CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-777

PHARMACOLOGY REVIEW

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:	**21-777**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**April 29, 2004**
PRODUCT:	**AMRIX® (Cyclobenzaprine HCl)**
INTENDED CLINICAL POPULATION:	**Patients with muscle spasm associated with musculoskeletal condition**
SPONSOR:	**ECR Pharmaceuticals** **P.O. Box 71600** **Richmond. VA 23255** **Contacts:** **Phone:** **Fax:** **e-mail:**
DOCUMENTS REVIEWED:	**Genotoxicity study reports**
REVIEW DIVISION:	**Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products (HFD-550)**
PHARM/TOX REVIEWER:	**Hamid R. Amouzadeh, Ph.D.**
PHARM/TOX SUPERVISOR:	**Josie Yang, Ph.D.**
DIVISION DIRECTOR:	**Brian Harvey, M.D., Ph.D.**
PROJECT MANAGER:	**Paul Balcer**

Date of review submission to Division File System (DFS): February 7, 2005

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approval

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling:

The sponsor has added the results of mutagenicity studies to the label. The description in the label is acceptable.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Cyclobenzaprine HCl was not mutagenic in *in vitro* bacterial reverse mutation assay, *in vitro* chromosomal aberration assay in CHO cells, and *in vivo* rat micronucleus assay.

B. Pharmacologic activity:

No studies were submitted.

Cyclobenzaprine HCl is a centrally acting muscle relaxant. It reduces tonic somatic motor activities by affecting both gamma and alpha motor neuron through its effects on the central nervous system at brain stem level.

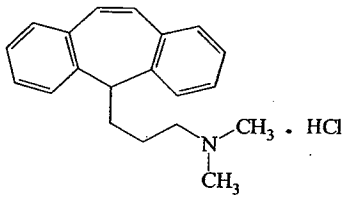
C. Nonclinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA Number:	**21-777**
Review Number:	One
Sequence Number:	N-000
Date and Type of Submission:	April 29, 2004, Commercial
Information to Sponsor:	No
Sponsor and/or Agent:	**ECR Pharmaceuticals** P.O. Box 71600 Richmond, VA 23255 Contacts: [redacted] Phone: [redacted] Fax: [redacted] e-mail: [redacted]
Reviewer's Name:	Hamid R. Amouzadeh, Ph.D.
Division Name:	Division of Anti-Inflammatory, Analgesic and Ophthalmic Drug Products
HFD:	550
Review Completion Date:	February 4, 2005

Drug Substance:

Trade Name:	**AMRIX®**
Non-proprietary Name:	Cyclobenzaprine HCl
Chemical Name:	1-Propanamine, 3-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-hydrochloride
CAS Registry Number:	6202-23-9
Structural Formula:	$C_{20}H_{21}N{\cdot}HCl$
Molecular Weight:	311.85
Manufacturer:	[redacted]
Chemical Structures:	

Relevant INDs/NDAs/DMFs: IND 62,261 and NDA 17-821 (HFD-550)

Drug Class: Centrally acting muscle relaxant

Intended Clinical Population: Patients with muscle spasm associated with musculoskeletal condition

Clinical Formulation: 15- and 30-mg capsules with the following composition:

Ingredient	*Amount (mg)/Capsules*		*Function*
	15-mg	*30-mg*	
Cyclobenzaprine HCl	15.0	30.0	Active substance
Sugar sphere NF, 20-25 mesh			
Opadry® clear YS-7006			
Ethylcellulose NF (ethocel standard 10 premium)			
Diethyl phthalate NF			
gelatin			

Route of Administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data Reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-777 are owned by ECR Pharmaceuticals or are data for which ECR Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 21-777 that ECR Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that ECR Pharmaceuticals does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-777.

Studies Reviewed within this Submission:

Report No.	*Study Title*	*GLP*	*Vol.: Page*
	Genetic Toxicology		
7469-100	*In Vivo* Mouse Micronucleus Assay with Cyclobenzaprine Hydrochloride	Yes	10: 71-95
7469-101	*Salmonella-Escherichia coli* Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay	Yes	10: 96-128
7469-102	Chromosomal Aberration in Chinese Hamster Ovary (CHO) Cells	Yes	10: 129-166

Studies <u>not</u> Reviewed within this Submission: None

2.6.2 PHARMACOLOGY

No studies were submitted.

