CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-431

Pharmacology Review(s)

MEMORANDUM
July 29, 2004

TO: File
FROM: Kenneth L. Hastings, Dr.P.H.

SUBJECT: NDA 21-431

I have reviewed the action package for Campral (acamprosate calcium) and concur with the pharmacology/toxicology review team that the product may be approved. I also concur that a standard mouse carcinogenicity bioassay should be conducted as a Phase 4 commitment. This product should be labeled as Pregnancy Category C as recommended by the review team. The product label is acceptable.

/S/

Kenneth L. Hastings, Dr.P.H.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluation II

FDA Center for Drug Evaluation and Research
Division of Anesthetic, Critical Care, and Addiction Drug Products
HFD-170, Room 9B-45, 5600 Fishers Lane, Rockville, MD 20857

MEMORANDUM TO FILE

Date:	July 16, 2004
To:	NDA 21-431, acamprosate calcium
From:	R. Daniel Mellon, Ph.D. Supervisory Pharmacologist, DACCADP
Subject:	Secondary Review of NDA 21-431 Acamprosate calcium

Overall Assessment:

I have carefully read Dr. Wasserman's review of the pharmacology/toxicology data submitted by Lipha Pharmaceuticals, Inc. in support of NDA 21-431 for acamprosate calcium. I have also read the pharmacology toxicology review of the original NDA submission by Dr. Kathleen Haberny and the secondary review by Dr. Timothy McGovern. Based on these reviews and careful consideration of the data presented, **I concur with Dr. Wasserman's assessment that, from the pharmacology and toxicology perspective, NDA 21-431 should be considered approvable**, provided the sponsor agrees to a Phase 4 commitment to complete a CAC approved carcinogenicity assessment in the mouse and agrees to a Pregnancy Category C.

Dr. Wasserman and I agree that there are two pharmacology toxicology issues that will require discussion with the sponsor prior to approval. Specifically,:

1. The sponsor should agree to a timeline for submission of a mouse carcinogenicity assessment protocol for CAC concurrence and initiation of the study.

2. The sponsor should agree to a Pregnancy Category C rather than the proposed Pregnancy Category of —

For details of the issues addressed in the first NDA review period, the reader is referred to the primary review by Dr. Kathleen Haberny and the secondary review by Dr. Timothy McGovern. This memorandum addresses only the material submitted in response to the not approvable letter previously issued.

Overview of Key Issues Addressed in Second Cycle Review:

The not approvable letter that was issued following the first cycle review (June 27, 2002) identified three pharmacology/toxicology deficiencies that were to be addressed or the second cycle review. These deficiencies were listed as items 18 through 20 of that not approvable letter and are reproduced below:

18. **Perform a one-month oral toxicity study**, including full histopathology, in dogs using adequate doses to either characterize the toxicity profile or achieve the maximum feasible dose.

19. **Repeat the gene mutation assay in Chinese hamster V79 cells and the chromosome aberration assay** using adequate dosing and procedures according to current standards.

20. **Repeat the carcinogenicity study in mice.** Either a standard two-year assay or an appropriate alternative model may be performed. The Agency encourages the submission of a study protocol with supporting data for concurrence of dose selection by our Executive Carcinogenicity Assessment Committee prior to initiation of the carcinogenicity study.

Prior to submitting the complete NDA package, the sponsor submitted amendment N031 (May 22, 2003). This submission outlined the sponsor's plan to submit their rationale why the mouse carcinogenicity study was an adequate assessment of acamprosate.

Issue 18: Demonstration of frank toxicity or maximum feasible dose in dog model.
To define the potential toxicity of acamprosate in the dog model, the sponsor submitted study EMD 171 482, a 4-week oral toxicology study in the dog model. This study increased the maximum dose of acamprosate administered to the dog to 3000 mg/kg/day from the previously studied maximum dose of 1000 mg/kg/day, described in the first NDA submission. The dose of 3000 mg/kg/day produced dose-related clinical signs of vomiting and diarrhea in the animals. Although these clinical signs preclude the identification of a NOAEL for the study, there were no histological changes noted to identify target organs of toxicity. However, the dose of 3000 mg/kg/day represents a 42-fold exposure margin (based on body surface area) above the maximum daily human dose. Pharmacokinetic comparison suggested that the study provides up to an 81-fold exposure margin based upon an area under the curve analysis. The high dose in this

study was chosen based upon a preliminary dose range finding studies that suggested that doses of 2500 and 5000 mg/kg/day produced severe and dose-related vomiting and diarrhea. At times, the vomitus and stool included a reddish material, which was not characterized by the sponsor.

Technically, the results of study EMD 171 482 did not identify histological changes indicative of toxicity to target organs; further, the highest dose tested appears to be close to if not at the maximum feasible dose. Further EPA protocols for 28-day rodent repeat-dose toxicology studies and 90-day rodent and nonrodent repeat dose toxicology studies indicate that the limit dose of 1000 mg/kg was generally considered adequate. As the highest dose tested was 3000 mg/kg/day, the dosing in the submitted study exceeded the limit doses described by the EPA for similar toxicity studies. Given the lack of clear toxicity, and the exposure margins (81-fold the systemic exposure in humans) for the high dose tested, any further studies in the dog model do not appear to be warranted and could be considered unethical. In my opinion, the deficiency has been adequately addressed.

Issue 19: Adequacy of dosing in two genetic toxicology studies. The sponsor submitted two new genetic toxicology studies, one in vitro mammalian chromosome aberration assay in human lymphocytes and one in vitro mammalian gene mutation assay in HPRT/V79 cells. The concentrations tested were adequate based upon the presence of precipitation of drug. The results indicated that acamprosate tested negative in both studies. I concur with Dr. Wasserman that the sponsor has provided two adequate genetic toxicology studies; therefore, this deficiency has been adequately addressed.

Issue 20: Requirement for carcinogenicity assessment in a second species. Rather than conduct a second carcinogenicity assessment as requested by the Agency, the sponsor provided an expert analysis of the original mouse study results and maintain that the mouse study should be considered acceptable. Dr. Wasserman reviewed the sponsor's submission and concluded that their response was not adequate. I concur with Dr. Wasserman's assessment that the original mouse study is not acceptable and that the sponsor should repeat the carcinogenicity assessment. For a new molecular entity intended for chronic use, this assessment should be completed at the time of NDA submission and would be required for NDA approval. Following considerable discussion within the Division, Dr. Wasserman and I agree that the assessment in the mouse model may be completed as a Phase 4 commitment for the following reasons:

1) There is considerable previous human experience. Acamprosate has been marketed in Europe for this indication since 1989, and although this alone does not demonstrate a lack of carcinogenic potential, the lack of any clear safety signal is reassuring,

2) the rat CA assessment did not identify an increase in tumor findings and was deemed acceptable by the Carcinogenicity Assessment Committee,

3) acamprosate is neither genotoxic nor clastogenic in the assays examined, although this alone does not demonstrate a lack of carcinogenic potential,

4) there was no evidence of proliferative lesions in the chronic toxicology studies, although this alone does not demonstrate a lack of carcinogenic potential,

5) the mouse study, although not adequate to capture a full assessment, did not show statistically significant increases in tumors at suboptimal doses (below the maximum tolerated dose), and

6) acamprosate shows efficacy in the indicated patient population and would therefore provide practitioners access to a third drug for the treatment of a very difficult-to-treat patient population (disulfiram and naltrexone are the only other approved therapeutic drugs for alcoholism).

The decision regarding the adequacy of the mouse study ultimately lies with the Division. However, the Division informally consulted the members of the CAC panel who reviewed the original mouse study as well as members of the current panel (Drs. Abigail Jacobs, Joseph Contrera, Joseph Sun and Timothy McGovern were members of the CAC panel that reviewed the carcinogenicity results in the rat and mouse model that were submitted for the original NDA submission.) The members of the CAC were provided with the meeting minutes from the first CAC meeting, the sponsor's outside expert review of the first mouse study, and Dr. Wasserman's review of the materials provided by the sponsor to address this deficiency. The Division also posed the following questions to the CAC panel members:

A. Based upon the argument put forth by the outside expert, do you still believe that the carcinogenicity study in the mouse model is inadequate?

B. Do you agree that under the circumstances, the Division should allow acamprosate to be approved with a commitment to conduct the CA assessment as a Phase 4 agreement

Drs. Jacobs, Sun, McGovern, and David Jacobson-Kram (Associate Director of Pharmacology and Toxicology, Office of New Drugs) provided their assessment via e-mail (NOTE: I have summarized the comments attributed to the individual reviewers).

Dr. Jacobs initiated the discussion by indicated that she felt the dosing in the mouse study was inadequate since it did not reach the maximum tolerated dose (MTD); that alone would make the study inadequate. Dr. Jacobs further noted that studies in the CD-1 mouse are able to be conducted for the full 2 years without survival problems. As such, the study may have been compromised by a confounding factor, necessitating early termination. Dr. Jacobs did not feel that the nematode infection was unusual. She further

noted that as the drug has been marketed in Europe for over 15 years, is efficacious, and fills a therapeutic need, the repeated carcinogenicity assessment requsted by the Division is justified and could be completed as a Phase 4 commitment, IF the sponsor obtained concurrence for a protocol and initiated the studies prior to the approval. Finally, Dr. Jacobs suggested the possibility of using [ ] transgenic mouse model.

Dr. McGovern agreed with the comments made by Dr. Jacobs. He indicated that if the genetic toxicology studies requested by the Division were adequate and negative, a Phase 4 submission could be justified. Drs. Jacobson-Kram and Sun noted that they concurred with Dr. Jacob's comments and recommendations.

Dr. Albert Defelice (member of CAC panel at the time of the Division's requested input) noted that he liked Jacob's suggestion of the [ ] model. However, he noted that although the study is inadequate the information obtained with the suboptimal dosing was still informative.

In short, the members of the CAC concur with Dr. Wasserman's analysis of the sponsor's evaluation of the original mouse study. I, too, agree with Dr. Wasserman's recommendation that an adequate carcinogenicity assessment in a mouse model, should be completed. Further, I agree that the sponsor may submit these data as Phase 4 commitment, should the NDA be approved.

Pregnancy Labeling: As noted in Dr. Habeny's review of the first NDA submission, Segment II (embryofetal development) studies in the rat found a treatment-related increase in the number of dams with malformed fetuses and an increase in the number of fetuses with malformations. Malformations included hydronephrosis, malformed iris, retroesophageal subclavian artery, and retinal dysplasia which were outside of the historical control range. Although the increase in malformations appeared to be driven by a high number of malformations in one litter, and were not noted in either mice or New Zealand White rabbits, there was an increase in the number of females with malformed fetuses (torsion of vertebrae and hydronephrosis) following acamprosate treatments at 400 mg/kg/day and higher.

In addition to the evidence of teratogenicity in rats and rabbits, the peri- and post-natal development studies detected a treatment-related increase in the incidence of still-born offspring and an increase in the number and percent of females with offspring dying after birth at 960 mg/day or greater, (approximately 2-fold the maximum recommended human daily dose on body surface area basis). These findings merit a Pregnancy Category C.

