reviewer, Dr. Haberny. Based on this study, the MTD would be expected to be at least 5-fold greater than the dose chosen for the high dose in the mouse carcinogenicity study. It should be noted that it is relatively unlikely that acamprosate exposure would be dramatically altered over the course of a 2-year bioassay as the compound is not highly metabolized and is excreted largely unchanged in the urine. Alterations in renal integrity and function with age could, however, produce significant alterations but this has not been demonstrated.

In summary, Dr. [] presents the following supports for his conclusion that the mouse carcinogenicity study is adequate for assessing human risk:

1. Negative results from all in vitro and in vivo genetic toxicology tests
2. No data indicating nematode infestation in the mouse carcinogenicity study had any experimental impact
3. No significant treatment-related neoplastic findings in the rat carcinogenicity study
4. No reported association with an increased incidence of neoplasia in human patients using acamprosate clinically in Europe (1 million patients exposed for variable durations over the last decade)
5. No significant treatment-related neoplastic findings in the mouse carcinogenicity study
6. Mouse carcinogenicity study relevance supported by a ~4.6-fold exposure ratio over the recommended human dose, therefore providing an adequate safety factor.

While I am in general agreement with the arguments made that the nematode infestation was unlikely to have affected the results of the mouse bioassay and that it is unlikely that the microscopic assessment of tissues from the LD & MD groups would have detected an increased tumor incidence in these groups, ultimately I believe the lack of the use of either a MTD dose or an exposure profile that approaches the 25-fold margin advocated in ICH guidelines fails to support the claim that the negative tumorigenicity found in the mouse bioassay is credible or would have relevance to the human as a means of risk assessment. The negative carcinogenicity study in the rat, the negative genetic toxicology studies submitted and the absence of evidence reporting a link between acamprostate usage in Europe and cancer does add to the general comfort surrounding use of this compound but this was known at the time of the initial evaluation of the submission and does not directly augment or enhance the mouse carcinogenicity assay which was the primary concern to which the Exec-CAC responded.

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APPENDIX A

Literature References Submitted Regarding Nematode Pathogenicity in Mice



[Flynn, 1973]

obat Docun [Percy & Barthold, 1993]

APPENDIX B

Literature References Submitted Regarding the Correlation of Gross Pathology vs. Microscopic Assessment for the Determination of Neoplastic Alterations in Tissues

obat Docu [Kulwich, Hardisty and Gilmore, 1980]



[Frith & Boothe, 1979]

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/s/

Adam Wasserman
6/16/04 07:30:54 PM
PHARMACOLOGIST

check my linkages

R. Daniel Mellon
6/16/04 09:13:35 PM
PHARMACOLOGIST

I concur with Dr. Wasserman's assessment. Note: Recommended labeling
changes have not been finalized.

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-431

Review number: 1

Sequence number: 000 / December 21, 2001 / Original Application

003 / February 6, 2002 / Amendment (Toxicology Study 537/059-D6154)

Information to sponsor: Yes (x) No ()

Sponsor: Lipha Pharmaceuticals, Inc., 1114 Avenue of the Americas, New York, NY 10036-7703

Manufacturer for drug substance: LIPHA – Usine de Meyzieu (Lipha Meyzieu Plant), 10 Boulevard de Lattre de Tassigny, ZI-69330 Meyzieu, France

Reviewer name: Kathleen Haberny, Ph.D.

Division name: Anesthetics, Critical Care, and Addiction Drug Products

HFD #: 170

Review completion date: June 10, 2002

Drug:

Trade names: AOTAL®, CAMPRAL®

Generic name: Acamprosate

Code name: None

Chemical names: 3-Propanesulfonic acid, 3-(acetylamino), calcium salt (CAS)

3-Acetamido-propanesulfonic acid

Calcium acetylaminopropane sulfonate

Calcium acetylhomotaurinate

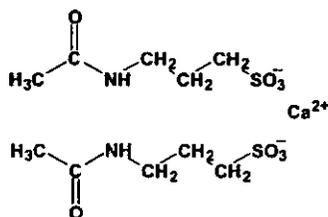
CAS registry number: 77337-76-9 (acid)

Mole file number: None

Molecular formula/molecular weight: C₁₀H₂₀N₂O₈S₂Ca / 400.48

C₅H₁₁NO₄S (acid) / 181.21

Structure:



Relevant INDs/NDAs/DMFs: IND 51,809; DMF (Amendment #3)

Drug class: Structural analog and agonist of gamma amino butyric acid (GABA)

Indication: [

]

Clinical formulation:

Ingredient	333 mg tablets Quantity per tablet (mg)	Quantity per tablet (%)	Function
Tablet Cores			
Calcium-acamprosate	333.0		Active Ingredient
Crospovidone equivalent, USP24-NF19)			
Microcrystalline cellulose or equivalent, USP24-NF19)			
Magnesium silicate (equivalent, In house specifications)			
Sodium starch glycolate (equivalent, USP24-NF19)			
Colloidal anhydrous silica or equivalent, USP24-NF19)			
Magnesium stearate USP24-NF19)			
Core weight			-
Tablet Coating			
Anionic copolymer of methacrylic acid and acrylic acid ethylester (in the form of an aqueous dispersion as Eudragit 1.30D or equivalent)			
Talc			
Propylene glycol			
Coating weight			-
Total Coated tablet weight	531.6	-	-

Route of administration: Oral (tablet)

Proposed use: Up to two 333-mg tablets, three times daily; total daily dose of 1998 mg

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability

NDA 21-431 is not approvable from a preclinical perspective.

B. Recommendation for Nonclinical Studies

1. The toxicity profile in a non-rodent species has not been characterized. The sponsor has committed to performing a 1-month oral toxicity study in dogs, using doses that are high enough to characterize the acamprosate toxicity or achieve the maximum feasible dose.
2. An *in vitro* gene mutation assay in Chinese hamster V79 cells and an *in vitro* chromosome aberration assay should be repeated using adequate concentrations and currently accepted methodology according to ICH guidelines.
3. The carcinogenicity study in mice should be repeated using adequate dose selection according to ICH criteria.

C. Recommendations on Labeling

A preliminary label review was performed and is detailed under Section X. The sponsor's [redacted] should be removed as the [redacted] was deemed invalid by the Executive Carcinogenicity Assessment Committee. The genotoxicity section should be updated once the requested studies are performed and reviewed. Also, the findings in the reproductive toxicology studies in rats, rabbits and mice, namely malformations in rats and rabbits and increased incidence of still-born fetuses in mice, indicate that the pregnancy category should be Category C as opposed to the sponsor's proposal for a Category —

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Acamprosate slightly increased spontaneous activity in rats and attenuated induced hyperactivity in mice. Acamprosate reduced blood pressure in spontaneously hypertensive rats only. Cardiovascular effects were minor in dogs, and included slight decreases in heart rate and respiratory rate, and slightly increased PR and QRS intervals when administered intravenously, and sporadic instances of 2nd degree auriculo-ventricular heart block and ventricular premature beat at lead II after 13, but not 26 weeks of treatment in dogs by the oral route. No effect on QT interval was observed.

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 pharmacokinetic studies showed peak plasma levels and AUC values increased less than dose proportionally, and there were no differences between males and females in the measured parameters. The T_{max} occurred from 0.5-2 hours after oral dosing in rats, rabbits and dogs, and decreased approximately 50% (from approximately 15 hours to 7-9 hours) with repeated dosing.

The toxicity following single dose intravenous, intraperitoneal and oral administration is considered low in rodents, and is attributed in large part to the calcium moiety. The oral median lethal doses (6-9 g/kg) are approximately 10x higher than the IV LD₅₀ values suggesting poor oral bioavailability in rodents. The clinical signs prior to the deaths were convulsions, cardiac arrest and GI tract congestion in rodents and hypotension and cardiac arrest in rabbits (intravenous acamprosate only), suggesting a causal relationship. Clinical signs of reduced motor activity, hypotonia, apathy, gastrointestinal and cardiovascular effects were observed.