2.6.2.1 Brief summary

The sponsor provided the following summary in response to Division's recommendation for updated information.

"The pharmacology of cyclobenzaprine HCl has been well established. Studies in animals showed a similarity of effects of cyclobenzaprine HCl and the structurally related tricyclic antidepressants. Cyclobenzaprine HCl reduced or abolished skeletal muscle hyperactivity in several animal models. Animal studies indicated that cyclobenzaprine HCl acts within the CNS, primarily at the brain stem as opposed to spinal cord level, although its action on the latter may contribute to its overall skeletal muscle relaxant activity. Evidence suggests that the net effect of cyclobenzaprine HCl is a reduction of tonic somatic motor activity, influencing both gamma and alpha motor systems."

2.6.2.2 Primary pharmacodynamics

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

No studies were submitted.

2.6.2.5 Pharmacodynamic drug interactions

No studies were submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

None

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

No studies were submitted.

2.6.4.1 Brief summary

The sponsor provided the following summary in response to Division's recommendation for updated information.

"The absorption, distribution, excretion, and metabolism of cyclobenzaprine HCl have been investigated in a variety of species. In studies involving rats, dogs,

rhesus monkeys, and humans, cyclobenzaprine HCl was well absorbed in all species after oral administration. In rats, elimination of the drug occurred primarily in the feces; but urinary excretion was predominant in dogs, monkeys, and humans. Cyclobenzaprine HCl was rapidly and widely distributed into rat tissues with the highest concentrations of drug being found in the small intestine, lung, kidney, and liver. Extensive biliary excretion of the labeled compound was observed in rats. The major metabolites of cyclobenzaprine HCl detected in rats were phenolic derivatives; but in humans, the major metabolites were 10, 11-dihydroxynortriptyline and cyclobenzaprine glucuronide. Only minor amounts of unchanged drug were present in the urine."

2.6.4.2 Methods of Analysis

No studies were submitted.

2.6.4.3 Absorption

No studies were submitted.

2.6.4.4 Distribution

No studies were submitted.

2.6.4.5 Metabolism

No studies were submitted.

2.6.4.6 Excretion

No studies were submitted.

2.6.4.7 Pharmacokinetic drug interactions

No studies were submitted.

Other Pharmacokinetic Studies

No studies were submitted.

2.6.4.9 Discussion and Conclusions

2.6.4.10 Tables and figures to include comparative TK summary

None

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

None

2.6.6 TOXICOLOGY

No studies were submitted except for three genotoxicity studies as recommended by the Division.

2.6.6.1 Overall Toxicology Summary

Genetic toxicology: Cyclobenzaprine HCl was not mutagenic in *in vivo* mouse micronucleus assay, *in vitro* bacterial reverse mutation assay, and *in vitro* chromosomal aberration assay in Chinese hamster ovary (CHO) cells.

2.6.6.2 Single-dose toxicity

No studies were submitted.

2.6.6.3 Repeat-dose toxicity

No studies were submitted.

2.6.6.4 Genetic Toxicology

Study Title: ***In Vivo Mouse Micronucleus Assay with Cyclobenzaprine Hydrochloride***

Key Finding: *Cyclobenzaprine HCl was not clastogenic.*

Report Number: 7469-100
Volume/Page: Vol. 10 pp. 71-95
Conducting Laboratory/Location: [redacted]
Study Period: May 29, 2003 – July 17, 2003
GLP Compliance: Yes
Quality Assurance: Yes
Test substance: Cyclobenzaprine HCl, Lot No. C14607986, [redacted] pure
Methods:
- **Species/Strain/Age/Sex/Weight:** Mouse ([redacted]), ♂ and ♀, 8-9 weeks old, 27-36 g (Both genders were used for dose-range finding and only males were used for micronucleus assay)
- **Route/Dose/Frequency:** Oral, 0, 125, 250, or 500 mg/kg once daily for dose range finding and 31.25, 62.4, or 125 for micronucleus assay)
- **Positive Control:** Cyclophosphamide (CP, ~ 80 mg/kg)
- **Formulation/Vehicle:** Water
- **Observations:** Clinical signs and mortality
- **Sampling Time:** 24 hours at 31.25 and 62.5 mg/kg and 24 and 48 hours at 125 mg/kg