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/s/

R. Daniel Mellon
7/16/04 05:58:14 AM
PHARMACOLOGIST

ADRA Review #1 of Action Package for NDA 21-431, Campral (acamprosate) Tablets

Reviewer: Lee Ripper, HFD-102
Date received in HFD-102: June 11, 2002
Date of Review: June 14, 2002

Date original NDA received: December 27, 2001
UF GOAL DATE: June 27, 2002

Indication: [ ]

Action type: AE
RPM: Lisa Basham-Cruz, x7-7420
Drug Classification: 1P
505(b)(1) application

Patent Info: No current relevant patent
Clinical Inspection Summary: AC 6/7/02, 1 site in Germany and 1 site in France inspected
DDMAC review of PI: Deferred until response to action letter
Debarment statement: AC
DMETS Review of Trade Name: AC. Trade name will need to be re-evaluated prior to AP.
EA: AC, CMC rev #1, p. 63

1. Financial disclosure information/review: The European studies were completed a number of years ago. The U.S. study was completed 1/28/99. The NDA, page 441, says "Disclosure statements are not applicable to this NDA. The clinical studies submitted in support for this NDA were conducted and completed prior to 2/2/99. However, reporting of SPOOS payments does apply to any payments between 2/2/99 and 1/28/00. The applicant needs to submit forms for this time period.

2. EER: Pending as of 6/12/02, two facilities in France, inspections scheduled for 6/5/02 and 6/18/02.

3. Safety Update: The electronic C&H listing doesn't show a SU submission. For several obvious reasons, it doesn't matter in this case, but we do need to remember to make sure that these are always submitted and reviewed.

4. I gave my comments on the draft letter to the RPM to incorporate into the next draft.

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/s/

Leah Ripper
6/14/02 05:59:06 PM
CSO

INTEROFFICE MEMO

TO: NDA 21-431, acamprosate
FROM: Timothy J. McGovern, Ph.D., Supervisory Pharmacologist
DATE: June 10, 2002

I concur with the pharmacologist's recommendation that the pharmacology and toxicology have not been adequately studied and that the drug is not approvable from a nonclinical perspective.

Pharmacology: Acamprosate demonstrated dose-dependent reduction in voluntary ethanol consumption in rodents by the oral and intraperitoneal routes, 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 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.

Safety pharmacology: 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.

General toxicology: Studies up to 6-months duration were performed in rats and dogs. In rats, effects on renal function including decreased urinary volume and significant increases in urine calcium were observed in a 3-month oral toxicity study. Other kidney effects included distension of kidney tubule sections from coaggulum accumulations attributed to early senile nephrosis. In the 6 month study, animals died between weeks 15 and 26 of dosing at the highest dose (2400 mg/kg). Associated renal lesions included vacuolation, calculi, tubular ectasia, pelvic distension, intracellular mineralization and epithelial atrophy. Other target organs included heart, brain and GI tract. Again an increase in urine calcium was noted. In the 6-month dog study, observations included diarrhea, cardiac abnormalities described earlier, increased urinary calcium. No definitive target organs were identified in dogs at doses up to 1000 mg/kg. The sponsor has committed to performing an additional toxicity study in dogs in order to characterize the toxicity profile in a non-rodent species.

Genetic toxicology: The genotoxic potential of acamprosate has not been fully evaluated. Acamprosate was negative for mutagenicity in the Ames test, and for clastogenicity in the chromosome aberration assay in human lymphocytes and in the in vivo mouse micronucleus test. Equivocal findings were observed in a point mutation assay using Chinese hamster V79 cells; results were negative with metabolic activation. The highest concentrations used in the chromosome aberration assay and point mutation assay using

Chinese hamster V79 cells and incubation times in the former assay with metabolic activation appear to be inadequate. The *in vitro* chromosome abberration assay and point mutation assay using Chinese hamster V79 cells should be repeated.

Carcinogencity: Acamprosate was negative for carcinogenicity in rats. The study in mice is considered to be inadequate to provide a definitive assessment of the carcinogenic potential. The results of the carcinogenicity studies were presented to the Executive CAC committee on March 19, 2002. The carcinogenicity study in mice is unacceptable due to inadequate dose selection, based on lack of evidence for an MTD. In addition, the mouse study results were confounded by nematode infestation and histopathology evaluation was conducted on an inadequate number of low- and mid-dose animals. The committee recommended that the sponsor repeat the mouse carcinogenicity study.

Reproductive toxicology: Acamprosate did not affect fertility in mice or rats at doses up to 2400 mg/kg or 1000 mg/kg, respectively. No effects on embryo-fetal development were observed in mice or New Zealand White rabbits at doses up 2400 mg/kg or 1000 mg/kg, respectively. However, developmental effects in rat pups were observed at doses of 300 mg/kg or greater and included malformed iris, retinal dysplasia, retroesophageal subclavian artery, and hydronephrosis. No effects were noted at the low-dose of 50 mg/kg. Hydronephrosis was also observed at a dose of 400 mg/kg and greater in Burgundy Tawny rabbits and at 1000 mg/kg in the rat fertility study. In peri- and post-natal studies, an increase in the number of maternal mice delivering still-born offspring and the number of still-born offspring was increased at doses of 960 and 2400 mg/kg. No effects were noted at the low dose of 320 mg/kg or in the study performed in rats up to 2000 mg/kg. The findings summarized above indicate that acamprosate should be classified as a Pregnancy Category C. However, the findings in animals should be considered in relation to known reproductive effects of ethyl alcohol, which include the characteristics of fetal alcohol syndrome (craniofacial dysmorphism, intrauterine and postnatal growth retardation, retarded psychomotor and intellectual development) and milder forms of neurological and behavioral disorders in humans.

Special toxicology: In a rat study to demonstrate the potential to induce the neurotoxic effect known as the "Olney Lesion", acamprosate produced no evidence of neuronal vacuolation, necrosis, or microglia in the retrosplenial and posterior cingulate cortices.

Based upon the above-mentioned results, the sponsor should perform studies to characterize to toxicity profile in a non-rodent species, repeat in vitro chromosome aberration and mutation assays with adequate dosing and methodologies, and repeat a mouse carcinogenicity study. The labeling should reflect the above-mentioned findings and recommendations.

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/s/

Timothy McGovern
6/10/02 09:22:30 AM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-431**
SERIAL NUMBER:	**2**
DATE RECEIVED BY CENTER:	**05/22/2003 (N 000 BP);**
	09/08/2003 (N 000 BZ)
DRUG NAME:	**Acamprosate**
INDICATION:	[redacted]
	]
SPONSOR:	**Lipha Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**N 000 BP; N 000 BZ**
REVIEW DIVISION:	**Division of Anesthetic, Critical Care and**
	Addiction Drug Products (HFD-170)
PHARM/TOX REVIEWER:	**Adam M. Wasserman, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Lisa Basham-Cruz**

Date of review submission to Division File System (DFS): 6/16/04

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This 2nd cycle submission of acamprosate (calcium acetylhomotaurinate) NDA 21-431 is approvable based on pharmacology/toxicology considerations.

B. Recommendation for nonclinical studies

A Phase 4 commitment to complete carcinogenicity assessment in a second species (mouse) will be required. If the sponsor decides to conduct a 2-year bioassay, dosing should utilized the maximum tolerated dose (or maximum feasible dose) determined from a 13-week dietary toxicity study in the mouse in order assure a drug exposures consonant with the ICH S1C guidance "Guideline for Industry: Dose Selection for Carcinogenicity Testing of Pharmaceuticals" (note: study . 138/88827: "AOTA-CA (Acamprosate) Sub Acute Toxicity To Mice By Dietary Administration for 13 Weeks" did not determine an MTD according to the initial review by Dr. Haberny). Alternatively, the sponsor may use a dose which will provide a 25-fold exposure margin between the mouse and the systemic exposure at steady state therapeutic dosing in humans. The sponsor may be able to conduct a 6-month transgenic mouse study to comply with this requirement; however, the exposure margins described would still need to be attained to support the safety of this compound and to allow for adequate risk assessment using this species. The sponsor is encouraged to submit a protocol with rationale for dosing and design to the Exec CAC for concurrence prior to the initiation of this study (Guidance for Industry -- Carcinogenicity Study Protocol Submissions (May 2002).

C. Recommendations on labeling

Specific details regarding the labeling of acamprosate within the Carcinogenesis, Mutagenesis, Impairment of Fertility section and the Pregnancy section are listed in the Suggested Labeling portion of the Overall Conclusions and Recommendations section of this review. The Carcinogenicity section of the label should not reference the original mouse carcinogenicity study as the Division has determined that the study is inadequate, an opinion that has been supported by the Executive Carcinogenicity Assessment Committee upon review of the expert opinion provided by the sponsor and the 28-day mouse toxicokinetic study. Statements describing the genetic toxicology studies completed for this 2nd cycle should be included in the final label.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

This 2nd Cycle NDA submission was in response to the "Not Approvable" decision sent by the Agency in a letter of June 27, 2002 which, among other issues, detailed several nonclinical issues which required resolution or response by the sponsor, as follows:

1. Perform a one-month oral toxicity study, including full histopathology, in dogs using adequate doses to either characterize the toxicity profile or achieve the maximum feasible dose.

2. Repeat the gene mutation assay in Chinese hamster V79 cells and the chromosome aberration assay using adequate dosing and procedures according to current standards.

3. Repeat the carcinogenicity study in mice. Either a standard two-year assay or an appropriate alternative model may be performed. The Agency encourages the submission of a study protocol with supporting data for concurrence of dose selection by our Executive Carcinogenicity Assessment Committee prior to initiation of the carcinogenicity study.

The sponsor responded to these requests in the second cycle by providing a 1-month repeated dose toxicity study in the dog, two *in vitro* genetic toxicity studies specified in the NA letter, 28-day repeated dose toxicokinetic study in the mouse and, *in lieu* of the repeat of the mouse carcinogenicity study, an expert evaluation of their original carcinogenicity study and an assessment of its adequacy for enabling the appropriate assessment of human risk. The results of these studies are summarized below:

In the one-month oral toxicology study, dogs were administered acamprosate at doses of 750, 1500 or 3000 mg/kg/day or placebo for 28 days by oral gavage in an attempt to characterize the toxicity of acamprosate in a non-rodent animal model using a top dose which would produce toxicity or represent the maximum feasible dose. Although one female animal was sacrificed on study day 2 due to poor condition, this was a low-dose animal which was determined to have aspirated vomit resulting in severe pneumonia. All other animals survived the dosing period. No target organs of toxicity were identified in surviving animals though dose-dependent GI distress, severe at the highest dose, was observed throughout the study and involved primarily multiple daily bouts of vomiting at higher doses and diarrhea throughout the treatment period. No treatment-related effects were observed on hematologic or clinical chemistry parameters. Urinary Ca^{2+} levels were not assessed, however; these had previously been observed to be increased significantly in the 26-week toxicity study in the dog performed for the 1st NDA cycle and was thought to reflect excretion of the test article (calcium acetylhomotaurinate). No treatment related effects were observed on cardiac function or conduction parameters, though 2nd degree AV block was observed in 3 animals in the MD treatment group (none in the HD group); of these, 2/3 animals had evidence of this anomaly within the pre-treatment baseline period. No treatment related changes were noted in organ weights, gross necropsy findings or in a histopathologic evaluation of tissues. Toxicokinetic assessment indicated that exposure was generally proportional to dose and not affected by

gender or repeated dosing. Although exposure appeared to become sub-proportional to dose at the highest dose tested (3000 mg/kg/day), a preliminary dose range-finding study in the dog with doses up to 5000 mg/kg/day x 10 days suggests dose-normalized exposure is relatively constant up to 5000 mg/kg/day. Peak plasma concentrations of acamprosate were reached within one hour of oral administration regardless of dose. Doses utilized represent 10 – 42-fold (body surface area-adjusted for a 50 kg individual) the maximum recommended human dose (MRHD) while systemic (AUC) exposures attained provide a 22 – 81-fold safety margin above human exposure at steady state therapeutic dosing. As in previous studies, a NOAEL could not be defined in the dog due to the presence of vomiting and diarrhea at the lowest dose tested, 750 mg/kg/day. The sponsor was requested to conduct this study using a dose which adequately characterized acamprosate toxicity or, barring significant toxicity, used the maximum feasible dose (MFD). Although the MFD was not used in this study – as higher concentrations of acamprosate could have been used to produce increased systemic exposure – and target organs of acamprosate toxicity were not clearly defined, the toxicity of acamprosate appears to be adequately characterized due to the severe vomiting and diarrhea observed with the 3000 mg/kg/day dose and the exposure margins between dog and human which were attained. Although technically, inadequate to fulfill the Division's request, ethically, there does not appear to be any reason to request a repeat of this study to include a 5000 mg/kg/day group.