The target organs of toxicity in the repeated oral dosing studies in rodents were the GI system, kidneys, brain and heart. Doses of up to 2 g/kg/day produced toxicity in rodents, including loose feces, altered water consumption, increased urinary calcium, decreased heart, liver and testes weights, and increased adrenal, kidney weights, increased plasma calcium, urea nitrogen and phosphorus, increased urinary calcium and acidity, and decreased urine volume. Histopathologic examination showed degenerative renal tubulopathy, tissue calcifications, hyperkeratosis, vaculation and thrombus in the cerebellum, stomach dysplasia, and cardiac myolysis in rats. 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 diarrhea in monkeys at 1 g/kg/day for seven days.

Acamprosate was negative for mutagenicity in the Ames test, and for clastogenicity in the in vitro chromosome aberration assay in human lymphocytes and in the mouse micronucleus test. The results in an in vitro mutagenicity assay with Chinese hamster V79 cells were equivocal. The in vitro chromosome aberration and mutagenicity assay with Chinese hamster V79 cells should be repeated due to the equivocal findings and inadequate dosing/methodology.

The results of the carcinogenicity studies in rats and mice were presented to the Executive CAC 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. Acamprosate was negative for carcinogenicity in the two-year dietary rat study at doses up to 400 mg/kg/day. The carcinogenicity study in mice is unacceptable because inadequate doses were used, the study results were confounded by nematode infestation, and histopathology evaluation was conducted on an inadequate number of mid- and high-dose animals.

No adverse effects on male and female fertility were observed in mice and rats. In the rats, oral acamprosate resulted in an increase in number of fetuses with malformations and in the number of dams with malformed fetuses. The treatment-related malformations were 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) were observed. Studies in New Zealand white rabbits did not produce any findings of concern at comparable doses. No acamprosate-induced perinatal or postnatal toxicity was observed in the Segment III studies in mice and rats although an increased incidence of still-born fetuses occurred at doses of 960 mg/kg or greater.

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.

B. Pharmacologic Activity

Acamprosate showed efficacy in reduction of voluntary ethanol consumption in rodents by the oral and intraperitoneal routes, 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.

C. Nonclinical Safety Issues Relevant to Clinical Use

Currently, the potential of the test drug to produce genetic damage and carcinogenic effects has not been fully evaluated. In addition, the toxicity profile in a non-rodent species has not been characterized. The sponsor will be requested to address these deficiencies.

III. Administrative

/S/

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Kathleen Haberny, Ph.D.

B. Supervisor signature: Concurrence - _____
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Introduction and Drug History: Acamprosate (Aotal, Campral) is being studied 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 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.

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. Overall, these studies demonstrated efficacy of acamprosate in increasing alcohol abstinence and time to first relapse. The clinical safety data indicate that acamprosate, at therapeutic doses (1332-1998 mg/d PO for up to 1 year) 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) for maintenance of alcohol abstinence, since 1989.

Studies Reviewed in this NDA:

General Toxicology:

Acamprosate: 3-Week oral toxicity study in rats. Determination of blood levels

Acamprosate: 28-day oral (dietary administration) toxicokinetic study in the rat

AOTA-Ca (Acamprosate) subacute toxicity to rats by dietary administration for 13 weeks

Three month repeat dose oral toxicity study of calcium acetylhomotaurinate (acamprosate) in rats followed by a thirty day reversibility period

AOTA-Ca (acamprosate) twenty-six week oral toxicity study in the rat followed by a six week reversibility period

Acamprosate: 2-Week oral toxicity study in mice. Determination of blood levels

AOTA-Ca (acamprosate) sub acute toxicity to mice by dietary administration for 13 weeks

AOTA-Ca (Acamprosate) - 4 week intravenous toxicity study in the beagle dog

Preliminary 4-week oral toxicity study in the dog

AOTA-Ca (acamprosate) twenty-six week oral toxicity study in the beagle dog

Seven day subacute toxicity study in the macaque monkey by oral administration of calcium acetylhomotaurinate (acamprosate)

Genetic Toxicology:

Mutagenicity study in salmonella typhimurium HIS according to the B.N. Ames technique on calcium acetylhomotaurinate (acamprosate)

Study to determine the ability of acamprosate to induce mutation in four histidine-requiring strains of salmonella typhimurium and two tryptophan-requiring strains of escherichia coli

Mutagenicity study using the HPRT locus mutation technique in Chinese hamster V79 cells (resistance to 6-thioguanine) on the product calcium acetylhomotaurinate (acamprosate)

Genotoxicity study investigating chromosome aberrations by metaphase analysis in human lymphocytes on the product calcium acetylhomotaurinate (acamprosate)

Mutagenicity study in the mouse using the micronucleus test on the product calcium acetylhomotaurinate (acamprosate)

Study to evaluate the potential of acamprosate to induce micronuclei on the polychromatic erythrocytes of CD-1 mice

Carcinogenicity:

Acamprosate: 91 week oral (dietary administration) carcinogenicity study in the mouse

Acamprosate: 104 week oral (dietary administration) carcinogenicity study in the rat

Reproductive Toxicology:

AOTA-Ca (acamprosate): fertility in the mouse

AOTA-Ca (acamprosate): Oral (gavage) fertility study in the rat (Segment I)

AOTA-Ca (acamprosate): Embryotoxicity study in the mouse

AOTA-Ca (acamprosate): Oral (gavage) range-finding study in the pregnant rat

AOTA-Ca (acamprosate): An oral (gavage) teratology study in the rat

AOTA-Ca (acamprosate): Oral (gavage) range-finding study in the pregnant rabbit

AOTA-Ca (acamprosate): Oral (gavage) teratology study in the rabbit

AOTA-Ca (acamprosate): Embryotoxicity study in the rabbit

Oral study of the effects of AOTA-Ca (acamprosate) on segment II of reproduction in the rabbit

AOTA-Ca (acamprosate): Peri-natal studies in the mouse

AOTA-Ca (acamprosate): Oral (gavage) peri and post-natal study in the rat

Special Toxicology:

Acamprosate and MK-801 neurotoxicity study by a single administration to CD rats

Studies Not Reviewed in this NDA: The results of the following studies were summarized in this NDA review:

Pharmacology:

Ethanol and amino acids in the central nervous system: assessment of the pharmacological actions of acamprosate

Reduction in voluntary alcohol consumption in drinker rats with acamprosate administered by the intraperitoneal route (I.P.)

Reduction in voluntary alcohol consumption in drinker rats with acamprosate administered per OS (PO)

Determination of the minimum active dose of acamprosate in “alcohol-preferring-rats”

Acamprosate and measurement of voluntary alcohol consumption in alcohol-dependent rats (pulmonary alcohol application) in a free choice situation

Effects of acamprosate on alcohol induced behavioral and morphological alterations following a pulmonary chronic alcoholization

Experiments on effects of acamprosate on free choice drinking of ethanol solutions by rats

Scientific report on the effects of acamprosate on consumption of ethanol solutions by rats and on the toxicity of ethanol

Investigation into the activity of calcium acetyl-homotaurinate (acamprosate) on hyperconsumption of ethanol in the dependent rat

Ethanol-induced hypermotility test

Effect on ethanol-induced hypomotility

Antagonism of withdrawal syndrome in C.57.BL mice

Protection against acetaldehyde toxicity

Antagonism by acamprosate of the effects of acetaldehyde administered I.V.