Cell Collection and Slide Preparation: The bone marrow (tibia) from 5 animals was flushed with fetal bovine serum and cells were collected, spread on slides, air-dried, fixed in methanol, stained with May-Grunwald solution followed by Giemsa, mounted, and coded for analysis.

Slide Analysis: "Slides were scored for micronuclei and the PCE to NCE cell ratio. The micronucleus frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micro nucleated PCEs from at least 2000 PCEs per animal. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least the first 500 erythrocytes per animal."

Assay Criteria: "The criteria for the identification of micronuclei were those of Schmid (1976). The unit of scoring was the micronucleated cell. The historical background frequency of rnicronucleated cells was expressed as percentage micro nucleated cells based on the number of PCEs analyzed. The historical background frequency of micronuclei in the [redacted] strain at this laboratory is about 0.0 to 0.4%, which is within the range reported in the published data (Salamone and Mavournin, 1994)."

Assay Acceptance Criteria:

Controls: For vehicle control, less than ~ 0.4% micronucleated PCEs and a group mean within the historical control range was considered acceptable. For positive control, statistically significantly higher ($p \leq 0.01$) number of micronucleated PCEs than the vehicle control and a group mean within the historical control range was considered acceptable.

High Dose: A dose producing signs of toxicity, mortality or reduction in PCE:NCE ratio, and/or a dose reaching the solubility limit.

Assay Evaluation Criteria: Analysis of variance on untransformed data was used when variances were homogeneous and ranked proportions were used when variances were heterogeneous. The Dunnett's t-test was used to determine significance.

Positive Response Criteria: "The criteria for a positive response were the detection of a statistically significant increase in micronucleated PCBs for at least one dose level, and a statistically significant dose-related response. A test article that did not induce both of these responses was considered negative. Statistical significance was not the only determinant of a positive response; the Study Director also considered the biological relevance of the results in the final evaluation."

Dosing Solution Samples: Not collected

Toxicokinetic Samples: Not collected

Results: In the dose-range finding study all of the animals at 250 and 500 mg/kg were found dead or were sacrificed in moribund condition within 24 hours after dosing. Treated animals were recumbent, hypoactive, ataxic, and had shallow or irregular respiration. Based on these clinical signs, doses of 31.25, 62.5, and 125 were chosen for the micronucleus assay. At these doses, animals had flattened posture and were slightly hypoactive. No statistically significant increases in micronucleated PCBs at any dose level and no bone marrow toxicity (i.e., no statistically significant decrease in the PCE:NCE ratios) were observed at any dose level of cyclobenzaprine hydrochloride. Cyclophosphamide induced statistically significant increases in micronucleated PCBs as compared to that of the vehicle controls.

Report's Conclusions: "The test article, cyclobenzaprine hydrochloride, was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of this assay."

Reviewer's Comments: The assay was performed according to an established method. Although plasma levels of cyclobenzaprine hydrochloride were not measured, exposure could be inferred from clinical signs. The reviewer concurs with the findings of the study. Dosing solutions should have been analyzed.