A repeat of the *in vitro* chromosomal aberration assay in human lymphocytes and the mammalian cell gene mutation assay in Chinese hamster V79/HRPT cells using adequate dosing and current methodology were provided in the current submission and reviewed for this 2nd cycle. Preliminary testing in human lymphocytes indicated acamprosate did not induce cytotoxicity with exposure up to 5000 µg/mL in the presence or absence of S9 metabolic activation though precipitate was observed at all concentrations ≥ 1580 µg/mL. Doses tested in the initial and confirmatory series of experiments were 158, 500 and 1580 µg/mL. Acamprosate at levels used did not induce polyploidy nor produce significant inhibition of mitosis except when incubated with 1580 µg/mL for 48-hr in the absence of S9; under this condition mitotic inhibition was 60% of negative control. No dose-related increases in aberrant cells were observed nor were increases in any tested group relative to negative control. The study is adequate to fulfill the Division's request.

Acamprosate was tested in the mammalian cell gene mutation assay using HRPT-defective hamster V79 cells; the assay submitted in the 1st cycle was judged equivocal and dosing was not adequately justified by cytotoxicity. The repeat of this assay submitted for this review cycle tested concentrations of up to 5000 µg/mL without S9 metabolic activation and up to 5000 µg/mL in the first series with 10% S9 mix and 2810 µg/mL in the second series using 5% S9 mix. Excessive cytotoxicity as measured by a decrease in cloning efficiency was observed at the highest concentration (5000 µg/mL) tested in the first series (> 90% cytotoxicity), thus necessitating a reduction in the highest concentration tested and a reduction in the concentration of S9 fraction in the test article solution in the second series to 2810 µg/mL and 5%, respectively. Although dosing of 2810 µg/mL produced only a 25% reduction in cloning efficiency and not the targeted 80 – 90% reduction in cloning efficiency specified by ICH guidelines as a criteria for

defining the top dose, the presence of a visible particulate at concentrations ≥ 1580 µg/mL led to two doses being tested above the solubility range which is considered to be adequate based on recommendations in the ICH S2A Guidance. A macroscopically visible precipitate was observed with all concentrations ≥ 1580 µg/mL in the presence of S9 metabolic activation but was not observed up to 5000 µg/mL when S9 was absent from the test material solution. In the absence of metabolic activation, no increase in mutation frequency was observed with 3 or 24 hr exposures up to an acamprosate dose of 5000 µg/mL (the limit dose of the assay). In the presence of metabolic activation, no increase in mutation frequency was observed up to 1580 µg/mL in the first series or 2810 µg/mL in the second series.

The sponsor did not repeat the mouse carcinogenicity bioassay, instead electing to submit an expert report reviewing the existing carcinogenicity study along with a 28-day repeat-dose toxicokinetic study in the mouse designed to establish the systemic exposure likely to have been achieved in the original mouse carcinogenicity study. The expert reviewer cited a number of factors supporting the argument that the original mouse carcinogenicity study was adequate for the determination of risk to humans, specifically: 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); and, 5) No significant treatment-related neoplastic findings in the mouse carcinogenicity study at an exposure which based on the 28-day toxicokinetic study recently conducted would represent an approximately 4.5-fold exposure margin over plasma acamprosate levels with therapeutic dosing in the human. Upon review, the arguments put forth in the expert review do not validate the conclusions of the original mouse study, which did not employ adequate dosing for a valid carcinogenicity assessment. Although not a requirement, members of the original and current Executive CAC reviewed the expert report in June of 2004 and concurred with this reviewer's conclusion, e.g., the mouse study did not reach adequate dosing for a valid bioassay. A summary of the original Exec CAC input regarding the carcinogenicity studies conducted can be found in Appendix 1 of this review.

B. Pharmacologic activity

Acamprosate has been shown to reduce the voluntary consumption of ethanol in ethanol-dependent but not ethanol-naïve rats after oral or intraperitoneal administration. Acamprosate also has been reported to decrease the relapse rate to ethanol consumption during prolonged abstinence periods in rats. Acamprosate has shown an ability to block several aspects of ethanol withdrawal-induced behaviors including hypermotility and withdrawal-associated place avoidance. Acamprosate functionally has been reported to antagonize the increase in glutamate release in the nucleus accumbens observed during ethanol withdrawal as well as withdrawal-induced *c-fos* expression in the hippocampus and cerebellum of rats. Acamprosate does not alter ethanol blood levels, ethanol-induced hypothermia, motor impairment or taste aversion. This compound does not substitute for

ethanol in non-clinical drug discrimination studies and does not produce signs of sedation.

The pharmacologic mechanism underlying the anti-craving effect of acamprosate has not been established definitively at this time. Although the strength of the evidence varies, four hypotheses have been discussed which are not mutually incompatible are as follows:

1) Interaction with the GABAergic system through $GABA_A$ and/or $GABA_B$ receptors
2) Interaction with the glutamatergic system through NMDA and/or metabotropic mGluR1 receptors
3) Inhibition of Ca^{2+} influx through NMDA receptors and/or voltage-gated Ca^{2+} channels, and,
4) Interaction with the inhibitory taurine neurotransmitter system in the CNS.

In general, the overarching hypothesis is that acamprosate restores the balance between excitatory and inhibitory neurotransmission which is disrupted by ethanol consumption and reflected in the subsequent withdrawal experience.

C. Nonclinical safety issues relevant to clinical use

Assessment of the nonclinical data submitted in support of acamprosate use for the maintenance of ethanol abstinence reveals few safety issues relevant to the clinical use of this product. Acamprosate is slowly absorbed and does not appear to be metabolized. The adverse effects noted in nonclinical repeat-dose toxicity studies occur at high dose multiples of the clinically therapeutic dose in humans and can be largely attributed to the Ca^{2+} moiety. There are two primary non-clinical safety issues related to clinical use of this drug product, specifically:

1) Acamprosate is a new molecular entity to the FDA, yet has only been adequately assessed for carcinogenic potential in one species. The sponsor should commit to completion of a study in a second species and should submit protocols to the CAC for concurrence within a reasonable timeframe of an approval action.
2) Acamprosate is a Pregnancy Category C drug. Reproductive toxicity was noted at doses that provide a body-surface area converted exposure margin of only 1.2-fold above the therapeutic dose in humans.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	21-431
Review number:	2
Sequence number/date/type of submission:	N 000 BP / 5-22-03 / Amendment #031 N 000 BZ / 9-08-03 / Amendment #033
Information to sponsor:	Yes ( ) No (X)
Sponsor and/or agent:	Lipha Pharmaceuticals, Inc. New York, NY 10036
Manufacturer for drug substance:	LIPHA – Usine de Meyzieu (Lipha Meyzieu Plant), ZI-69330, Meyzieu, France
Reviewer name:	Adam M. Wasserman, Ph.D.
Division name:	Division of Anesthetic, Critical Care and Addiction Drug Products
HFD #:	170
Review completion date:	**June 16, 2004**

Drug:

Trade name: CAMPRAL® (also AOTAL® in France, Sobrial in South Africa, and Zulex in Spain)

Generic name: Acamprosate

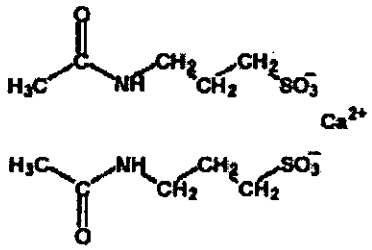
Code name: EMD 171 482

Chemical name: Calcium acetylhomotaurinate
Calcium acetylaminopropane sulfonate
3-Acetamido-propanesulfonic acid
3-Propanesulfonic acid, 3-(acetylamino), calcium salt (CAS)

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

Molecular formula/molecular weight: $C_{10}H_{20}N_2O_8S_2Ca$ / 400.48
$C_5H_{11}NO_4S$ (acid) /181.21

Structure:



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

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

Indication: [redacted]

Clinical formulation:

Ingredient	333 mg tablets Quantity per tablet (mg)	Quantity per tablet (%)	Function
Tablet Cores			
Calcium-acamprosate	333.0		Active Ingredient
Crospovidone (USP24-NF19)			
Microcrystalline cellulose (or equivalent, USP24-NF19)			
Magnesium silicate (equivalent,)			
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 L 30D or equivalent)			
Talc			
Propylene glycol			
Coating weight			
Total Coated tablet weight	531.6		

There are no pharmacology toxicology concerns related to excipients in this drug product.

Route of administration: Oral (tablet)

Proposed use: Up to two 333 mg tablets, three times daily for a total daily dose maximum of 1998 mg.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise. Additionally, portions of the text for this review were taken directly from the Original NDA review by Dr. Kathleen Haberny completed on June 10, 2002. These sections are indicated by fully justified paragraphs which are offset on both left and right sides from this reviewer's contributions.

Studies reviewed within this submission:

Study Title	Document #	Volume	Review / Date
Toxicology			
EMD 171 482 – 4 Week Oral Toxicity Study in Beagle Dogs	T8838	1	N21-431 BP, 6/04
Pharmacokinetics and Toxicokinetics			
Acamprosate: 28-Day Oral (Dietary Administration) Toxicokinetic Study in the Mouse	0537/060	2	N21-431 BP, 6/04
Genetic Toxicology Studies			
EMD 171 482 – *In vitro* mammalian chromosome aberration test (human lymphocytes)	T15446	2	N21-431 BP, 6/04
EMD 171 482 – *In vitro* mammalian cell gene mutation test (HPRT/V79)	T15444	2	N21-431 BP, 6/04

Studies not reviewed within this submission (Previously reviewed for Original [redacted] by Dr. Kathleen Haberny):

Study Title	Document #	Volume	Review / Date
Toxicology			
Acamprosate: 3-Week oral toxicity study in rats. Determination of blood levels	91.07.AOT.001.RP4	13	N21-431, 6/02
Acamprosate: 28-day oral (dietary administration) toxicokinetic study in the rat	537/059	1	N21-431, 6/02 (Amendment #003)
AOTA-Ca (Acamprosate) subacute toxicity to rats by dietary administration for 13 weeks	[redacted] 139/88834	14	N21-431, 6/02
Three month repeat dose oral toxicity study of calcium acetylhomotaurinate (acamprosate) in rats followed by a thirty day reversibility period	1097	14	N21-431, 6/02
AOTA-Ca (acamprosate) twenty-six week oral toxicity study in the rat followed by a six week reversibility period	602201.1986	15	N21-431, 6/02
Acamprosate: 2-Week oral toxicity study in mice. Determination of blood levels	91.05.AOT.001.SP3	13	N21-431, 6/02
AOTA-Ca (acamprosate) sub acute toxicity to mice by dietary administration for 13 weeks	[redacted] 138/88827	13	N21-431, 6/02
AOTA-Ca (Acamprosate) - 4 week intravenous toxicity study in the beagle dog	35191	18	N21-431, 6/02
Preliminary 4-week oral toxicity study in	408231	17	N21-431, 6/02