Ethanol withdrawal test (acamprosate: 400 mg/kg)

Ethanol withdrawal test (acamprosate: 400 and 800 mg/kg)

Action of acamprosate on metabolism of ethanol in the rat

Binding to GABA_A and GABA_B receptors

Effect on cGMP levels

Nipecotic acid binding

Synaptosomal uptake of neuromediators

Pentetrazole-induced convulsions in the mouse

Effect on bicucullin-induced convulsions

Antagonism of acamprosate on excitatory amino-acids responses in bovine adrenal chromaffin cells

Mechanism of action of acamprosate. Part I. Characterization of spermidine-sensitive acamprosate binding site in rat brain

Mechanism of action of acamprosate. Part II. Ethanol dependence modifies effects of acamprosate on NMDA receptor binding in membranes from rat cerebral cortex

In vitro and in vivo effects of acamprosate on glutamate transmission

Central effects of acamprosate: Part I. Acamprosate blocks the glutamate increase in the nucleus accumbens microdialysate in ethanol withdrawn rats

Calciumdiacetylhomotaurinate (Ca-AOTA) decreases the action of excitatory amino acids in the rat neocortex in vitro

Acamprosate (calcium acetylhomotaurinate) decreases postsynaptic potentials in the rat neocortex: possible involvement of excitatory amino acid receptors

Acamprosate (calcium acetylhomotaurinate) enhances the N-methyl-D-aspartate component of excitatory neurotransmission in rat hippocampal CA1 neurons in vitro

The anti-craving drug acamprosate reduces C-Fos expression in rats undergoing ethanol withdrawal

Acamprosate and alcohol: III. Effects on alcohol discrimination in the rat

Investigation of spontaneous motility in the mouse by the actimetry method

Investigative behavior in the mouse in a free situation

Investigation of food and water consumption in animals treated with acamprosate and its derivatives

Effect on rectal temperature in the mouse

Amphetamine/chlordiazapoxide interaction in the mouse

Antagonism of morphine agitation

Interaction with harmaline-induced trembling

Open field test

Evasion test

Traction test
 Possible hypnotic activity
 Investigation of potentiation of barbiturate narcosis
 4-Plate test
 Investigation of the effect of acamprosate on aggressive behavior in response to electric shock in the rat
 Mouse-killing behavior in the rat
 Investigation of yohimbine cross-toxicity in the mouse
 Investigation of reserpine antagonist action in the mouse
 Antagonism of apomorphine-induced erect posture, stereotypy and hypothermia in the mouse
 Forced swimming test
 Tail suspension test
 Interaction with oxotremorine
 Investigation of interaction with apomorphine-induced stereotypy in the rat
 Investigation of amphetamine-induced stereotypy in the rat
 Antagonism of amphetamine group toxicity
 Investigation of prochlorperazine (PCPZ) interaction
 Effect on picrotoxin-induced convulsions
 Effect on strychnine-induced convulsions
 Manifestations of gallamine triiodoethylate-induced behavior modifications in the rat
 Influence of acamprosate on acetylpyridine-induced behavior modifications in the rat
 Effect on kainic acid-induced, wet-dog-shaking sign
 Investigation of possible "anti petit-mal" activity
 Investigation of interaction with tranlycypromine/L-tryptophan combination
 Interaction of MAOI and L-tryptophan in rats pre-treated with a blocking dose of PCPA
 Interaction of MAOI and L-tryptophan in rats pre-treated with a non-blocking dose of PCPA
 Investigation of possible modifications of tranlycypromine/L tryptophan combination-induced hyperactivity-hyperthermia syndrome in rats pre-treated with PCPA (non-blocking dose) and acamprosate
 Investigation of analgesic activity by the phenylbenzoquinone (PBQ) method
 Investigation of analgesic activity by the hot-plate method
 Potentiation of morphine analgesia by the phenylbenzoquinone (PBQ) method
 Tail-burn analgesia
 Action of acamprosate I.V. on cardiovascular parameters in the non-anaesthetised normotensive rat
 Action of acamprocate I.P. on cardiovascular parameters in the spontaneously hypertensive rat
 Action of acamprosate in combination with adrenaline I.V. on cardiovascular parameters in the non-anesthetized normotensive rat
 Investigation of anti-arrhythmic action in the mouse
 General activity profile in the dog following I.V. administration of acamprosate
 Sodium nicotinate-induced flush effect
 Stabilizing action on the erythrocyte membrane
 Anti-inflammatory activity on carrageenin-induced oedema in the rat
 Action of acamprosate on ovalbumin-induced generalized oedema
 Investigation of antagonism of barium chloride-induced contractions on isolated rat duodenum

Investigation of antagonism of histamine dihydrochloride-induced contractions on isolated guinea pig ileum

Investigation of antagonism of acetylcholine-induced contractions on rat duodenum

Acamprosate drug interactions

Reinforcing and discriminative stimulus effects of Ca Acetyl Homotaurine in animals

The transport of ^{14}C -acamprosate across CACO-2 monolayers

Supplement report on acamprosate experiments with confluent monolayers of CACO-2 epithelial cells

Pharmacokinetics and Toxicokinetics:

The effect of dose level on the absorption and excretion of AOTA-Ca (Acamprosate) in the rat and the dog

Study of bioavailability and linearity of kinetics in the rat after single administration

Acamprosate plasma kinetics in dogs after a single oral or intravenous administration of doses between 25 and 400 mg/kg

Pilot studies: Acamprosate plasma levels and urinary elimination after intravenous and oral administration of 20 and 31.7 mg/kg in dogs

Pilot studies: Acamprosate plasma levels and urinary elimination after oral administration of 100 mg/kg in rats

Studies with [^{14}C]AOTA-Ca (acamprosate) in rabbits

Plasma kinetics of acamprosate in the rat after oral administration of single and repeat doses of 100 mg/kg

The metabolism and pharmacokinetics of [^{14}C]-AOTA-Ca (acamprosate) in dogs

Plasma kinetics of acamprosate in the dog after single and repeated oral administration of the dose: 100 mg/kg/day measurement of urinary elimination

Binding of ^{14}C acamprosate to plasma proteins. Comparison rat-dog-human

The absorption, tissue distribution and excretion of ^{35}S -AOTA-CA (Acamprosate) in the rat and the dog

Kinetics of plasma and tissue radioactivity in the rat after single oral administration of 100 mg/kg of ^{14}C acamprosate

The disposition of [^{14}C]-AOTA-Ca (Acamprosate) in pigmented rats

Study of placental transfer in the rat kinetics of plasma and tissular radioactivity in the pregnant and non-pregnant female after single oral administration of ^{14}C Acamprosate at the dose of 100 mg/kg

Cytochrome P450 inhibitory and induction properties of acamprosate: an in vitro study using human liver microsomes and hepatocytes

Pilot study rats: Excretion and metabolism after administration of ^{14}C AOTA Ca (acamprosate)

Analysis of the major radioactive components in urine and faeces from rats and man following oral administration of [^{14}C]AOTA-Ca (acamprosate)

Passage into milk in the rat. Kinetics of radioactivity in plasma and milk of lactating females after single oral administration of 300 mg/kg of ^{14}C Acamprosate

General Toxicology:

Determination of the LD₅₀ of acamprosate following oral administration to mice for 14 days

Determination of the LD₅₀ of acamprosate following intravenous administration to mice for 14 days

Single dose toxicity study in mice with calcium acetylhomotaurinate (Acamprosate) calcium chloride, homotaurine, sodium acetylhomotaurinate
Determination of LD₅₀ of acamprosate following intravenous administration to rats for 14 days
Determination of the LD₅₀ of acamprosate following oral administration to rats for 14 days
Single dose toxicity study in rats with calcium acetylhomotaurinate (acamprosate), calcium chloride, homotaurine, sodium acetylhomotaurinate
Minimal lethal dose of calcium acetylhomotaurinate and calcium chloride via intravenous infusion in the rabbit
Toxicity of a single dose of calcium acetylhomotaurinate tablets in the rabbit
Nitrosatability of acamprosate

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Pharmacodynamics and Mechanism of Action

Effects on Alcohol Consumption: The results of pharmacodynamic studies in male Long Evans rats showed significant, dose-dependent decreases (24%, 33%, and 44% at 50, 100, and 200 mg/kg/day IP for 15 days) in voluntary alcohol consumption. This effect was inhibited by co-administration of bicuculline, a gamma-aminobutyric acid (GABA) antagonist, suggesting that the mechanism of effect of acamprosate involves GABA transmission. In another study, reduction of ethanol consumption in "drinker" Long Evans rats (with experimentally induced ethanol dependence), ethanol self-administration was significantly decreased (8%-35%) from days 15 to 29 when acamprosate (1%) was continuously administered in drinking water for 29 days. Administration of three compounds related to acamprosate, homotaurine, sodium acetylhomotaurinate and calcium chloride, at comparative doses, had no effect on ethanol consumption in rats. The minimum active acamprosate dose in Long-Evans rats was 25 mg/kg/d PO in a 3-week study at doses from 10-25 mg/kg/day acamprosate. In male Wistar rats made alcohol-dependent in a pulmonary alcohol exposure model, 400 mg/kg/d oral acamprosate significantly reduced voluntary alcohol consumption. In another study in Wistar rats, acamprosate (50-400 mg/kg/d PO) significantly and dose-dependently decreased the duration of alcohol consumption (approximately 21, 11, 9 and 1 days at 50, 100, 200, and 400 mg/kg/day, respectively). Reduction of ethanol consumption by acamprosate at 50-4000mg/L PO was demonstrated in ethanol-dependent (1-5 ml/day in acamprosate-treated rats compared to 4-9 ml/day in the controls over a 10-day treatment period) but not in non-dependent Wistar rats. Acamprosate at 50 mg/kg IP was effective in reducing alcohol consumption in alcohol-dependent male Wistar rats following a single acute administration.