Study Title: ***Salmonella-Escherichia coli Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay***

Key Findings: *Cyclobenzaprine HCl was not mutagenic.*

Report Number: 7469-101
Volume/Page: Vol. 10 pp. 96-128
Conducting Laboratory/Location: [redacted]
Study Period: June 9 – October 7, 2003
GLP Compliance: Yes
Quality Assurance: Yes
Methods:

Strains: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2*uvr*A

Test substance: Cyclobenzaprine HCl, Lot No. C14607986, [redacted]% pure

Concentrations: Dose-Range Finding Assay (TA100 and WP2*uvr*A only): 6.67 – 5000 µg/plate
Initial Assay: 3.33-1000 µg/plate with S9 and 1-1000 µg/plate without S9 for *Salmonella* and 10 - 2000 µg/plate with and without S9 for *E. coli*
Confirmatory Assay: 23.5 -1500 µg/plate with S9 and 11.8 – 750 without S9 for *Salmonella* and 23.5 - 1500 µg/plate with and without S9 for *E. coli*

Assay Performed: Plate incorporation
Incubation: 52 ± 4 hours at 37 ± 2 °C
Vehicle: Water was used for cyclobenzaprine HCl. Vehicle for positive controls was not reported.
Metabolic Activation System: Rat liver S9 (Aroclor 1254-induced, male)
Positive Controls:

Strain	*S9*	*Substance*	*µg/plate*
TA 98	-	2-nitrofluorene	1
	+	Benzo[a]pyrene	2.5
TA 100	-	Sodium azide	2.0
	+	2-aminoanthracene	2.5
TA 1535	-	Sodium azide	2.0
	+	2-aminoanthracene	2.5
TA 1537	-	ICR-191	2.0
	+	2-aminoanthracene	2.5
WP2 *uvr*A	-	4-Nitroquinoline-N-oxide	1.0
	+	2-aminoanthracene	25

Number of Replicates: One for dose range finding and three for initial and definitive assays
Counting Method: Automatic or manual
Dosing Solution Samples: Not analyzed

Evaluation of Results:

Criteria for a Valid Assay: (as described in the report)

Tester Strain Integrity:

1. To demonstrate the presence of the *rfa* wall mutation, *Salmonella typhimurium* tester strain cultures exhibited sensitivity to crystal violet.
2. To demonstrate the presence of the pKM101 plasmid, cultures of tester strains TA98 and TAl00 exhibited resistance to ampicillin.
3. To demonstrate the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), the tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. The acceptable ranges for the mean vehicle controls were as follows:

TA 98	8-60
TA100	60-240
TA1535	4-45
TA1537	2-25
WP2*uvr*A	5-40

Tester Strain Culture Density: To demonstrate that appropriate numbers of bacteria are plated, the density of tester strain cultures was greater than or equal to 0.5×10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 0.5×10^9 bacteria per mL.

Positive Control Values in the Absence of S9 Mix: To demonstrate that the tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.

Positive Control Values in the Presence of S9 Mix (S9 Mix Integrity): To demonstrate that the 89 mix was capable of metabolizing a promutagen to its mutagenic form(s), the mean value of the positive control for a respective tester strain in the presence of the 89 mix exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both the integrity of the S9 mix and the ability of the tester strain to detect a mutagen.

Cytotoxicity: A minimum of three non-toxic doses was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn which was not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Assay Evaluation Criteria: Once the criteria for a valid assay had been met, responses observed in the assay were evaluated.

Tester Strains TA98, TA100, and WP2*uvr*A: For a test article to be considered positive, it had to produce at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Tester Strains TA1535 and TA1537: For a test article to be considered positive, it had to produce at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle

control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Results: (as described in the report) All criteria for a valid study were met.

Dose-Range Finding Study: Cytotoxicity was observed with both tester strains in the presence and absence of S9 mix as evidenced by a dose-related decrease in the number of revertants per plate and a reduction or disappearance of the bacterial background lawn.

Mutagenicity Assay: In the initial mutagenicity assay, all data were acceptable and no positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix. In the confirmatory assay, 2.9-fold increase in the mean number of revertants per plate was observed with tester strain TA98 in the absence of S9 mix at one concentration. However, this increase was not clearly dose-dependent and all observed mean revertant values for TA98 in the absence of S9 mix were within the acceptable vehicle control range. In addition, a similar increase was not observed in the initial assay. For these reasons, this increase was considered spurious and was not evaluated as a positive response.

Report's Conclusions: "The test article, cyclobenzaprine HCl, did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9)."