Study Title	Document #	Volume	Review / Date
the dog			
AOTA-Ca (acamprosate) twenty-six week oral toxicity study in the beagle dog	509215	17	N21-431, 6/02
Seven day subacute toxicity study in the macaque monkey by oral administration of calcium acetylhomotaurinate (acamprosate)	1605	18	N21-431, 6/02
Genetic Toxicology	83058	19	
Mutagenicity study in salmonella typhimurium HIS according to the B.N. Ames technique on calcium acetylhomotaurinate (acamprosate)	537/50	19	
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*	85065	19	
Mutagenicity study using the HPRT locus mutation technique in Chinese hamster V79 cells (resistance to 6-thioguanine) on the product calcium acetylhomotaurinate (acamprosate)	86002	19	
Genotoxicity study investigating chromosome aberrations by metaphase analysis in human lymphocytes on the product calcium acetylhomotaurinate (acamprosate)	84008	19	
Mutagenicity study in the mouse using the micronucleus test on the product calcium acetylhomotaurinate (acamprosate)	537/51	19	
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	6894-537/27	20 – 24	
Acamprosate: 104-week oral (dietary administration) carcinogenicity study in the rat	7062-537/26	25 – 29	

Study Title	Document #	Volume	Review / Date
Reproductive Toxicology:			
AOTA-Ca (acamprosate): fertility in the mouse	1578	30	
AOTA-Ca (acamprosate): Oral (gavage) fertility study in the rat (Segment I)	6688-537/22	30	
AOTA-Ca (acamprosate): Embryotoxicity study in the mouse	1578	32	
AOTA-Ca (acamprosate): Oral (gavage) range-finding study in the pregnant rat	6158-537/20	32	
AOTA-Ca (acamprosate): An oral (gavage) teratology study in the rat	6385-537/21	32	
AOTA-Ca (acamprosate): Oral (gavage) range-finding study in the pregnant rabbit	6172-537/24	33	
AOTA-Ca (acamprosate): Oral (gavage) teratology study in the rabbit	6381-537/25	33	
AOTA-Ca (acamprosate): Embryotoxicity study in the rabbit	1578	33	
Oral study of the effects of AOTA-Ca (acamprosate) on segment II of reproduction in the rabbit	2273	34	
AOTA-Ca (acamprosate): Peri-natal studies in the mouse	1578	34	
AOTA-Ca (acamprosate): Oral (gavage) peri- and post-natal study in the rat	6494-537/23	35	
Special Toxicology:			
Acamprosate and MK-801 neurotoxicity study by a single administration to CD rats	203/000127	35	
Studies conducted by Sponsor or referenced from the published literature which were summarized in 1st Cycle NDA review but were not reviewed			
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			

Study Title	Document #	Volume	Review / Date
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 bicuculline-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			

Study Title	Document #	Volume	Review / Date
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 tranylcypromine/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 tranylcypromine/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			

Study Title	Document #	Volume	Review / Date
Reinforcing and discriminative stimulus effects of Ca Acetyl Homotaurine in animals			
The transport of 14C-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 [14C]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 35S-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 14C 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 LD_{50} of acamprosate following oral administration to mice for 14 days			
Determination of the LD_{50} 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 LD_{50} of acamprosate following intravenous administration to rats for 14 days			
Determination of the LD_{50} 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			

Study Title	Document #	Volume	Review / Date
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			

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacodynamic studies in animal models provide clear data suggesting the potential utility of acamprosate for the proposed indication. Acamprosate (calcium acetylhomotaurinate) has been demonstrated to reduce voluntary consumption of alcohol in the rat. Similarly, published studies have provided evidence that acamprosate is associated with reduced rates of relapse to ethanol use in detoxified human subjects (Pelc et al., 1992 & 1995; Paille et al., 1995; Sass et al., 1996; Tempesta et al., 2000), an observation which has been replicated in rats undergoing periods of prolonged abstinence from ethanol (Spanagel et al., 1996; Heyser et al., 1998). Acamprosate administration does not alter ethanol concentration in plasma and does not substitute for ethanol in drug discrimination studies (Spanagel et al., 1996; Grant and Woolverton, 1989). Acamprosate does not alter ethanol-induced hypothermia, motor-impairment or taste aversion. That acamprosate can attenuate ethanol consumption and reduce the propensity to relapse into drinking is supported by several observations of a functional antagonism of ethanol-related behavior or response; specifically, acamprosate has been shown to reduce ethanol withdrawal-induced hypermotility (Dahchour and De Witte, 1999), place avoidance, glutamate release in the nucleus accumbens (Dahchour et al., 1998) as well as reportedly blocking the increase in *c-fos* expression in the hippocampus and cerebellum seen during periods of ethanol withdrawal (Putzke et al., 1996). The exact mechanism(s) by which acamprosate is believed to function as an anti-craving compound is currently unknown but is generally thought to involve a restoration of excitatory-inhibitory balance within the central nervous system. Studies which have investigated specific potential mechanisms of action have centered on several possibilities which may not be mutually exclusive, which are described below:

1) Interaction with GABA receptors

Although acamprosate has been demonstrated to increase GABA levels in a rat synaptosomal preparation and that the $GABA_A$ receptor antagonist, bicuculline, can block the inhibitory effect of acamprosate on ethanol consumption in ethanol-dependent rats (Boismare et al., 1984), binding studies indicate acamprosate weakly interacts with $GABA_A$ receptors at high concentrations. Mild inhibition of muscimol binding was only demonstrated when acamprosate levels exceeded 100 μM (unpublished report from sponsor – CEREP #892958/AC). This is somewhat surprising as homotaurine (of which acamprosate is a derivative) is a direct $GABA_A$ agonist. *In vitro* electrophysiological studies have demonstrated that acamprosate does not alter the $GABA_A$ receptor mediated inhibitory post-synaptic potential (IPSP) or resulting chloride influx (Zeise et al., 1993). A recent study confirmed this observation and presented evidence that acamprosate may also be an antagonist at presynaptic $GABA_B$ receptors (Berton et al., 1998).

2) Interaction with EAA receptors

Strong evidence has accumulated suggesting acamprosate alters the activity of excitatory amino acid receptors, particularly the NMDA receptor. An antagonist action at NMDA receptors has been suggested (Olive, 2002), but other studies have demonstrated the inability of acamprosate to attenuate the neurotoxic effects of NMDA agonists (Mayer et al., 2002) in culture. This may

be dependent on previous ethanol exposure as acamprosate has been shown to block glutamate-induced neurotoxicity only when cells were previously exposed to ethanol but had no effect on glutamate-induced neurotoxicity when cells were naïve to ethanol and exposed to glutamate challenge (al Qatari et al., 2001). Several authors have published reports of acamprosate binding to the polyamine site of the NMDA receptor (al Qatari et al., 1998; Naassila et al., 1998; Popp and Lovinger, 2000) though the functional consequences of this is uncertain and may depend on the site within the CNS the NMDA is found and the specific subunits comprising the NMDA receptor as published reports suggest that acamprosate is an agonist (Berton et al., 1998; Madamba et al., 1996), a weak antagonist (Zeise et al., 1990, 1993; Rammes et al., 2001; Allgaier et al., 2000) or has no effect on NMDA mediated neurotransmission (Popp and Lovinger, 2000) in different brain regions. An intriguing finding was recently described by Rammes and colleagues (2001) in which a single administration of acamprosate altered subtype expression of NMDA receptors and increased NMDA receptor expression in specific regions of the cortex but not in the medulla. Most recently, acamprosate has been shown to inhibit the binding of the mGlur1 agonist *trans*-ACPD and block *trans*-ACPD neurotoxicity in neuronal cultures obtained from hippocampal slices (Harris et al., 2002).

3) Interaction with Ca^{2+} -channels

Recent research indicates that acamprosate can inhibit Ca^{2+} influx through both voltage-gated calcium channels as well as through NMDA receptors in cultured rat mesencephalic neurons (Allgaier et al., 2000).

4) Interaction with taurine

Evidence that acamprosate may exert anti-craving effects through an interaction with taurine, an amino acid which has been shown to have CNS activity is more speculative. Ethanol and taurine both exert a positive allosteric effect on $GABA_A$ and glycine receptors, both of which allow influx of Cl^- into the neuron. Ethanol has been demonstrated to elevate taurine levels in the CNS although the functional effect(s) of this is unknown. Acamprosate is also observed to increase taurine levels in the CNS and at high concentrations displaces taurine binding (Olive, 2002). Taurine has also been observed to inhibit the intestinal transport of acamprosate (Mas-Serrano et al., 2000).

2.6.2.2 Primary pharmacodynamics

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 50-4000 mg/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.

Mechanism of action:

Effects on Inhibitory Amino Acid Neurotransmission

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 (KD = 1.66 ± 0.19 and Bmax = 52.7 ± 10.8 where Bmax = number of receptor sites, compared to KD = 0.905 ± 0.2 and Bmax = 26.3 ± 8.0 in the controls), reduced the rate of GABA uptake (Vmax = 0.747 ± 0.3 compared to 1.9 ± 0.44 in the control) and increased the affinity of the transporter for GABA (KM = 137 ± 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 (GABAA antagonist, 7.5 mg/kg SC).

Effects on Excitatory Amino Acid Neurotransmission

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).

Drug activity related to proposed indication:

The pharmacologic mechanism underlying the anti-craving effect of acamprosate has not been established definitively at this time. Although the strength of the evidence varies, four hypothesis have been discussed which are not mutually incompatible: 1) interaction with the GABAergic system through $GABA_A$ and/or $GABA_B$ receptors; 2) interaction with the glutamatergic system through NMDA and/or metabotropic mGluR1 receptors; 3) inhibition of Ca^{2+} influx through NMDA receptors and/or voltage-gated Ca^{2+} channels; and, 4) interaction with the inhibitory taurine neurotransmitter system in the CNS. In general, the overarching

hypothesis is that acamprosate restores the balance between excitatory and inhibitory neurotransmission which is disrupted by ethanol consumption and reflected in the subsequent withdrawal experience.

2.6.2.3 Secondary pharmacodynamics

Acamprosate has been shown to decrease sensitization to the locomotor-stimulating effect of morphine and decreases conditioned place aversion produced by naloxone-precipitated morphine withdrawal but does not alter morphine drug discrimination or rates of stress-induced relapse to heroin self-administration in preclinical models (reviewed in Olive, 2002).

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.

2.6.2.4 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 after acamprosate in the number of explorations and movements (400-800 mg/kg PO) in rats. Oral acamprosate (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 by acamprosate 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 that acamprosate interacts with serotonergic systems in rats; the conclusions suggested antagonism of serotonin effects during high serotonin activity and potentiation of serotonin effects during low serotoninergic activity. Acamprosate at 500 and 800 mg/kg IP antagonized hyperthermia, hyperactivity and death (indices of excessive serotonin activity) resulting from tranylcypromine/L-tryptophan at 20 and 250 mg/kg IP respectively. Acamprosate at 200 and 400 mg/kg IP, when administered with tranylcypromine, induced hyperactivity that was antagonized by parachlorphenylalanine, 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 instance of 2nd degree atrioventricular (AV) heart block was observed at baseline in 1/4 control females, one instance of 2nd degree AV 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 instances of 2nd degree AV 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 no instances of cardiovascular alterations were observed in any group at week 26.

The non-approvable letter sent by the Agency requested the sponsor to conduct *in vitro* studies designed to assess the potential for acamprosate to interact with ion-channel function in cardiac tissue as part of the safety testing requirements for new chemical entities and first-in-class drugs. These studies were conducted for the 2nd cycle of this NDA by the sponsor and a general assessment of cardiac toxicity in *in vitro* and *in vivo* preclinical models exposed to acamprosate with subsequent extrapolation to human safety was also provided.