Effects on Acute Ethanol and Acetaldehyde Toxicity: Acamprosate at 25 and 50 mg/kg IP had no effect on the acute toxicity of ethanol, but did reverse the analgesic effect of ethanol at 50 mg/kg IP, in male Wistar rats. Hyperactivity in mice was inhibited by up to 61% during the 60 minute period after ethanol administration by acamprosate at 100-800 mg/kg PO and by homotaurine at 100-400 mg/kg PO to a similar extent, but calcium chloride (111-444 mg/kg PO) and sodium acetylhomotaurinate (400 mg/kg PO) had no effect. Acamprosate at 200 mg/kg PO, but not homotaurine, sodium acetylhomotaurinate or calcium chloride, reversed alcohol effects indicative of central nervous system (CNS) depression (e.g. staggering, muscle spasms) in the mouse. Both acamprosate (100-300 mg/kg PO) and sodium acetylhomotaurinate (300 mg/kg PO) significantly increased survival in rats after administration of a lethal oral dose of acetaldehyde (792 mg/kg) compared to saline control animals. In mice, acamprosate (100-400 mg/kg PO) reversed the hypoactive effects of 100 mg/kg intravenous acetaldehyde. Homotaurine, sodium acetylhomotaurine and calcium chloride had no effect on acetaldehyde-induced hypoactivity in these animals.

Effects on Alcohol Withdrawal Syndrome: Acamprosate (400 and 800 mg/kg PO and IP), but not oral homotaurine (400 mg/kg) significantly decreased the number of head twitches, an index of alcohol withdrawal in mice. Head twitches decreased 19.5%, 43.4%, 36.0%, 42.7%, and

50.8% at 30, 60, 90, 120, and 150 minutes at 400 mg/kg PO acamprosate, and 42.0%, 53.3%, 34.45, 45.6%, and 49.2% at the same timepoints at 800 mg/kg PO acamprosate. Acamprosate (100 mg/kg IP), but not homotaurine (50 mg/kg IP), calcium chloride (29 mg/kg IP) and sodium acetylhomotaurinate (80 mg/kg IP), decreased convulsions (means of 1.2, 1.0, 1.8, 1.8, 2.2, 2.2, 2.2, 2.2, and 2.9 compared to control means of 1.2, 1.6, 2.2, 2.8, 2.8, 2.8, 2.6, 2.6, and 2.8 at 1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, and 6 hours after removing ethanol), and other indices of alcohol withdrawal (tremors, hyperexcitability and tail rigidity) in mice. This effect was antagonized by bicuculline (2 mg/kg IP) but not bicuculline methobromide (2 mg/kg IP) suggesting a central GABAergic mechanism for acamprosate inhibition of ethanol withdrawal effects.

Effects on Ethanol Metabolism: Acamprosate (200 mg/kg IP) slightly decreased absorption of 2.4 g/kg intragastric ethanol by when administered 30 minutes prior to ethanol, and decreased the rate of elimination of alcohol by 18% in Long Evans rats.

General Pharmacology

Mechanism of Action: In *in vitro* studies, acamprosate displaced GABA at both GABA type A and type B receptors. Cerebellar cGMP levels, measured by radioimmunoassay, decreased approximately 50% after both acute (300 mg/kg IP) and repeated (150 mg/kg/d IP, 12 days) acamprosate administration, in an *ex vivo* study in rats. Other studies in rats showed that acamprosate (150 mg/kg/d IP for 10-12 days) increased nipecotic acid (marker for cerebral GABA uptake sites) uptake sites ($K_D = 1.66 \pm 0.19$ and $B_{max} = 52.7 \pm 10.8$ where B_{max} = number of receptor sites, compared to $K_D = 0.905 \pm 0.2$ and $B_{max} = 26.3 \pm 8.0$ in the controls), reduced the rate of GABA uptake ($V_{max} = 0.747 \pm 0.3$ compared to 1.9 ± 0.44 in the control) and increased the affinity of the transporter for GABA ($K_M = 137 \pm 60$ compared to 469 ± 96 in the control) in the corpus striatum, and increased GABA uptake in the thalamus (mean 52150 ± 1571 fmol/mg protein compared to 42754 ± 4920 fmol/mg protein in the controls at 30 hours on day 10). In mice, acamprosate at 400 and 800 mg/kg IP prolonged survival time (23.28 and 22.83 minutes, respectively, compared to 13.11 minutes in the controls), and at 800 mg/kg IP delayed the onset of convulsions (latency to onset of convulsions 6.22 minutes compared to 2.97 minutes in the controls), but did not prevent death in response to the GABAergic antagonist pentetrazole. In comparison, the barbiturate phenobarbitone fully antagonized the lethality by pentetrazole administration. In mice, acamprosate (400 mg/kg IP) delayed (latency of death 2015 ± 279 seconds compared to 1029 ± 56 seconds in the controls), but did not prevent, mortality in response to bicuculline (GABA_A antagonist, 7.5 mg/kg SC).

Effects on Excitatory Amino Acids: In bovine adrenal cell cultures, acamprosate (10 nM - 1 mM administered 30 min before, but not simultaneously with NMDA or homocysteic acid) inhibited NMDA receptor stimulated catecholamine release. Acamprosate did not inhibit catecholamine release induced by nicotinic receptor activation or high potassium concentration. Acamprosate did not displace MK-801 from NMDA receptor binding sites and did not prevent glutamate-induced MK-801 binding in rat brain membranes. Acamprosate (10 mM - 0.1 mM) increased glutamate receptors in the hippocampus and striatum in rat brain synaptosomes and in *in vivo* experiments (100 mg/kg/day for 2 weeks).

Summary of Pharmacology

In rats, oral (400 mg/kg/d) and intraperitoneal (50-200 mg/kg/d for 15 days) acamprosate reduced voluntary ethanol consumption and reversed some effects of acute ethanol and acetaldehyde toxicity and alcohol withdrawal (at 50-400 mg/kg/d PO). Alcohol-induced hyperactivity in mice was inhibited by acamprosate at 100-800 mg/kg PO. Continuous administration of 1% acamprosate in drinking water for 29 days, or a single acute IP administration of 50 mg/kg 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 appeared 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.

Pharmacology conclusions: Acamprosate demonstrated dose-dependent reduction in voluntary ethanol consumption in rodents by the oral and intraperitoneal routes, with an onset of action at 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, perhaps by restoring the inhibition/excitation balance that may be altered by chronic alcohol consumption.

II. SAFETY PHARMACOLOGY:

Neurological effects:

Acamprosate at doses up to 400 mg/kg IP, and related substances homotaurine (up to 1000 mg/kg IP), sodium acetylhomotaurinate (up to 800 mg/kg IP) and calcium chloride (up to 400 mg/kg IP), had no effect on motor activity in mice. Acamprosate at doses up to 400 mg/kg IP and related substances homotaurine (up to 100 mg/kg IP), sodium acetylhomotaurinate (up to 850 mg/kg IP) and calcium chloride (up to 400 mg/kg IP) had no effect in mice on normal exploratory behavior in a free situation. Acamprosate, and the three related substances at up to 800 mg/kg PO for 11 days each, had no effect on food and water consumption in mice. Acamprosate induced transient hypothermia in mice at 100 mg/kg IP and sustained hypothermia at 220 mg/kg IP. Acamprosate antagonized amphetamine ($ED_{50} = 650$ mg/kg), chlordiazepoxide ($ED_{50} = 650$ mg/kg), and morphine-induced hyperactivity (400 mg/kg IP or 800 mg/kg PO) in mice; the related substances had little or no effect on this parameter. In rats, acamprosate (ED_{50} 450 mg/kg IP) and calcium chloride (ED_{50} 304 mg/kg IP) antagonized harmaline-induced hyperactivity. Acamprosate demonstrated no sedative or muscle relaxant effects measured by number of reaches (at 200-800 mg/kg PO) in rats, time of first escape or number of escapes (except during the last 2 minutes) in an evasion test (at 200-800 mg/kg PO) in mice, or recovery

from placement on a support by the front paws (at 200-800 mg/kg IP) in mice. Homotaurine increased reaches and number of movements at 800 mg/kg PO. There was a significant increase afteracamprosate in the number of explorations and movements (400-800 mg/kg PO) in rats. Oralacamprosate (up to 1000 mg/kg) and its derivatives had no effect on hypnotic activity or pentobarbitone-induced narcosis in mice. Acamprosate and its derivatives had no anxiolytic effects in the 4-plate test in mice (100-400 mg/kg IP), fighting behavior in rats after electroshock (25-200 mg/kg IP) or mouse-killing behavior in the isolated magnesium-deficient rat (200-400 mg/kg IP).