Reviewer's Comments: The assay was performed based on an accepted method and the acceptance criteria were met. All the requisite strains were used and the genotypic characteristics of them were verified. The sterility of test materials was also verified. 2-Aminoanthracene was used as the positive control in the presence of S9 for all strains except TA98. Use of 2-Aminoanthracene as the only positive control in the presence of S9 is not adequate to demonstrate the metabolic capacity of the S9 preparation. Benzo[a]pyrene was used as the positive control with the TA98 in the presence of S9. Although the metabolic capacity of the S9 batch was not reported, positive findings with benzo[a]pyrene is an indication of the metabolic capability of the S9 batch. All vehicle control and positive control values were within historical control ranges of the conducting laboratory. The reviewer concurs with the findings of the study. Dosing solutions should have been analyzed.

Study Title:	***Chromosomal Aberration in Chinese Hamster Ovary (CHO) Cells***
Key Findings:	*Cyclobenzaprine HCl was not clastogenic.*
Report Number:	7469-102
Volume/Page:	Vol. 10 pp. 129-166
Conducting Laboratory/Location:	[redacted]
Study Period:	May 29 – July 21, 2003
GLP Compliance:	Yes
Quality Assurance:	Yes

Methods:

Cells: Chinese hamster ovary (CHO) cells

Test substance: Cyclobenzaprine HCl, Lot No. C14607986, [redacted] pure

Assay Design:

Assay	*Time*		*Concentration (μg/mL)*	
	Incubation	*Harvest*	*Tested*	*Scored*
Initial				
- S9	~ 3 hrs	~ 20 hr	23.7, 33.9, 48.4, 69.2, 98.9, 141, 202, 288, 412, 500, 840, 1200, 1720, 2450, and 3500	23.7, 33.9, and 48.4
+ S9	~ 3 hrs	~ 20 hr		23.7, 33.9, 48.4, and 69.2
Confirmatory				
- S9	~ 20 hr	~ 20 hr	1.88, 3.75, 7.50, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, and 50.0	20.0, 25.0, 30.0, and 35.0
+ S9	~ 3 hrs	~ 20 hr	20.0, 30.0, 40.0, 50.0, 55.0, 60.0, 65.0, 70.0, and 80.0	40.0, 50.0, 55.0, and 60.0

Vehicle: Water

Negative Control: Culture medium

Positive Controls: Mitomycin C (MMC, 0.75 and 1.5 Φg/mL for 3-hr treatment without S9 and 0.2 and 0.4 Φg/mL for 20-hr treatment) and cyclophosphamide (CP, 7.5 and 12.5 Φg/mL for 3-hr treatment with S9)

Metabolic Activation System: Rat liver S9 (Aroclor 1254-induced)

Replicates: Two

Preparation of cells: After incubation with the test substance, cells were collected, swollen in a hypotonic solution (75 mM KCl), fixed in absolute methanol:glacial acetic acid (3:1, v/v), air-dried, mounted, and stained with 5% Giemsa solution.

Dosing Solution Samples: Not collected

Evaluation of Results: (as described in the report)

Scoring of Cells: Twenty five to one hundred cells from each replicate culture from at least three concentrations were examined for chromosomal aberration (coded). Percent polyploidy and endoreduplication were also determined.

Assay Acceptance Criteria: An assay was considered acceptable for evaluation of test results only if all of the following criteria were satisfied. The metabolic activation and nonactivation sections of the aberrations assay were independent units and would be repeated independently, as needed, to satisfy the acceptance criteria.

Acceptable Controls: The negative (untreated) and vehicle control cultures must contain less than approximately 5% cells with aberrations. The positive control result must be significantly higher ($p \leq 0.01$) than the vehicle controls.

Acceptable High Dose: If the aberration results are negative and there is no significant reduction (approximately ≥ 50%) in mitotic index, the assay must include the highest applicable dose (a target dose of 10 mM or 5 mg/mL, whichever is lower) or a dose exceeding the solubility limit in culture medium.

Testing will be conducted at insoluble dose levels when a well dispersed suspension in culture medium is obtained that does not settle rapidly.