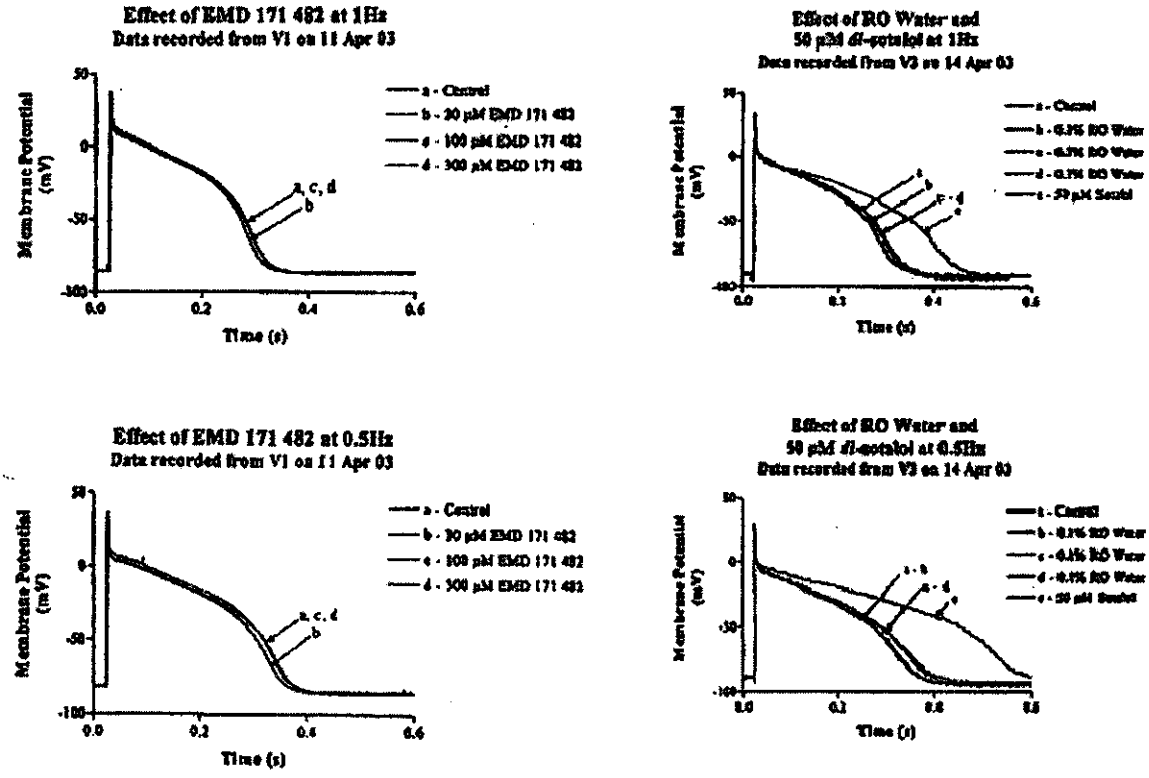
Acamprosate interaction with critical voltage-gated K^+-channels was assessed using HEK-293 cells stably transfected with the human *ether-a-go-go related gene* the product of which (the HERG channel) is responsible for the repolarization of the ventricular cardiac action potential (Study report DCLG1032, conducted by [ ]
Blockade of I_K through this channel is associated with the propensity to develop Long QT Syndrome and the potential fatal Torsades de Pointes. Acamprosate was tested at concentrations of 30, 100 and 300 µM and compared with the vehicle (negative) control which was water and a positive reference compound E-4031 at 100 nM. 4 cells were tested with each concentration of acamprosate and 4 cells were exposed to negative and positive control substances. Acamprosate exposure did not produce inhibition of HERG tail current at any concentration tested while negative and positive controls produced the expected response as can be seen in the table reproduced below:

Effect of Acamprosate, RO water and E-4031 on HERG Tail Current

Treatment	Tail Current (% Control)	Tail Current (% Control; vehicle corrected)*
0.1% RO Water	86.2 ± 3.8	-
30 µM Acamprosate	84.4 ± 1.7	97.9 ± 2.0
100 µM Acamprosate	90.6 ± 4.2	105.1 ± 4.9
300 µM Acamprosate	84.1 ± 4.2	97.5 ± 4.9
100 nM E-4031	9.2 ± 1.4	10.7 ± 1.6

A second *in vitro* study was performed in the Canine Purkinje Fiber model (Study report DCLG1033, conducted by [ ]) in which freshly isolated ventricular Purkinje fibers from the heart of beagle dogs were exposed to acamprosate at a concentration of 30, 100 or 300 µM or the vehicle control reverse osmosis purified water (0.1% final concentration) or the positive reference compound *dl*-sotalol hydrochloride (selective for the I_{Kr} current) at a final concentration of 50 µM and the effects on the action potential paced at 0.5, 1 or 3 Hz was assessed by intracellular patch clamp methods. The pacing of Purkinje cells at 3 Hz was utilized to study any potential frequency-dependent interaction of acamprosate with voltage-gated sodium channels in the tissue which would be reflected by a reduced maximum

rate of depolarization (MRD). Slower pacing was designed to reveal any repolarization-prolonging effects of the drug (i.e. increased action potential duration, APD) and would also reveal changes in MRD, upstroke amplitude and resting membrane voltage. Exposure of Purkinje fibers to acamprosate at concentrations of 30, 100 or 300 μM did not produce any dose-related changes in APD, MRD, upstroke amplitude or resting membrane voltage at frequencies of 1 or 0.5 Hz though 1 out of 4 acamprosate-treated cells demonstrated a dose and inverse frequency-dependent APD prolongation (APD_{90} of 10, 26 and 38% at 30, 100 and 300 μM, respectively). This was felt to be partly due to a time-dependent increase in APD which was observed in 2/4 vehicle treated cells (up to 18% prolongation of APD). When stimulation frequency was increased to 3 Hz, no difference in the MRD was observed when comparing vehicle and acamprosate (300 μM)-treated cells though a small decrease was obtained in both conditions (no positive control was assessed in this assay, however). In contrast, *dl*-sotalol produced a dose-dependent and inverse frequency-dependent prolongation of APD (~ 39% at 1 Hz, ~ 58% at 0.5 Hz) without evidence of any other effects on the action potential. See figure below for comparisons of APD with exposure to acamprosate, vehicle and *dl*-sotalol.



Taken together, these results suggest that acamprosate is not likely to adversely affect voltage-gated K^+ or Na^+ channels of ventricular Purkinje cells at concentrations up to 300 μM.

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.

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 atrioventricular heart block and ventricular premature beats in lead II in the ECG evaluations in the 26-week study in dogs.

Further evaluation through *in vitro* studies of potential acamprosate interaction with HERG cardiac ion channels and Purkinje fiber action potentials were submitted in this 2nd cycle of the NDA and indicated acamprosate had little effect at concentrations up to 300 μM in either of these preclinical models.

> Acamprosate effects on gastrointestinal function were evaluated in two *in vitro* models which demonstrated acamprosate had no effects on barium chloride and acetylcholine-induced contractions, and only slightly antagonized histamine dihydrochloride-induced contractions in isolated rat duodenum.

Safety pharmacology conclusions:

> 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 atrioventricular block and premature ventricular beats in lead II after 13, but not 26 weeks of treatment in dogs.

Follow-up in vitro studies of potential acamprosate interaction with HERG cardiac ion channels and Purkinje fiber action potentials indicated acamprosate had little effect at concentrations up to 300 μM in either of these preclinical models. As the plasma concentration of acamprosate at therapeutic steady-state dosing is approximately usually between 2 – 6 μM, this represents a 50 – 150-fold excess over the likely human exposure and suggests acamprosate is not likely to affect cardiac conduction with therapeutic dosing. Acamprosate had mild inhibitory effects on duodenal contractility observed only when stimulated by histamine but was without effect when contractility was elicited by acetylcholine.

2.6.2.5 Pharmacodynamic 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.

In summary, 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.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

No tabulated summaries of pharmacology studies were provided by the sponsor in this submission.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Absorption of acamprosate occurs slowly through the GI tract after oral dosing in all species tested including humans. Bioavailability is low in rats and limited in dogs with evidence of a dose-limiting saturable process when given as an oral gavage to dogs. Capsule and tablet formulation improves the bioavailability in dogs. The PK 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 Tmax 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. No evidence for gender-specific differences in the pharmacokinetic behavior of acamprosate has been found in any tested species when administered as an oral gavage though a dietary study in the mouse (but not in the rat) found that

females were exposed to higher concentration of acamprosate through this route. Acamprosate given orally slowly is distributed from the GI tract to liver, kidney, lymph nodes and bone marrow with evidence in lungs, adrenal gland and bile within 24 hr. Once in circulation, acamprosate can cross the blood-brain-barrier and is detected in the CNS. Acamprosate has also crosses the placental barrier and enters breast milk. Little protein binding is observed in nonclinical and human plasma samples (<10% in general). Acamprosate is not metabolized in any species tested, including human, and has not been observed to induce or inhibit the cytochrome P450 mixed function oxidase system in *in vitro* hepatocyte or liver microsomal preparations. Oral administration of acamprosate is primarily excreted in the feces of nonclinical species in the rat with only ~ 25% eliminated renally though in dog this is a slightly more important route. Elimination is complete by 5 days after oral dosing with the majority of drug excreted within 24 hr of dosing. Some billiary excretion has been observed in the dog model. Human elimination appears similar with the great majority of the oral dose excreted in the feces suggesting a limited absorption from the GI tract. No accumulation of acamprosate has been observed with repeated dosing in the rat or dog.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 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.

2.6.4.4 Distribution

In male Sprague-Dawley and Long Evans rats, the greatest concentrations of radioactivity from ^{35}S-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 nonpregnant 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 ^{35}S-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).

2.6.4.5 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 μM). No inducing potential by acamprosate was found at the concentration of 100 μM on the CYP1A2 and 3A4 enzymes in human hepatocytes.

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2.6.4.6 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)

* Most radioactivity due to ^{45}Ca-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) ^{35}S-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 ^{35}S-acamprosate, with most radioactivity recovered in the first 24 hours after dosing. Oral administration of ^{35}S-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 ^{45}Ca acamprosate in rats, due to incorporation of the ^{45}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 ^{14}C-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 ^{14}C-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 ^{14}C acamprosate at concentrations of 0.25, 1, and 10 mcg/ml. The results of the binding assay are presented in the following table:

Plasma Protein Binding of ^{14}C Acamprosate (Mean Percent Radioactivity Bound)

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.

2.6.4.7 Pharmacokinetic drug interactions

Acamprosate has not been demonstrated to alter the action or disposition of any drug through a pharmacokinetic interaction. However, a study conducted in the rat model indicated that due to an active secretion component to renal elimination of unchanged acamprosate, inhibitors of tubular secretion such as probenecid may elevate plasma acamprosate levels and increase elimination half-life (Zornoza et al., 2002) though it is not clear that an active tubular secretion occurs in humans. A recent clinical study suggests that naltrexone coadministration with acamprosate enhances acamprosate absorption, bioavailability, decreases T_{max} and increases

systemic exposure without altering elimination half-life of the compound nor influencing the pharmacokinetics of naltrexone or its active metabolite 6-β-naltrexol (Mason et al., 2002).

2.6.4.8 Other Pharmacokinetic Studies

Study Title: Acamprosate: 28-Day Oral (Dietary Administration) Toxicokinetic Study in the Mouse

Key study findings:

- 28-day dietary administration of acamprosate 400 mg/kg/day to male and female mice resulted in a systemic exposure of 21,372 and 33,512 ng•h/mL, respectively. The average systemic exposure in mice was 27,442 ng•h/mL which represents a 4.6-fold exposure margin over human exposure observed with therapeutic dosing.
- Female exposure to acamprosate was approximately 57% greater than acamprosate exposure in male mice.
- No differences in plasma Ca^{2+} concentrations were observed between animals receiving acamprosate and control animals.
- No overt toxicity was observed at this dose over the 28 days of the study.