In tests of anti-depressant activity,acamprosate increased yohimbine toxicity in mice (ED_{50} = 275 mg/kg IP and 5900 mg/kg PO) suggesting antidepressant activity at alpha and beta-adrenergic receptors, and antagonized reserpine-induced hypothermia but not ptosis in mice (100-800 mg/kg IP) suggesting beta-adrenergic activity. However,acamprosate (100-400 mg/kg IP) and derivatives had no effect on the apomorphine-induced righting reflex, stereotypy and hypothermia in mice, and thus demonstrated no antidopaminergic activity of many antidepressants. In additional tests of antidepressant activity,acamprosate had no effects on agitation time in non-escapable water (at 10-400 mg/kg IP and 400 mg/kg PO), immobility time and increase in movement energy and power upon suspension by the tail (10-400 mg/kg IP and 400 mg/kg PO), and inhibition of oxotremorine-induced hypothermia (at 400 mg/kg IP) in mice, although lower doses (100 and 200 mg/kg IP) resulted in a tendency toward antagonism of oxotremorine hypothermia. Thus,acamprosate does not have antidepressant properties.

In tests of neuroleptic activity,acamprosate had no effect on apomorphine stereotypy (at 200-800 mg/kg IP) or amphetamine-induced stereotypy (at 100-400 mg/kg IP) in rats, but did reduce amphetamine mortality to 16% and 66% (at 400 and 800 mg/kg IP respectively) in mice. Acamprosate (200 or 400 mg/kg IP or 1000 mg/kg PO) had no effect on prochlorperazine catatonia in rats, and thus demonstrated no dopaminergic or parasympatholytic activity.

Acamprosate (200-400 mg/kg IP) was not anticonvulsant against picrotoxin-induced or strychnine-induced seizures in mice. However,acamprosate (112-896 mg/kg IP) did antagonize gallamine triiodoethylate-induced twitch, convulsions and death, which result from anoxia, in mice. Calcium chloride (130 mg/kg IP) and sodium acetylhomotaurinate (at 920 mg/kg IP) were also protective in this test. Acamprosate (100-200 mg/kg IP) and sodium acetylhomotaurinate (200 mg/kg IP) reduced acetylpyridine-induced tremor in rats, but not other indices of acetylpyridine-induced cerebellar lesions including "rolling", hypoactivity and motor weakness. The shaking response to kainic acid-induced hippocampal cytotoxicity in rats was significantly reduced byacamprosate at 850 mg/kg PO and 380 mg/kg IP and by homotaurine at 100 and 200 mg/kg IP. Acamprosate had no effect on gamma-hydroxybutyrate-induced EEG changes characteristic of petit mal activity, in male rabbits.

The results of several studies suggested thatacamprosate interacts with serotonergic systems in rats; the conclusions suggested antagonism of serotonin effects during high serotonin activity and potentiation of serotonin effects during low serotonergic activity. Acamprosate at 500 and 800 mg/kg IP antagonized hyperthermia, hyperactivity and death (indices of excessive serotonin activity) resulting from tranlycypromine/L-tryptophan at 20 and 250 mg/kg IP respectively. Acamprosate at 200 and 400 mg/kg IP, when administered with tranlycypromine, induced

hyperactivity that was antagonized by parachlorophenylalanine, a serotonin synthesis inhibitor.

Acamprosate at 400 mg/kg IP antagonized phenylbenzoquinone induced writhing and potentiated morphine analgesia in mice. On the other hand, acamprosate at 400 mg/kg IP had no effect on hot-plate reaction time in mice, and at doses up to 800 mg/kg PO had no effect in the tail-burn analgesia test in rats.

Cardiovascular effects:

Acamprosate had no effect on blood pressure and heart rate 20 minutes after dosing in conscious normotensive rats at 50 mg/kg IV and from 15-120 minutes after dosing in anesthetized (ethyl carbamate at 1.25 g/kg IP) normotensive rats at 200 mg/kg PO. Acamprosate had no effect on epinephrine-induced (0.5 mcg/kg IV coadministered with acamprosate) tachycardia in conscious normotensive rats at 50 mg/kg IV measured 2 minutes after dosing, and no effect on chloroform-induced (by inhalation 10 minutes after acamprosate administration) fibrillation in mice at doses from 200 - 800 mg/kg IP. Acamprosate decreased blood pressure at 500 (12%) and 1000 (8%) mg/kg IP and decreased heart rate at 500 mg/kg IP (9%) in spontaneously hypertensive rats.

Anesthetized (pentobarbitone) mongrel dogs administered acamprosate at 30, 100, 330, and 1000 mg/kg IV showed dose-related decreased heart rate (up to -16% baseline) and increased respiratory rate at all doses, and slightly increased PR interval and QRS interval at all doses. No effects on QT interval were noted. In that study, the mortality rate was 60% at 330 mg/kg IV and 100% at 1000 mg/kg IV. Cardiovascular effects possibly related to acamprosate treatment were also observed in the 26-week oral toxicity study in the beagle dog, described under Repeated Dose Toxicology below. One 2nd degree auriculo-ventricular heart block was observed at baseline in 1/4 control females, one 2nd degree auriculo-ventricular heart block was observed 90 min after the first dose and one 1st degree block was observed before administration in week 13 in 1/4 high dose male dogs, one ventricular premature beat at lead II before administration was observed in week 13 in another high dose male dog, and several 2nd degree auriculo-ventricular blocks were observed before administration in week 13 in 1/4 high dose female dogs. There were no treatment related cardiovascular effects at the low and mid- doses, and in any group in week 26.

Other:

General Pharmacologic Activity:

The sponsor reported that up to the maximum tolerated dose, acamprosate at 100 mg/kg IV had little effect on blood pressure, heart rate, ECG, respiratory rate, diuresis, choleresis, duodenal motility and rectal temperature in dogs. Acamprosate, at 125-1000 mg/kg PO, inhibited sodium nicotinate-induced peripheral vasodilation in guinea pigs, demonstrated by attenuation of sodium nicotinate-induced increase in ear temperature and reddening. Acamprosate prevented heat and hypotonic medium induced hemolysis in isolated rabbit erythrocyte membrane (ED₅₀ = 1 mmol/l).

Anti-Inflammatory and Anti-Allergic Activity:

Acamprosate, at doses of 200-800 mg/kg PO, was not anti-inflammatory in the carrageenin-induced edema test in Sprague-Dawley rats. Acamprosate was slightly anti-allergic at 400 mg/kg/d PO in the ovalbumin-induced generalized edema test in rats.

In Vitro Effects on Smooth Muscle Contraction:

Acamprosate had no effects on barium chloride-induced rat duodenum contractions and acetylcholine-induced rat duodenum contractions at 10^{-6} to 10^{-3} g/l, and only slightly antagonized histamine-induced contractions in isolated guinea pig ileum at 10^{-3} g/l.