Acceptable Number of Doses: The assay must include at least three analyzable concentrations.

Assay Evaluation Criteria: After completion of microscopic analysis, data were decoded. The following factors are taken into account in evaluation of the test article data:

- The number and percentages of aberrant cells excluding gaps (-g)
- The number and percentages of aberrant cells including gaps (+g)
- Evidence of a dose-response relationship

The experimental unit is the cell, and therefore the percentage of cells with structural aberrations was the basis for evaluation.

Statistical Analysis: Cochran-Armitage test for linear trend and Fisher's Exact Test were used to compare the percentage of cells with aberrations in treated cells to the results obtained for the vehicle controls. Statistical analysis was also performed for cells exhibiting numerical aberrations (polyploidy and endoreduplication) in order to indicate significant ($p \leq 0.01$) increases in these events as indicators of possible induction of numerical aberrations; however, the test articles were evaluated only for structural aberrations and not for numerical aberrations by this protocol.

Evaluation of a Positive Response: The test article was considered positive for inducing chromosomal aberrations if a significant increase (the difference was considered significant when $p \leq 0.01$) in the number of cells with chromosomal aberrations is observed at one or more concentrations. The linear trend test evaluated the dose responsiveness. A dose-response should be observed if a significant increase was seen at one or more concentrations.

Evaluation of a Negative Response: A test article was considered negative for inducing chromosomal aberrations if no significant increase was observed in the number of cells with chromosomal aberrations at any of the concentrations.

Equivocal Evaluation: Although most assays give clearly positive or negative results, in rare cases the data set would preclude making a definitive judgment about the activity of the test article. Results might remain equivocal or questionable regardless of the number of times the assay is repeated.

Results: (as described in the report)

Initial Chromosomal Aberration Assay:

In the assay without metabolic activation, Reductions of 17%, 49%, 58%, and 100% was observed in mitotic indices of the cultures treated with 23.7, 33.9, 48.4, and 1200 µg/mL, respectively, as compared with the vehicle control cultures. Due to excessive toxicity, only dead cells or no cells were present on the slides prepared from the cultures dosed with 69.2, 1720, and 2450 µg/mL and from one of the cultures dosed with 1200 µg/mL. Chromosomal aberrations were analyzed from the cultures treated with of 23.7, 33.9, and 48.4 µg/mL. No significant increase in cells with chromosomal aberrations or polyploidy was observed in the cultures analyzed. A significant increase in endoreduplication was observed in the cultures treated with 33.9 and 48.4 µg/mL.

In the assay with metabolic activation, no Reduction was observed in mitotic indices of the cultures treated with 48.4 and 69.2 µg/mL as compared with the vehicle control cultures. Due to excessive toxicity, only dead cells were present on the slides prepared from the cultures dosed with 1200 and 1720 µg/mL. Chromosomal aberrations were analyzed from the cultures treated with of 23.7, 33.9, 48.4, and 69.2 µg/mL. A significant increase in cells with chromosomal aberrations was observed in the cultures treated with 69.2 µg/mL (a dose with a 71 % reduction in the cell monolayer confluence). No

significant increase in polyploidy was observed in the cultures analyzed. A significant increase in endoreduplication was observed in the cultures treated with 33.9, 48.4, and 69.2 µg/mL.

Confirmatory Chromosomal Aberration Assay: In the assay without metabolic activation, a reduction of 8% was observed in mitotic indices of the cultures treated with 35.0 µg/mL as compared with the vehicle control cultures. Chromosomal aberrations were analyzed from the cultures treated with 20.0, 25.0, 30.0, and 35.0 µg/mL. The cultures treated with 35.0 µg/mL had a 43% reduction in cell monolayer confluence and the next, very closely spread dose of 40.0 µg/mL had excessive cytotoxicity. Thus 35.0 µg/mL was a valid high dose for analysis. No significant increase in cells with chromosomal aberrations or endoreduplication was observed in the cultures analyzed. A significant increase in polyploidy was observed in the cultures treated with 20.0 µg/mL.