Reviewer: Adam M. Wasserman, Ph.D.
Study no: 0537/060 Submitted in NDA 21-431 BP Amendment #031
Volume and page #: Volume 2, Attachment #5
Conducting laboratory and location:
Test Facility (Animal Treatment): [redacted]

Test Site (Toxicokinetics): [redacted]

Date of study initiation: 2/12/2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity: Acamprosate, Lot #1, Batch # M242B, — % purity, Expiry date: 2/29/2004. Test article was not radiolabeled.

Formulation/vehicle: Test article was administered orally through diet. Animals had access *ad libitum* to the dietary formulations for at least 28 days. Test diets prepared weekly and reserve samples retained, deep-frozen, from each batch used in the study. Test article was weighed and ground with a small amount of diet and added directly to diet mix in a mixing drum which was set in motion for 5 minutes. The control article and vehicle for the test article was Rat and Mouse Maintenance Diet No. 1, Ground Fine, supplied by: [redacted]

Methods (unique aspects): No unique methods.
Dosing:

Species/strain: —:CD-1(ICR)BR mice obtained from [redacted]
#/sex/group or time point (main study): 24 mice/sex/group (toxicokinetic study)
Satellite groups used for toxicokinetics or recovery: None
Age: 8 weeks of age at start of dosing
Weight: ♂: 27.5 – 41.5 g; ♀: 24.1 – 31.3 g
Doses in administered units: 0 or 400 mg/kg/day
Route, form, volume, and infusion rate: Admixture in diet

Observations and times:

Clinical signs: Daily observation, weekly physical examination
Body weights: Prior to treatment on Day 1, weekly thereafter
Food consumption: Weekly, calculated as g/animal/week
Ophthalmoscopy: Not performed
EKG: Not performed
Hematology: Not performed
Clinical chemistry: $[Ca^{2+}]$ determined from the plasma of returned TK samples (conducted at [redacted]
Urinalysis: Not performed
Gross pathology: Not performed
Organs weighed: Not performed
Histopathology: Not performed
Toxicokinetics: Blood samples taken from 3 animals/sex/group on Day 29 at the following time-points (GMT): 6:00, 9:00, 12:00, 15:00, 18:00, 21:00, 24:00, (Day 30) 3:00. Samples obtained by cardiac puncture under halothane anesthesia.
Other:

Results:

Mortality: No mortality observed in any group
Clinical signs: No treatment-related effects observed
Body weights: No treatment-related effects observed
Food consumption: No significant differences between treatment groups though acamprosate ♀ demonstrated approximately 10% greater food consumption than control ♀ as well as a slightly higher food consumption (by body weight) than the acamprosate ♂; the secondary consequence being that female intake of acamprosate was slightly above the intake of their male counterparts as can be seen in the table below:

Test article consumption:

Interval (weeks)	Mean Compound Consumption (mg/kg/day) by Treatment Group			
	Controls (♂)	Acamprosate (♂)	Controls (♀)	Acamprosate (♀)
1 – 4	0	407.7	0	433.2
% nominal	-	102	0	108

Clinical chemistry: No treatment-related effects observed on plasma Ca^{2+} concentrations; values in animals receiving acamprosate were not different from controls and all were within background historical levels.

Test article formulation analysis: Achieved concentrations sampled from chow prepared in Weeks 1 and 4 were within the intended range of 85 – 110% of nominal (85 – 102% in female treated group; 93 – 103% in male treated group). Analytical procedure validated in study 537/59 (28-Day Rat Toxicokinetic Study).

Toxicokinetics: Acamprosate was detected in the plasma of all animals in the acamprosate treatment groups with little differences within groups or between genders. Peak plasma levels for both males and females were found between the 3:00 a.m. and 9:00 a.m. time-points.

Table 1: Acamprosate Exposure after 28 Days Dietary Administration (Individual mouse data)

	female				male			
Time (hours)	animal	conc (ng/mL)	mean	SD	animal	conc (ng/mL)	mean	SD
6:00 AM	73	836			25	1030		
	74	1957	1710	780	26	1199	1544	749
	75	2337			27	2404		
9:00 AM	76	1084			28	667		
	77	1783	1643	504	29	1680	1050	550
	78	2062			30	803		
12:00 PM	79	664			31	820		
	80	904	1006	402	32	726	871	177
	81	1449			33	1068		
3:00 PM	82	1043			34	460		
	83	1006	1255	400	35	986	877	353
	84	1717			36	1185		
6:00 PM	85	1201			37	489		
	86	918	1230	327	38	1151	1024	484
	87	1570			39	1432		
9:00 PM	88	676			40	632		
	89	1810	1025	662	41	424	491	121
	90	587			42	419		
12:00 AM	91	1353			43	548		
	92	1401	1293	147	44	409	530	113
	93	1128			45	632		
3:00 AM	94	2333			46	536		
	95	1860	2009	280	47	760	736	189
	96	1835			48	913		

Table 2: Acamprosate Exposure after 28 Days Dietary Administration (Group data)

Dose	Sex	C_{max} (ng/mL)	AUC_{0-24} (ng•h/mL)	T_{max} (h)
400 mg/kg/day	Male	—	21,372	6:00 AM
	Female	—	33,512	3:00 AM
	Male-Female	—	27,442	6:00 AM

The lower limit of quantitation (LLQ) of this method was — ng/mL acamprosate for the assay.

Summary of individual study findings:

Results of this 28-Day dietary administration of acamprosate 400 mg/kg/day were designed to provide supplementary information for the assessment of the adequacy of dosing in the 2-year mouse carcinogenicity bioassay conducted previously. In the carcinogenicity study, the MTD could not be used to support adequacy of dosing as an MTD had not been established in a 13-week repeat-dose toxicity study and the high dose administered in the diet during the carcinogenicity study was 400 mg/kg/day. This study, therefore, was completed to provide an estimation of the drug exposure that was likely achieved in the 2 year bioassay versus the plasma levels considered to be therapeutic in humans (data obtained from the Acamprosate NDA). As can be seen in the above tables, dietary administration of acamprosate at approximately 400 mg/kg/day to male and female mice produced detectable levels of drug in all animals at all time-points. Peak acamprosate concentrations were seen at different times for males and females but roughly occurred between 3:00 AM and 6:00 AM. Females exhibited higher plasma exposure over 24 hr (~57% above males). No signs of overt toxicity were observed in this study and body weight and food consumption were not affected by 28-day treatment with 400 mg/kg/day acamprosate in the diet. No differences in plasma calcium concentrations were observed between treated and untreated mice, either. The average AUC value observed in mice, 27,442 ng•h/mL, represents a 4.6-fold exposure margin over the steady-state therapeutic concentration of acamprosate in humans.

Acamprosate was studied as a 28-day oral (dietary administration) toxicokinetic study in the rat (Study no. 537/059; Amendment #003, reviewed by Dr. Kathleen Haberny. Sprague-Dawley rats received 0, 25, 100 or 400 mg/kg/day of acamprosate admixed into diet. Blood samples for toxicokinetic analysis were taken at 3 hr intervals from selected members of each dose group starting at 6 a.m. on day 28 and ending at 3 a.m. on Day 29 of the study. Serum calcium was also assessed. One HD female died on Day 28, cause was not determined. No other mortalities or treatment-related effects observed in any animal at any dose.

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

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.

2.6.4.9 Discussion and Conclusions

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 lacrymal glands in beagle dogs. Acamprosate crossed the blood brain barrier with highest brain concentrations appearing at 30 minutes after dosing. The brain:plasma AUC ratio was 0.17. Acamprosate also crossed the placenta, 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 ^{35}S acamprosate appeared in the feces while most of the radioactivity after dosing with oral ^{45}Ca-acamprosate was measured in the carcass, suggesting incorporation of the calcium moiety into bone. Protein binding was low in rat, dog, and human plasma, with higher percent binding in rat (overall mean approximately 13.5%) than in dog (2.4%) and human (6%) samples. Acamprosate pharmacokinetics were studied in mice, rats, rabbits and dogs. Dietary administration of acamprosate at 100 mg/kg/d resulted in Cmax levels below the limit of detection in most mice. Oral acamprosate at 100 mg/kg/d produced peak plasma levels of[ ]mg/l in rats and[ ] mg/l in dogs. In comparison, in clinical studies, the proposed 1998 mg/d dose for 8 days resulted in a

steady state Cmax of — mg/l. The increases in Cmax and AUC values were slightly less than dose proportional in the 28-day oral study in rats, with no differences between males and females in the PK parameters. The times to peak plasma concentrations after 100 and 400 mg/kg/d were approximately 0.5 hour in rats and 2 hours in dogs. After 1000 mg/kg/d PO in rabbits, the Cmax of — mcg eq/ml was detected at 2 hours. The Tmax decreased by approximately 50% with repeated dosing in humans.

The AUC, determined by using the last measurable time point after single dose acamprosate at 400 mg/kg PO, was 44 mg•h/l in rats and 240 mg•h/l in dogs. After 1000 mg/kg/d PO in rabbits, total exposure (AUC) over 24 hours was 522.1 µg eq•h/ml. In humans the AUC measured from 0-24 hours at steady state after 1998 mg/d for 18 days was 6884 ng•h/l. The half-life of acamprosate after administration of single oral doses of 400 mg/kg was 31 hours in rats and 2.4 hours in beagle dogs. In humans, the half-life at steady state after oral treatment at the dose of 666 mg was 17 hours.

A 28-day dietary administration of 400 mg/kg/day acamprosate to male and female mice, conducted in support of the submitted mouse carcinogenicity study, produced a C_{max} in males and females of approximately 1.5 µg/mL and 2.0 µg/mL and an AUC of 21.3 µg•h/mL and 33.5 µg•h/mL, respectively. Thus under these conditions, females were observed to have a greater systemic exposure (30% by C_{max}, 57% by AUC) than males. Female food consumption was slightly higher than males by body weight (~ 6%) but this does not appear to explain this finding. No assessment of dose accumulation was made in the study and no overt toxicity was observed in mice receiving this dose. The systemic exposure produced by this dose of drug administered through the diet represents a 4.6-fold margin above that observed in humans at steady state receiving therapeutic dosing at 1998 mg/day.

PK/TK conclusions:

Oral bioavailability of acamprosate is variable in animals but is generally low. Distribution is primarily to the gastrointestinal tract, kidney and liver, however, acamprosate does cross 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 PK studies show peak plasma levels and AUC values increase in a less than dose proportional manner, and there were no differences between males and females in the measured parameters. The Tmax occurs from 0.5-2 hours after oral dosing in rats, rabbits and dogs, and decreases approximately 50% (from approximately 15 hours to 7-9 hours) with repeated dosing.

2.6.4.10 Tables and figures to include comparative TK summary

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

Acamprosate Exposure after 28 Days Dietary Administration to Mice (Individual mouse data)

	female				male			
Time (hours)	animal	conc (ng/mL)	mean	SD	animal	conc (ng/mL)	mean	SD
6:00 AM	73	636			25	1030		
	74	1957	1710	760	26	1199	1544	749
	75	2337			27	2404		
9:00 AM	76	1064			28	667		
	77	1783	1643	504	29	1680	1050	550
	78	2062			30	803		
12:00 PM	79	664			31	820		
	80	904	1008	402	32	726	871	177
	81	1449			33	1068		
3:00 PM	82	1043			34	480		
	83	1006	1255	400	35	996	877	353
	84	1717			36	1155		
6:00 PM	85	1201			37	489		
	86	916	1230	327	38	1151	1024	484
	87	1570			39	1432		
9:00 PM	88	676			40	632		
	89	1810	1025	662	41	424	491	121
	90	587			42	419		
12:00 AM	91	1353			43	548		
	92	1401	1293	147	44	409	530	113
	93	1128			45	632		
3:00 AM	94	2333			46	536		
	95	1660	2009	260	47	760	736	189
	96	1835			48	913		

Acamprosate Exposure after 28 Days Dietary Administration to Mice (Group data)

Dose	Sex	C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)	T_{max} (h)
400 mg/kg/day	Male	—	21,372	6:00 AM
	Female	—	33,512	3:00 AM
	Male-Female	—	27,442	6:00 AM

The lower limit of quantitation (LLQ) of this method was —ng/mL acamprosate for the assay.