Drug Interactions:

Drug interactions studies were conducted using oral acamprosate at doses of 100, 200, and 400 mg/kg, and drugs likely to be co-administered in the treatment of alcohol abuse. The parameters studied included mortality, and attenuation or antagonism of the pharmacologic effect. No interactive effects were observed in the pentylenetetrazole-induced convulsions test with the anticonvulsants, phenobarbitone (25 mg/kg PO), sodium valproate (280 mg/kg PO), and diazepam (1.7 mg/kg/ PO) in male Wistar rats. No interactive effects were observed in the reserpine-induced ptosis and hypothermia test using the antidepressant imipramine (15-25 mg/kg PO) and potentialization of 5HTP effects (tremors, head twitches, spreading of hind limbs) with the antidepressant fluvoxamine (10 mg/kg PO) in female mice.

In drug interaction tests with several anxiolytics in female mice, the 4-plate test evaluated removal of behavioral inhibition to electric shock. Acamprosate, at doses of 100, 200, and 400 mg/kg PO had no effect on meprobamate (130 mg/kg PO) anxiolysis. Acamprosate showed slight antagonism of the anxiolytic effect at 400 mg/kg/ PO, but not at 100 and 200 mg/kg PO against chlorazepate dipotassium (1 mg/kg/ PO), slight antagonism at 100, 200 and 400 mg/kg PO against diazepam (2.5 mg/kg/ PO), and slight potentiation of atrium (200 mg/kg PO) at 400 but not at 100 and 200 mg/kg PO. The positive interactive effects observed in this test were not statistically significant. Interactive effects with the neuroleptics were measured by apomorphine-induced climbing in female mice. Acamprosate had no effects on haloperidol (0.4 mg/kg IP), sulpiride (45 mg/kg IP) and chlorpromazine (4.5 mg/kg IP) inhibition of climbing at doses of 100-400 mg/kg PO). Acamprosate slightly antagonized tiapride (100 mg/kg IP) inhibition of apomorphine-induced climbing at 400, but not at 100 and 200 mg/kg PO.

Acamprosate at doses of 200 and 400 mg/kg PO slightly attenuated the sleep delay and sleep duration effects by butobarbitone (140 mg/kg PO) in female mice, and the effect on blood pressure by the hepatic ethanol metabolism inhibitor, disulfiram (100 mg/kg PO), in rats. The interactions were not statistically significant.

Summary of Safety Pharmacology

In studies on neurological effects, acamprosate increased spontaneous activity in rats but had no effect on normal motor activity or exploratory behavior in mice. Acamprosate induced sustained

hypothermia, antagonized amphetamine, chlordiazepoxide and morphine-induced hyperactivity, and antagonized harmaline-induced tremors in the rodent studies. The meaning of these interactions is not clear. Acamprosate had no anxiolytic effects, no effects on hypnotic activity or pentobarbitone-induced narcoses, no effects on food and water consumption, no sedative or muscle relaxant effects, and no effects on fighting behavior after electroshock. Also, there was 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. 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. 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.

Safety pharmacology conclusions:

In conclusion, acamprosate had negligible central nervous system activity except for a slight increase in spontaneous activity in rats and attenuation of induced hyperactivity in mice. No cardiovascular effects were noted in normal rats, but acamprosate reduced blood pressure in spontaneously hypertensive rats. Cardiovascular effects were minor in dogs, and included slight decreases in heart rate and respiratory rate, and slightly increased PR and QRS intervals when administered intravenously; no effects on QT interval were noted. Oral administration induced sporadic instances of 2nd degree auriculo-ventricular heart block and ventricular premature beat at lead II after 13, but not 26 weeks of treatment in dogs.

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.

III. PHARMACOKINETICS/TOXICOKINETICS:

Absorption:

In male Wistar rats, the bioavailability of acamprosate at doses of 50, 200 and 400 mg/kg by oral gavage was 7%, 16%, and 15%, respectively. The bioavailability of oral acamprosate (25, 100 and 400 mg/kg) in male beagle dogs was approximately 61%, 26% and 13%, respectively. In another study comparing the capsule (20 or 31.71 mg/kg) and non-enteric-coated tablet (20 or 31.71 mg/kg) forms in male dogs, the oral bioavailability of acamprosate was 35%-112% in capsule form, and 21%-61% in tablet form. No data was collected on acamprosate absorption in female animals.

Distribution:

In male Sprague-Dawley and Long Evans rats, the greatest concentrations of radioactivity from ³⁵S-labeled acamprosate (20 mg/kg PO) outside the gastrointestinal (GI) tract were in the liver (11.1 mcg eq/g) and kidney (20.2 mcg eq/g) at 2 hours, lymph nodes (4.58 mcg eq/g) at 1 hour and bone marrow (2.97 mcg eq/g) at 4 hours. At 24 hours post-drug administration, all remaining radioactivity was found in the GI tract, kidneys, liver and lungs. In male Wistar rats given acamprosate at 100 mg/kg by oral gavage, radioactivity was highest in the GI tract (94.2% at 2 h and 41.3% at 48 h). Radioactivity measurements in kidney were 12.1, 33.7 and 3.44 mcg eq/g at 30 min, 24 h and 48 h respectively, and in liver the concentrations were 8.65 mcg eq/g and 0.57 mcg eq/g at 4 and 48 h. Radioactivity concentrations were higher in the kidneys and liver than in plasma. In brain, the radioactivity levels were highest at 30 min (0.755 mcg eq/g) and declined over 6 h. The brain/plasma area ratio was 0.17.

In pregnant Wistar female rats, plasma radioactivity was 5.28 mg eq/l at 30 min and 0.24 mg eq/l at 48 h, similar to that in non-pregnant female rats. Amniotic fluid radioactivity concentrations were lower than in plasma (0.5 to 0.04 mg eq/l at 6 and 48 h respectively). In pregnant and non-pregnant female rats, the concentrations of radioactivity were higher in kidney and liver than in plasma. The placenta/plasma AUC ratio was 0.2, fetus/plasma ratio 0.43 and amniotic fluid /plasma ratio 0.2.

After 20 mg/kg oral ³⁵S-labeled acamprosate administration in male beagle dogs, most radioactivity was in the GI tract. Highest concentrations in other organs were detected at 1 hour post-drug administration; kidney and liver concentrations were 232.8 and 11.4 mcg eq/g respectively. At 24 h, radioactivity was detected in the adrenal glands (1.02 mcg eq/g), bile (0.59 mcg eq/l), liver (10.2 mcg eq/g) and kidneys (8.87 mcg eq/g). At 120 h, radioactivity was detected in the lacrimal glands (0.456 mcg eq/g).

Metabolism:

No evidence of acamprosate metabolism was found in fasting and non-fasting male Wistar rats administered ^{14}C -acamprosate 100 mg/kg PO and 100 mg/kg IV. No metabolites of acamprosate were found in urine, feces or plasma in male pigmented rats administered ^{14}C -acamprosate at 20 mg/kg PO, White rabbits administered ^{14}C -acamprosate at 100 and 1000 mg/kg PO, and dogs administered ^{14}C -acamprosate at 100 mg/kg PO. In an *in vitro* assay on potential metabolic inhibitory properties in human liver microsomes, acamprosate had no inhibitory effect on CYP1A2, 2C9, 2C19, and 6, 2E1, and 3A4 enzymatic activities at the concentration tested (100 mcM). No inducing potential by acamprosate was found at the concentration of 100 mcM on the CYP1A2 and 3A4 enzymes in human hepatocytes

Excretion:

The results of the preclinical excretion studies are summarized in the following table.