In the assay with metabolic activation, no reduction was observed in mitotic indices of the cultures treated with 60.0 µg/mL as compared with the vehicle control cultures. Chromosomal aberrations were analyzed from the cultures treated with of 40.0, 50.0, 55.0, and 60.0 µg/mL. The cultures treated with 60.0 µg/mL had a 29% reduction in cell monolayer confluence and the next dose of 65.0 µg/mL had complete cytotoxicity. Thus 60.0 µg/mL was a valid high dose for analysis. No significant increase in cells with chromosomal aberrations or polyploidy was observed in the cultures analyzed. A significant increase in endoreduplication was observed in the cultures analyzed.

Evaluation of Positive Responses: (as described in the report): The positive response that was observed in the initial assay with metabolic activation was at a single extremely cytotoxic dose level, 69.2 µg/mL. Complete cytotoxicity was observed at 65.0 µg/mL in the confirmatory assay. Thus the slight increase in chromosomal aberrations was at a single dose with severe cytotoxicity with 86 % reduction in cell monolayer confluence. No visual evidence of cytotoxicity was observed at the next dose, 48.4 µg/mL, which had no evidence of clastogenicity. This raises the strong possibility that clastogenicity observed at 69.2 µg/mL is associated with cytotoxicity, possibly due to secondary effects of cytotoxicity. Therefore this observation is not relevant biologically, since this is probably induced indirectly only above a certain threshold concentration (Annstrong et al., 1992; Galloway, et al., 1998; Galloway, 2000, Hilliard et al., 1998; Kirkland and Muller, 2000). This is borne by the negative response seen in the confirmatory assay at a similar dose (60.0 µg/mL). The response observed at a single high dose always raises the possibility of cytotoxicity inducing clastogenicity. The biological relevance of this response is, therefore, very debatable.

In addition, a positive response was observed in endoreduplication in the initial assay without metabolic activation and in the assays with metabolic activation. There is no clear understanding of the mechanism or meaning of the induction of endoreduplication (Sutou and Arai, 1975; Sutou, 1981). Also, there is a lack of a dose response over a wide range of doses. Thus, the significance of e observation of endoreduplication is debatable since the increase observed was probably not due to any potential of the test article to inhibit mitotic processes.

Under nonactivaition conditions, the sensitivity of the cell cultures for induction of chromosomal aberrations is shown by the increased frequency of aberrations in the cells exposed to mitomycin C, the positive control agent. The test article, cyclobenzaprine

hydrochloride, is considered negative for inducing chromosomal aberrations under nonactivation conditions.

The successful activation by the metabolic system is illustrated by the increased incidence of cells with chromosomal aberrations in the cultures induced with cyclophosphamide, the positive control agent. The test article, cyclobenzaprine hydrochloride, was considered negative for inducing chromosomal aberrations under activation conditions.

The results of the assays are summarized in the following table.

Assay	*Treatment Time (hr)*	*Monolayer Confluency (%)*	*Mitotic Index (%)*	*Endoreduplicated cells (n)*	*Polyploid cells (n)*	*Cells with Aberration (%)* - *Gaps*	*Cells with Aberration (%)* + *Gaps*
Initial							
- S9							
Negative Control	3	100	17.3	0.0	0.0	0.0	0.0
Vehicle Control	3	100	18.8	0.0	0.0	0.5	2.0
23.7 µg/mL	3	100	15.6	2.5	0.0	0.0	2.0
33.9 µg/mL	3	100	9.6	**10.0***	0.0	2.0	2.0
48.4 µg/mL	3	86	7.9	**5.5***	0.0	2.5	5.5
+ S9							
Negative Control	3	100	8.8	0.5	0.0	0.0	1.5
Vehicle Control	3	100	9.3	2.0	0.0	0.0	0.5
23.7 µg/mL	3	100	-	4.5	0.0	0.0	0.0
33.9 µg/mL	3	100	-	**8.5***	1.5	0.5	1.5
48.4 µg/mL	3	86	9.7	**14.0***	0.5	**3.5**	**5.5**
69.2 µg/mL	3	29	14.1	**8.5***	0.0	**18.7***	**21.3***
Confirmatory							
- S9							
Negative Control	20	100	15.1	0.0	1.5	0.0	0.0
Vehicle Control	20	100	14.5	0.0	1.5	0.0	3.0
20.0 µg/mL	20	86	-	0.0	**11.0***	1.5	3.5
25.0 µg/mL	20	86	-	0.0	3.0	0.5	**5.0**
30.0 µg/mL	20	57	-	0.0	5.5	0.0	**9.0**
35.0 µg/mL	20	57	13.3	1.0	2.5	1.0	**5.0**
+ S9							
Negative Control	3	100	13.5	0.0	2.5	0.5	1.5
Vehicle Control	3	100	10.7	0.0	1.5	0.0	1.0
40.0 µg/mL	3	100	-	4.0	1.0	0.5	2.0
50.0 µg/mL	3	100	-	**7.5***	6.0	0.5	2.5
55.0 µg/mL	3	86	-	**4.0***	3.5	0.5	**3.5**
60.0 µg/mL	3	71	21.9	**8.0***	3.0	1.0	**5.5**