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]
Cmax (mg/l)	—	—	—	—	—
Tmax (h)	0.5	0.25	0.30	1	0.5
AUC Ct (mg.h/l)	2.94	14.55	14.12	36.84	44.16
AUC $_{0-\infty}$ (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.

* Cmax: Maximum plasma acamprosate concentration achieved; Tmax: Time at which Cmax 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 hr 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]
Cmax (mcg eq/ml)	—	—
Tmax (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.

*Cmax: Maximum plasma acamprosate concentration achieved; Tmax: Time at which Cmax occurred; AUC: Area under plasma acamprosate time curve.

In female rabbits, plasma acamprosate decreased to baseline levels over 120 hr. Acamprosate was excreted in urine at 53-56% and in feces at 23-24% of radioactive dose administered. 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]
Cmax (mg/l)	[				]
Tmax (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.

*Cmax: Maximum plasma acamprosate concentration achieved; Tmax: Time at which Cmax 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 hr, elimination half-life was 0.77 hr, clearance was 0.23 L/h/kg and the 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:

Oral Acamprosate 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				
Cmax (mg/l)	Capsule			
	Tablet			-
Tmax (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

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Human Pharmacokinetic Parameters: Comparisons across Acamprosate 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
Tmax (h)	1.4	1.5	1.4	1.2
Cmax (ng/ml)	[redacted]			[redacted]
$AUC_{0-\infty}$ (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, Tmax: Time to peak plasma concentration, Cmax: Peak plasma concentration, AUC: Area under the plasma acamprosate 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
Cmax (mg/l)	162±21	523±57	471±39	279±88	481±46	481±49
Tmax (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. 2000 mg/d respectively.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

No tabulated summary of pharmacokinetic studies was provided by the sponsor.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

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 LD_{50} values suggesting poor oral bioavailability in rodents. Oral and IV LD_{50} 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.

A follow-up 1-month study of acamprosate at higher oral doses (up to 3000 mg/kg/day) intended to identify target organs of toxicity in dogs again failed to reveal this information though no NOAEL could be identified due to vomiting and diarrhea beginning at the lowest dose tested (750 mg/kg/day) and which demonstrated dose-related increases in frequency and severity. Acamprosate administration to monkeys for a period of 7 days also caused diarrhea.

Genetic toxicology:

The sponsor conducted several studies which were reviewed by Dr. Haberny in the first cycle of this NDA. The following table summarizes the genetic toxicology studies previously conducted and reviewed:

Study #	Study Type	Test System	Doses Used	Results	Notes
83058	Ames Assay	*S. typhimurium*	50 – 5000 µg/plate	Negative	E. Coli or TA102 not used
537/50	Ames Assay	*S. typhimurium* *E. coli*	8 – 5000 µg/plate	Negative	
85065	*In vitro* mammalian cell gene mutation assay	V79 Hamster Lung Cells	100 – 3000 µg/mL	Positive results at 300 µg/mL in one test; 100, 1000 and 3000 µg/mL in a second test (both without metabolic activation); 3rd test did not demonstrate revertants at any concentration. No revertants with metabolic activation in any study.	Dosing was deemed inadequate due to an absence of precipitate and lack of cytotoxicity
86002	*In vitro* chromosomal aberration assay	Human Peripheral Blood Lymphocytes	10 – 300 µg/mL	Negative	Dosing in –S9 condition was not adequate
84008	*In vivo* mouse micronucleus assay	Mouse bone marrow	3000, 6000 mg/kg PO	Negative	
537/51	*In vivo* mouse micronucleus assay	Mouse bone marrow	500 – 2000 mg/kg PO	Negative	

The sponsor was informed that due to the equivocal findings found in the *in vitro* cell gene mutation assay in V79 cells and the lack of dose adequacy in both this study and the *in vitro* chromosomal aberration assay these should be repeated.

These studies were submitted as N 000 BP (amendment #031) on 5/23/03. The repeat of the *in vitro* chromosomal aberration assay with human lymphocytes (study # T15446) utilized doses up to 1580 µg/mL as this was a dose which was observed in a preliminary study to demonstrate precipitate without evidence of mitotic inhibition in the presence or absence of metabolic activation. No biologically significant increase in chromosomal aberrations nor evidence for polyploidy were observed with acamprosate up to the limiting dose of 1580 µg/mL in the presence or absence of metabolic activation at any time-point. Thus, acamprosate appears to be non-clastogenic in this *in vitro* assay. The repeat of the *in vitro* cell gene mutation assay in HPRT-deficient V79 cells (study # T15444) utilized acamprosate doses up to 5000 µg/mL both in the presence and absence of S9 metabolic activation (3 hr and 24 hr incubation, respectively) in the 1st series and dosing up to 5000 µg/mL (without metabolic activation) and 2810 µg/mL (with metabolic activation) in the 2nd series assessment with exposures of 3 hr in both conditions. Precipitate was noted at all concentrations ≥ 1580 µg/mL and in the presence of metabolic

activation in the 1st series (10% S9 for 3 hr incubation) the concentration of 5000 µg/mL was considered cytotoxic; therefore, a lower concentration still capable of producing precipitate was chosen as the top dose (2810 µg/mL) and a reduction in the concentration of S9 mix was used (10% S9 1st series, 5% S9 2nd series). Acamprosate did not induce 6-TG-resistant colonies (i.e. mutants) in the absence of metabolic activation up to 5000 µg/mL in either series. Acamprosate did not produce increases in mutation frequency in the presence of metabolic activation in either series as well, and though a slight increase in mutants relative to negative controls (2-fold increase) was noted, this was well within the historical range of values for negative controls in this lab. Thus in light of the previous findings as reviewed by Dr. Haberny in the 1st cycle submission combined with the two studies repeated for the 2nd cycle, acamprosate appears to be negative in tests for genotoxicity.

Carcinogenicity:

Carcinogenicity of acamprosate was evaluated in two 2-year rodent bioassays reported and reviewed for the 1st cycle of this NDA. The studies were completed prior to the current policy recommending that sponsor's obtain Executive Carcinogenicity Assessment Committee (Exec CAC) concurrence with the carcinogenicity protocols prior to conducting these studies (studies were conducted over the period 1989 – 1991). In the original NDA submission, the Exec CAC concluded the dosing strategy used in the rat study was adequate, though dosing appeared more appropriate for males due to evidence of overall toxicity (decreased body weight, increased incidence of tail sores, renal pelvic mineralization) while the highest dose tested in female rats was considered "marginally" acceptable. Subsequent investigations conducted to determine the MTD in 13-week (dietary) repeated dose toxicity studies revealed that the MTD dose in rats was judged to be 1000 mg/kg/day. The top dose used in the rat carcinogenicity study (400 mg/kg/day) thus represents between 1/3 – 1/2 of the MTD. Dosing in the mouse carcinogenicity study was deemed inadequate due to the lack of the use of a MTD dose based on an apparent lack of overt toxicity. The Exec CAC supported Dr. Haberny's conclusions 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." This opinion and recommendation was communicated to the sponsor on April 25, 2002.

Rather than complete the mouse carcinogenicity assessment, the sponsor elected to submit an outside expert opinion on the validity of the original mouse bioassay. Dr [ ] supported the sponsor's contention that the mouse carcinogenicity study should be useful and adequate to support the risk assessment and safety of acamprosate in clinical use when examined in light of the additional studies submitted for the second cycle. For additional support, the sponsor conducted and provided a 28-day toxicokinetic study of dietary administration of acamprosate in the mouse ([ ] study #0537/060) in order to provide an estimate of the exposure margin between mice in the 2-year bioassay and the expected systemic exposure in humans following therapeutic use of the drug product. The equivalent dose of acamprosate used in the 2-year bioassay was given to mice for 28 days through the same route (i.e. dietary) and clinical signs and toxicokinetics were assessed. No overt signs of clinical toxicity were observed in the mice

at this dose and the systemic exposure produced (AUC_{0-24} was 21,372 ng•h/mL and 33,512 ng•h/mL for male and female mice, respectively). The therapeutic exposures observed clinically with t.i.d. dosing in humans is reportedly 5905 ng•h/mL (NDA 21-431 Vol 36 p.70). Thus, the acamprosate exposure in the mouse at the highest dose of 400 mg/kg/day in the carcinogenicity study submitted is approximately 3.6-fold to 5.6-fold greater than that reported in humans at steady state at clinically therapeutic levels. Although several of the arguments made by Dr. [redacted] in support of the adequacy of the mouse carcinogenicity study are credible, ultimately the mouse carcinogenicity bioassay is still considered unacceptable due to a MTD dose not being utilized as the high dose and the toxicokinetic demonstration that the exposure in mice was no greater than 6-fold that expected to be observed in humans. This exposure margin does not reach the level described in the ICH S1C guidance, which recommends a 25-fold exposure margin as an alternative dosing strategy. Thus, acamprosate has been appropriately evaluated in the rat but not the mouse for carcinogenic potential.

Reproductive toxicology:

No new reproductive toxicology studies were submitted for this 2nd cycle review of acamprosate. The sponsor submitted the required studies for the 1st cycle, which were reviewed at that time and specific details can be found in the review by Dr. Habeny. The results of these studies are summarized briefly below.

Segment I (Fertility and Early Embryonic Development): Acamprosate was tested in mice and rats for effects on fertility with oral administration of the compound. At doses up to 2400 mg/kg/day in the mouse (5-fold the maximum recommended human dose, MHRD) and 1000 mg/kg/day in the rat (4-fold the MRHD) no effects on fertility of the F_0 or F_1 generation were observed nor was there evidence of maternal toxicity at these doses.

Segment II (Embryo-Fetal Development): Reproductive studies designed to assess acamprosate for potential embryofetal toxicity and teratogenicity utilized mice, rats and rabbits (New Zealand and Burgundy-Tawny strains). Mice were tested with dosing of the pregnant dam during the critical gestational window (Days 6-14) with doses of acamprosate up to 2400 mg/kg/day (5-fold the MRHD) without evidence of fetal abnormalities. Pregnant rats were administered acamprosate at doses of 50, 300 or 2000 mg/kg/day (0.2-, 1.2- or 8-fold MRHD) during gestational days 6-15. Examination of resulting fetuses indicated both a treatment-related increase in dams with malformed fetuses (1, 3, 4 and 4 in the 0, 50, 300 and 2000 mg/kg/day groups) and a treatment related increase in the number of **malformed fetuses** (1, 3, 12, and 10 in the 0, 50, 300 and 2000 mg/kg/day groups). Malformations most commonly seen in the Medium Dose (MD) and High Dose (HD) groups were: **hydronephrosis, malformed iris, retroesophageal subclavian artery, retinal dysplasia, edema, abnormal carotid artery, anophthalmia and polydactyly**. Thus in the rat, the target organs for embryofetal teratogenicity are the kidney, the eye and the vascular system with a NOAEL equivalent to approximately 0.2-fold the MRHD.