Species	n	Drug	Route	Dose (mg/kg)	Time Interval (h)	Urinary Excretion (% Radioactivity Recovered)	Fecal Excretion (% Radioactivity Recovered)
Male Wistar Rats (non-fasted)	1	^{14}C -acamprosate	Oral	100	72	10.6 (10.23/first 24 h)	Remainder
Male Wistar Rats (fasted)	1	^{14}C -acamprosate	Oral	100	72	13.6 (11.81/first 24 h)	Remainder
Male Wistar Rats	1	^{14}C -acamprosate	IV	100	72	90.5 (87.3/first 8 h)	8.56
Male Sprague-Dawley Rats	3	^{35}S -acamprosate	IV	40	120	>90 (all within 24h)	Approx 6
Male Sprague-Dawley Rats	3/grp	^{35}S -acamprosate	Oral	40,200, 1000	120	Approx 24,22,25	Approx 76,78,77
Male Sprague-Dawley Rats	3	^{45}Ca -acamprosate*	IV	40	120	<6	Not available
Male Sprague-Dawley Rats	3/grp	^{45}Ca -acamprosate*	Oral	40,200, 1000	120	0	Approx 30
Male Sprague-Dawley Rats	10	^{35}S -acamprosate	Oral	20	120	11.3-14.5 (most in first 24h)	85.6-89.4 (most in first 24h)
Male Wistar Rats	8	Acamprosate	Oral	100	72	8.3 (6.4 w/in 24 h)	Not available
Female New Zealand White Rabbits	5	^{14}C -acamprosate	Oral	100	120	56.2+11.1 in cage wash	23.4
Beagle Dogs	2	Acamprosate	IV	31.71	24	68.9&90.3 (50% in first 2h)	Not available
Beagle Dogs	2	Acamprosate	Oral capsule	31.71	24	64.6&36.6	Not available
Beagle Dogs	2	Acamprosate	Oral tablet	31.71	24	44.6&37.6	Not available
Male Beagle Dogs	3	^{35}S -acamprosate	IV	40	120	>90 (most in first 24 h)	Not available
Male Beagle Dogs	3/grp	^{35}S -acamprosate	Oral	40,200, 1000	120	35.1, 31.8, 35	56.4, 51, 35

Beagle Dogs	3/sex	¹⁴ C-acamprosate	Oral	100	168	22-41 (no differences between sexes)	59-72 (no differences between sexes)
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*Most radioactivity due to ⁴⁵Ca-acamprosate found in bone residue.

Urinary excretion was 90% after intravenous acamprosate at 40 mg/kg, and 22%-25% after oral acamprosate at 40, 200 and 1000 mg/kg in male Sprague-Dawley rats. In male beagle dogs, urinary excretion was 90% after intravenous (40 mg/kg) and 32%-35% after oral (40, 200 and 1000 mg/kg) administration. Excretion of radioactivity was complete by 120 hours after intravenous (40 mg/kg) and oral (40, 200, 1000 mg/kg) ³⁵S-acamprosate in both rats and dogs. More than 91% radioactivity was excreted in the urine after IV dosing in both species, and 24% and 35% radioactivity was excreted in urine and 76% and 56% in feces after oral dosing in rats and dogs, respectively. In another study in Sprague-Dawley and Long-Evans rats, 85.64%-89.40% radioactivity was recovered in the feces and 11.28%-14.47% radioactivity was recovered in the urine over 120 hours after dosing with 20 mg/kg oral ³⁵S-acamprosate, with most radioactivity recovered in the first 24 hours after dosing. Oral administration of ³⁵S-acamprosate at 20 mg/kg in dogs resulted in up to 66.04% total radioactivity excreted in the feces and up to 47.88% excretion in urine by 120 hours after dosing, and up to 3.68% excretion in bile by 24 hours after dosing. By comparison, most of the radioactivity was retained after dosing with ⁴⁵Ca acamprosate in rats, due to incorporation of the Ca into bone. In comparison, human elimination of intravenous acamprosate is primarily renal (90% within 24 h). After oral administration (1320 mg) in humans, 11% of the acamprosate dose was recovered in urine and 88% in feces over five days, suggesting limited absorption from the gastrointestinal tract.

No radioactivity was detected in expired air of rats administered 100 mg/kg oral and intravenous ¹⁴C acamprosate. Urinary and fecal acamprosate excretion was unaffected by fasting state.

Excretion of acamprosate into milk was measured in female Wistar rats after a single oral dose of ¹⁴C-acamprosate (dose not provided in summary), 6-8 days postpartum. Peak radioactivity occurred in plasma (16.4 mcg eq/ml) and whole blood (11.5 mcg eq/ml) at 1-2 hours and fell to 0.23 and 0.29 mcg eq/ml respectively over 24 hours. Acamprosate was detected in the milk and maximum radioactivity concentration occurred in milk (5.25 mcg eq/ml) at 4 hours. The milk/plasma ratio was 0.33 at 2 hours, 1.34 at 4 hours, 1.0 at 6-8 hours, and 3.8 at 24 hours.

Plasma Protein Binding:

Protein binding was evaluated in plasma samples from Wistar rats, Beagle dogs and healthy human volunteers administered ¹⁴C acamprosate at concentrations of 0.25, 1, and 10 mcg/ml. The results of the binding assay are presented in the following table:

Species	0.25 mcg/ml	1 mcg/ml	10 mcg/ml
Rat	29.33	2.63	8.45
Dog	4.42	1.2	1.68
Human	3.9	9.98	4.05

Protein binding was low in rat, dog, and human plasma, with higher percent binding in rat (overall mean approximately 13.5%) than in dog (2.4%) and human (6%) samples.

Other studies:

Study title: ACAMPROSATE: 28 DAY ORAL (DIETARY ADMINISTRATION) TOXICOKINETIC STUDY IN THE RAT

Key study findings:

- One high dose (400 mg/kg/day) female rat died; cause of death unknown
- No other toxicity by acamprosate demonstrated in this study
- Slightly less than dose proportional increases in Cmax and AUC values in males and females
- No differences between males and females in the TK parameters
- Results demonstrated systemic exposures that were 0.2X, 0.7X, and 2.3X the MRHD of 1998 mg/day on an AUC basis for the total drug.

Study no: 537/059

Volume # Amendment #003 Volume 1, and page #: 1

Conducting laboratory and location: Animal treatment:

TK analysis:

Date of study initiation: August 30, 2001

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot # M242B, radiolabel None, and % purity: %

Formulation/vehicle: Admixture in the diet

Methods (unique aspects):

Dosing:

Species/strain: ~ CD(SD)IGSBR rats

#/sex/group or time point (main study): 12/sex/dose: toxicokinetic study

Satellite groups used for toxicokinetics or recovery: none

Age: 42 days

Weight: 303.0-367.4 g males, 193.2-237.7 g females

Doses in administered units: 0, 25, 100, 400 mg/kg/day

Route, form, volume, and infusion rate: Admixture in diet

Observations and times:

Clinical signs: Daily

Body weights: Weekly

Food consumption: Weekly

Clinical chemistry: Serum Calcium at the time of blood sampling for TK analysis

Toxicokinetics: Day 28 2.5 ml blood samples at 6:00, 9:00, 12:00, 15:00, 18:00, 21:00, 24:00, and at 3:00 on day 29

Results:

Mortality: There was one death in a female at 400 mg/kg/day on Day 28.

Clinical signs: No treatment-related effects

Body weights: No treatment-related effects

Food consumption: No treatment-related effects

Clinical chemistry: No treatment-related effects

Toxicokinetics: The results of the toxicokinetic analysis are presented in the following table:

Results of 28-Day Oral Acamprosate Toxicokinetic Study in the Rat

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		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

Summary of individual study findings:

There was a death in one female rat at 400 mg/kg/day; no other toxicity observed in this study. The results of the toxicokinetic evaluation showed slightly less than dose-proportional increases in Cmax and AUC values in both males and females. There were no differences in males and females in peak plasma acamprosate levels and acamprosate exposure (AUC). The results demonstrated exposure levels at 0.2X, 0.7X, and 2.3X the MRHD of 1998 mg/day on an AUC basis.

Selected results of the preclinical and clinical pharmacokinetic studies are presented in the following tables for comparison.

Pharmacokinetic Parameters in Mice^A

Parameter*	100 mg/kg/d (dietary, 1 day)	100 mg/kg/d (dietary, 15 days)	400 mg/kg/d (dietary, 1 day)	400 mg/kg/d (dietary, 15 days)
Cmax (mg/l)	Below detection limit	Below detection limit	[]
Tmax (h)	-	-	23	21

^A Study No. 91.05.AOT.001.SP3, 1992, Vol. 9, p. 337.

*Cmax: Maximum plasma acamprosate concentration achieved; Tmax: Time at which Cmax occurred

The pharmacokinetic parameters for oral acamprosate in male Wistar rats are presented in the following table:

Pharmacokinetic Parameters in Rats

Parameter*	50 mg/kg PO (Single dose) ^A	100 mg/kg PO (Single dose) ^B	100 mg/kg PO (Steady state) ^B	200 mg/kg PO (Single dose) ^A	400 mg/kg PO (Single dose) ^A
C _{max} (mg/l)	L				J
T _{max} (h)	0.5	0.25	0.30	1	0.5
AUC _{0-t} (mg.h/l)	2.94	14.55	14.12	36.84	44.16
AUC _{0-∞} (mg.h/l)	4.27	8.91 (24 h)	12.26 (24 h)	39.78	73.85
Half-life (h)	2.1	-	-	22.7	30.7
Bioavailability(%)	7	-	-	16	15

^A Study No. MET/AOTA-Ca/R-90-6,1991, Vol. 8, p. 162.