* Significantly different from vehicle control at $p \leq 0.01$

Dosing Solution Analysis: Not done

Report's Conclusions: "Cyclobenzaprine hydrochloride was considered negative for inducing chromosomal aberrations in CHO cells with and without metabolic activation."

Reviewer's Comments: The assay was performed according to an established method. Cultures were reported to be mycoplasma-free and the stability of the karyotype was verified. Monolayer confluence and mitotic index (compared to vehicle control) were reported as measures of cytotoxicity. Percent of cells with aberration (with and without gap), polyploidy, and endoreduplications were within historical control ranges except for the percent endoreduplication in treated cultures which were significantly higher than the vehicle control. The biological relevance of increased endoreduplication is not known. The dosing solution should have been analyzed and the osmolality of the cultures should have been measured. Because the positive finding occurred at a toxic concentration and it was not duplicated in the confirmatory assay and the results from bacterial reverse mutagenicity assay and mouse micronucleus assay were negative, the positive finding was not considered biologically significant. The reviewer concurs with the report's conclusion.

2.6.6.5 Carcinogenicity

No studies were submitted.

2.6.6.6 Reproductive and developmental toxicology

No studies were submitted.

2.6.6.7 Local tolerance

No studies were submitted.

2.6.6.8 Special toxicology studies

No studies were submitted.

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

None

2.6.7 TOXICOLOGY TABULATED SUMMARY

None

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

This is a 505(b)(2) application. The sponsor's justification for this classification is cited below.

"The formulation of cyclobenzaprine used in AMRIX is novel in that it provides a different release profile compared to immediate release formulations of cyclobenzaprine, including Flexeril. This change in release profile is supportive of a different dosing regimen from the previously approved formulations and thus qualifies for 505(b)(2) classification. The dosing recommendation for AMRIX will be once per day, compared to the three times per day dosing regimen approved for the immediate release products currently available. Five phase I studies of the pharmacokinetics have been conducted by the sponsor and are included in this submission, supporting the plasma profile of a once-a-day product. In addition, the safety and efficacy of AMRIX have been demonstrated in two controlled clinical trials contained in this NDA submission."

During a pre-NDA meeting on April 29, 2003, the sponsor's inquired about the adequacy of a literature search to provide non-clinical information. The Division considered this acceptable and recommended genotoxicity testing if no information on the gentotoxic potential of cyclobenzaprine HCl was found in the literature. The sponsor provided summaries of pharmacology and non-clinical pharmacokinetic information in addition to study reports from three genotoxicity studies. The genotoxicity studies were reviewed in this NDA. Cyclobenzaprine HCl was not genotoxic. The sponsor's description of the genotoxicity findings in the proposed label were considered acceptable.

Unresolved toxicology issues (if any): None

Recommendations: Approvable

Suggested labeling: The sponsor's mutagenicity part of the label is acceptable.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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

/s/

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2/9/05 03:40:45 PM
PHARMACOLOGIST

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