The sponsor further studied acamprosate in two strains of rabbit, New Zealand and Burgundy-Tawny, to further characterize potential embryofetal toxicity. In the New Zealand rabbit, acamprosate dosing up to 1000 mg/kg/day (8X MRHD) over days 7-19 of gestation produced

mild maternal toxicity reflected in decreased weight gain and food consumption especially at the high dose but no treatment-related embryofetal developmental effects were observed at any dose. In contrast, Burgundy-Tawny rabbits administered acamprosate up to 800 mg/kg/day (6X MRHD) over gestation days 8-16 demonstrated embryofetal toxicity and teratogenicity reflected in slightly decreased fetal weights at the MD (400 mg/kg/day; 3X MRHD) and HD and a non-dose-related but treatment-related increased incidence of **fetal malformations**, almost exclusively findings of **hydronephrosis** in 6.5% (6/92) of examined fetuses at the MD, 2.1% (2/94) observed at the HD. No findings of hydronephrosis were observed in control or LD (200 mg/kg/day; 1.5X MRHD) fetuses. One MD fetus was observed to have torsion of the vertebrae but this was not observed at any other dose studied or in controls. The target organ in embryofetal Burgundy-Tawny rabbits is clearly the kidney. In light of the positive teratogenic response found in the kidney of the Burgundy-Tawny rabbit, the New Zealand rabbit study was repeated using a dose of 800 mg/kg/day over gestation days 8-16 in order to replicate the conditions of the previous study. Results of this study confirmed that embryofetal toxicity is not observed in the New Zealand rabbit at a dose corresponding to 6-fold the therapeutic dose of acamprosate used in humans.

Segment III (Prenatal and Postnatal Development, Including Maternal Function): Reproductive toxicity studies designed to assess potential pre- and postnatal developmental alterations due to acamprosate exposure were conducted in the mouse and rat. Pregnant mice were administered acamprosate in doses up to 2400 mg/kg/day (5X MRHD) starting at gestational day 15 and continuing through postnatal day 28. A dose-dependent increase in the incidence of **still-born births** as well as the number of dams with **offspring dying after birth** was observed in the MD (960 mg/kg/day; equivalent to 2X MRHD) and HD groups. These findings were not observed in the LD (320 mg/kg/day; equivalent to 0.7X MRHD) group, however. No treatment-related effects on behavioral measures or maternal performance were found in the F_1 generation. The NOAEL for adverse pre/postnatal development was considered to be 320 mg/kg/day (0.7X MRHD). In the rat model, pregnant dams were exposed to acamprosate doses up to 2000 mg/kg/day (8X MRHD) and, except for higher pup weights in the MD and HD groups, no treatment-related effects on pre- and postnatal development of the F_1 generation were found. The NOAEL therefore was considered to be greater than 2000 mg/kg/day.

In summary (and all MRHD are body surface area-adjusted to a 50 kg individual), acamprosate was noted to have no effects on fertility measures in the mouse and rat with a NOAEL > 4-fold the MRHD. The potential for teratogenicity with exposure during critical periods of embryofetal development was revealed to be species- and even strain-specific. No evidence of embryofetal toxicity was observed in mouse studies using doses up to 5-fold the MRHD and in New Zealand rabbit studies with doses up to 8-fold the MRHD. In contrast, exposure to acamprosate in pregnant rats resulted in fetal malformations of the kidney, eye and vascular system at doses that approximate the MRHD (1.2-fold) with a NOAEL less than the clinical dose (0.2-fold MRHD). In contrast to the findings in New Zealand rabbits (which was replicated in a separate study), exposure to acamprosate in pregnant Burgundy-Tawny rabbits produced fetal malformations that were almost exclusively characterized by hydronephrosis and observed at doses which were 3-fold the MRHD. The NOAEL for this study was approximately 1.5-fold MRHD. Peri- and postnatal studies of acamprosate in mice and rats with dosing equivalent to > 5-fold the MRHD

detected no alterations in behavior or function but an increase in still-born offspring was observed in the mouse study in all but the lowest dose tested, representing approximately 0.7-fold the expected clinical dose.

Special toxicology:

The sponsor conducted a study designed to assess the potential for acamprosate to elicit "Olney Lesions", a specific form of CNS toxicity which has been demonstrated to occur with exposure to NMDA antagonists in rats and is specific to the retrosplenial and posterior cingulated cortex. Rats were administered 2000 mg/kg acamprosate by oral gavage, sacrificed and brain histology evaluated. No evidence of these lesions was found in contrast to the positive control used in this study, MK-801 5 mg/kg s.c., in which clear evidence of neurotoxicity was observed.

2.6.6.2 Single-dose toxicity

Acamprosate
Mean Lethal Dose, LD_{50}

Species Study (Vol.)	Route		
	P.O.	**I.P.**	**I.V.**
Mouse ***(CD1, Swiss OFI)***			
90.02.AOT.001.SP1 (Vol 11)	8.37 g/kg		
90.03.AOT.001.SP2 (Vol 11)			771 mg/kg
1100.22.02.84 (Vol 12)	7.7 g/kg	1.5 g/kg	720 mg/kg
Rat ***(Sprague-Dawley)***			
90.AOT.001.RP2 (Vol 12)			730 mg/kg
90.04.AOT.001.RP3 (Vol 12)	6.16 g/kg		
1100.22.02.84 (Vol 12)	9.34 g/kg	1.25 g/kg	
Rabbit ***(Burgundy Tawny)***			
1100.22.02.84 (Vol 12)			2.23*
1602.26.11.86 (Vol 12)	0.6 g/kg§		

*Minimum Lethal Dose with slow infusion; §, non-lethal dose (produced wet stools only).

Single dose toxicity studies were conducted in mouse (two strains), rat and rabbit. A high degree of consistency was observed between toxicity (LD_{50}) values between the mouse and rat; the rabbit studies were conducted in a different manner (slow i.v. infusion and a single lower p.o. dose) so these should be judged slightly differently from the other rodent species. Nevertheless, a similar constellation of clinical signs and symptoms and gross findings were observed between species and routes of administration with toxic doses of acamprosate. Typically, these consisted of decreases in body weight and food consumption during the course of extended (14-day) observations, decreased motor activity, ataxia, muscular hypotonia and an observation of "general paralysis". GI congestion was observed in rats and mice with p.o and i.v. dosing, respectively. Oral dosing in rats at very high doses produced respiratory changes described as dyspnea in one study and hypernea in the other. All species through all routes of administration had death attributed to cardiac arrest although this diagnosis was not verifiable. Evidence of

cardiac changes including hypotension (mouse and rabbit), pulmonary hyperemia (mouse), bradycardia (mouse) were described at lower doses or leading up to death. Convulsions were seen in the mouse and rat with i.v. dosing but were not seen with oral or intraperitoneal dosing. Gross pathology in mouse decedents revealed hardened, contracted heart and blood in the auricles. A study in rabbit in which a sublethal dose of acamprosate (600 mg/kg) was given orally produced only wet stools. More specific information can be obtained in the original review of this NDA by Dr. Haberny.

2.6.6.3 Repeat-dose toxicity

The current resubmission contains a 1 month oral toxicity study in dog using doses higher than that used in the original 1 month and 26 week oral toxicity study in this species. This will be reviewed in detail but a summary of the previously submitted studies is presented briefly below:

Repeat-dose toxicology studies were conducted in the mouse, rat, dog and monkey. The following table lists the studies which were submitted with the original NDA and which were reviewed by Dr. Haberny:

Summary of Repeated Dose Toxicology Studies on Acamprosate

Species/Strain	Acamprosate Dose (mg/kg/d)	Duration	Mortality	Clinical Signs	Clinical Pathology	Organ Weight Pathol:Gross& Micro	NOAEL/ EL (mg/kg)	Reference
Mouse (CD1 VAF) n=12/sex/dose	0,100,400 dietary	15 d	0	None	Not Determined	Not Determined	-	Study 91.05.AOT.001. SP3 Vol. 13
Mouse (CD1) 10/sex/dose	0,500,1000,1500,2000 dietary	13 wk	0	Incr water consumption	Incr urinary Ca & P	1000: decr brain, heart, liver, spleen, testes wt, 2000: decr brain, heart wt	0/500	Study [illegible] 138/8827 Vol. 13
Rat (Sprague-Dawley) 18/sex/dose	0,100,400 dietary	3 wk	0	Incr body wt gain in females, decr body wt gain in males	Plasma Ca determined only. No effect	Not Determined	-	Study 91.07.AOT.001. RP4 Vol. 13
Rat (Sprague-Dawley) 10/sex/dose	0, 500, 1000, 2000 dietary	13 wk	0	≥500: loose feces ≥1000 incr water consumption	≥500 decr urinary vol ≥1000 incr urinary CA	≥500: watery, pale GI contents ≥1000: decr liver wt 2000: incr adrenal wt, decr heart & ovary wts	0/1000	Study [illegible] 139/88834 Vol. 14
Rat (Sprague-Dawley) 9/sex/dose	0,320,960, 2400 PO	90 d	0	Salivation, liquid diarrhea at high dose	None	≥960: adrenal wt, 2400: abs gonad wt, 2400 recovery: distended kidney tubule sections	0/320	Study 1097 Vol. 14

Table continued

Species/Strain	Acamprosate Dose (mg/kg/d)	Duration	Mortality	Clinical Signs	Clinical Pathology	Organ Weight Pathol:Gross& Mlcro	NOAEL/EL (mg/kg)	Reference
Rat (Sprague-Dawley) 15-30/sex/dose, reversibility: 5-10/sex/dose	0,320,960, 2400 PO	26 wk	5M & 17F at 2400	Dose related soft/liquid feces, transient ptyalism, water consumption, piloerection, hypothermia, incr water consumption,	320: urine acidity , incr urine protein &hemoglobin, 960: , urinc acidity , urine protein & hemoglobin , 960,2400: incr blood urea N , Ca , serum P , incr urine Ca	960: incr heart, adrenal&kidney wts , cerebellum vacuolation 2400: heart& spleen, adrenal wt, degen renal tubulopathy, renal-cardiac-digestive-vascular calcifications, hyperkeratosis, stomach dysplasia, cardiac myolysis in rats that died	0/320	Study 602201 Vol. 15
Dog (Beagle) 3/sex/dose	0,25,100, 200 IV	4 wk	0	25-200: vomiting, salivation, swelling/induration at injection site	200: incr serum Ca , decr P	No effect	0/25	Study 35191 Vol. 18
Dog (Beagle) 2/sex	1000 PO	4 wk	0	Liquid diarrhea, decr body wts (5-7%)	None	No effect	-	Study 509215 Vol. 17
Dog (Beagle) 4/sex	0,250,500, 1000 PO	26 weeks	0	Dose related diarrhea, severe at HD Cardiac rhythm/conduction abnormalities in several HD dogs	Dose related incr urine Ca , decr serum P, incr serum Cl at HD	No effect	0/250	Study 509215 Vol. 17
Monkey (Macaque) 2 male, 1 female	1000 PO	7 days	0	Diarrhea, decr body wts (5-6%)	None	No effect	-	Study 1605 Vol. 18

In CD-1 VAF mice administered target doses of 100 and 400 mg/kg/day (0.2x and 0.8x the MRHD on a BSA basis) by admixture in the diet for 2 weeks, mean acamprosate intake was 125-137 and 534-564 mg/kg/day in the low and high dose animals, respectively. Wounds and hair loss were observed in the high dose groups, and increased food consumption and body weight gains in both dose groups. Plasma sampling demonstrated that acamprosate was well absorbed in mice; steady plasma levels indicated that sampling can be conducted at any time of day.

In a 13-week oral (dietary) toxicity study in CD-1 mice (doses of 500, 1000, 1500 and 2000 mg/kg/day; 1x, 2x, 3x, and 4x the MRHD on a BSA basis), findings included