^B Study No. MET/AOTA-Ca/R-90-7,1991, Vol. 9, p. 1.

* C_{max}: Maximum plasma acamprosate concentration achieved; T_{max}: Time at which C_{max} occurred; AUC: Area under plasma acamprosate time curve; AUC CT: last time point which could be measured.

The results of study MET/AOTA-Ca/R-90-7 show no differences in acamprosate plasma kinetics with repeated dosing for up to 9 days at 100 mg/kg/day PO, except for an increase in the C_{max} at 0.5 hours after administration. Therefore, there was no accumulation of acamprosate during the 9-day treatment period.

The pharmacokinetic parameters of oral acamprosate in rabbits are presented in the following table:

Pharmacokinetic Parameters in Rabbits

Parameter*	100 mg/kg PO (Single dose) ^A	1000 mg/kg PO (Single dose) ^A
C _{max} (mcg eq/ml)	L	J
T _{max} (h)	1.5	2
AUC ₀₋₂₄ (mcg eq.h/ml)	64.6	522.1

^A Study #41, Report No. 9194,1993, Vol. 9, p. 37.

*C_{max}: Maximum plasma acamprosate concentration achieved; T_{max}: Time at which C_{max} occurred; AUC: Area under plasma acamprosate time curve.

In female rabbits, plasma acamprosate decreased to baseline levels over 120 hours. Acamprosate was excreted in urine at 53%-56% and in feces at 23%-24%. A single radioactive component was identified to be identical to acamprosate. Acamprosate levels in whole blood were lower than in plasma.

The pharmacokinetic parameters of oral acamprosate in Beagle dogs are presented in the following table. Systemic exposure increased sub-proportionally with dose following single dosing and there was no indication of drug accumulation with repeated dosing.

Pharmacokinetic Parameters in Beagle Dogs

Parameter*	25 mg/kg PO (Single Dose) ^A	100 mg/kg PO (Single Dose) ^A	100 mg/kg PO (Single Dose) ^B	100 mg/kg PO (Multiple Dose) ^B	400 mg/kg PO (Single Dose) ^A
C _{max} (mg/l)	1				1
T _{max} (h)	2.8	3.5	2	2	1.8
AUC Ct (mg.h/l)	66.36	114.6	235	202	239.7
Half-life (h)	2.5	6.4	5.8 (to 24 h)	9.8 (to 24 h)	2.4

^A Study MET/AOTA-Ca C-91-8, 1991, Vol. 8, p. 209.

^B Study MET/AOTA-Ca C-92-14, 1992, Vol. 9, p. 250.

*C_{max}: Maximum plasma acamprosate concentration achieved; T_{max}: Time at which C_{max} occurred; AUC CT: last time point which could be measured.

Intravenous administration of 25 mg/kg acamprosate in dogs resulted in a mean peak plasma concentration of 177 mg/l at the first sampling time point of two minutes; the distribution half-life was 0.17 hours, elimination half life was 0.77 hours, clearance was 0.23 l/h/kg and volume of distribution was 0.251 l/kg.

The pharmacokinetic parameters of acamprosate, comparing the oral capsule and oral non enteric-coated tablet forms in Beagle dogs, are presented in the following table:

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Oral Acamprostate Pharmacokinetic Parameters in Beagle Dogs Comparing Capsule and Tablet Forms*

Kinetic Parameter		Administered Doses		
		31.71 mg/kg		20 mg/kg
		Dog -	Dog -	Dog -
Plasma				
C_{max} (mg/l)	Capsule			
	Tablet			
T_{max} (h)	Capsule	5	3	3
	Tablet	4	2	-
AUC_{0-t} (mg.h/l)	IV	79.7	124.1	102.4
	Capsule	89.7	80.2	35.9
	Tablet	48.3	26.6	-
Half-life (h)	IV	0.9	1.1	1.5
Clearance (l/h/kg)	IV	0.4	0.255	0.193
Bioavailability (%)	Capsule	112	65	35
	Tablet	61	21	-
Urine				
% 24 hour	IV	68.9	90.3	ND
	Capsule	64.6	36.6	ND
	Tablet	44.6	37.6	-
Renal Clearance (l/h/kg)	IV	9.274	0.23	ND
	Capsule	0.228	0.145	ND
	Tablet	0.293	0.45	-

*Study MET/AOTA-Ca C-88-1 and 88-2, 1989, Vol. 8, p. 275.

ND: Not determined

Human Pharmacokinetic Parameters: Comparisons across Acamprostate Doses (Single oral dose, aqueous solution)*

Parameter ¹	333 mg	666 mg	1332 mg	2664 mg
K (1/h)	0.4	0.056	0.06	0.05
T_{max} (h)	1.4	1.5	1.4	1.2
C_{max} (ng/ml)				
AUC_{0-∞} (ng.h/ml)	1155	5442	7197	12624
T_{1/2} (h)	1.9	13	12.5	14.5
Cl_R (l/h)	23.8	7.7	12.9	16.9

* Study Report No. 298/17927, 1991

¹ K: Elimination rate constant, T_{max}: Time to peak plasma concentration, C_{max}: Peak plasma concentration, AUC: Area under the plasma acamprostate time curve to infinity, T_{1/2}: Half-life, Cl_R: Renal Clearance.

Human Pharmacokinetic Parameters: Comparisons across Dosage Forms and Schedules*

Parameter	1998 mg/d (2x 333 mg tablets t.i.d., PO) Day 1	1998 mg/d (2x 333 mg tablets t.i.d., PO) Day 8+17	1998 mg/d (2x 333 mg tablets t.i.d., PO) Day 9+18	2000 mg/d (2x 500 mg tablets b.i.d., PO) Day 1	2000 mg/d (2x 500 mg tablets b.i.d., PO) Day 8+17	2000 mg/d (2x 500 mg tablets b.i.d., PO) Day 9+18
C _{max} (mg/l)	162±21	523±57	471±39	279±88	481±46	481±49
T _{max} (h)	15.2±2.2	7.1±1.3	8.96±1.9	18.6±1.9	7.2±1.3	9.5±2.1
AUC ₀₋₂₄ (ng.h/ml)	1802±217	7365±871	6884±661	2096±479	6691±676	6204±726
Half-life (h)	-	17±3 (Day 19-23)	-	-	14±2 (Day 19-23)	-

*Comparative PK results at steady state (n=24 healthy s), report entitled *Comparative bioavailability study to compare pharmacokinetic parameters under steady state conditions of two acamprosate treatments (666 mg acamprosate T.I.D. vs. 1000 mg acamprosate B.I.D.) In 24 healthy male volunteers, 1995*

In humans, bioavailability was decreased when acamprosate was administered in enteric coated tablets in comparison to acamprosate in aqueous solution. Additional clinical pharmacokinetic studies found no effects of gender, history of alcoholism, ethanol co-administration (0.9 g/kg), disulfiram (500 mg), diazepam (5 mg), imipramine (50), and hepatic disease on acamprosate pharmacokinetic parameters. Renal impairment resulted in decreased plasma clearance and renal clearance of acamprosate and food decreased bioavailability of acamprosate. Acamprosate had no effect on ethanol kinetic parameters in humans. From the AUC data, bioequivalence was demonstrated for acamprosate at 333 mg and 500 mg tablet strengths over nine days at 1998 vs 2000mg/d respectively.

Summary of ADME/Pharmacokinetics

Acamprosate bioavailability by the oral route was variable, and usually low in the 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 lacrimal 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, resulting in a placenta:plasma AUC ratio of 0.2, fetus:plasma ratio of 0.43, and amniotic fluid:plasma ratio of 0.2 after oral dosing in rats. 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 while intravenous acamprosate is primarily excreted renally, 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 ³⁵S acamprosate appeared in the feces while most of the radioactivity after dosing with oral ⁴⁵Ca