

the number of true stillbirths, and underestimate the number of true postnatal offspring deaths. However, this does not change the fact that there is a dose-related increase in perinatal mortality.

Issues #3a (corpora lutea and preimplantation loss data) and # 3c (apparent discrepancies in data for three F₁ females) refer to an effect of treatment on the reproductive parameters in F₁ animals. The assessment of an effect of treatment on the reproductive parameters of F₁ females should have included assessments of corpora lutea and preimplantation loss for all animals in which mating was confirmed. It appears that neither assessment was conducted. It is not unreasonable to assume that there was no effect of treatment on preimplantation parameters for the F₁ females that delivered litters since there was no effect of treatment on the mean number of implantation sites per group for this subset of F₁ females. For this same subset of F₁ females, there is no clear effect of treatment on reproductive parameters.

There were two F₁ females (low dose female #B73509 and high dose female #B73557) that while noted to be pregnant, had not delivered a litter by gestational Day 26 and were sacrificed (as per protocol). Additional data were requested for these animals (Phase 4) (issue #3c, above). A reevaluation of the study protocol suggests that no additional relevant data exists for these animals. It is not clear why the protocol would not have required that the sponsor conduct an evaluation of the reproductive organs from these two animals. These data would have provided useful information. Without further data, it has to be assumed that these animals had total litter losses (preimplantation loss, early post-implantation loss or a combination of both). Since total litter losses did not occur in any other F₁ females in any group, it does not provide a strong signal for a dose-related finding.

In addition, further review of the data in the original study report indicates that no further data are needed for mid dose F₁ female # B73526 (as requested in issue #3c), in order to adequately label this drug with regard to reproductive toxicities.

Conclusion: Based on a reevaluation of the existing data and consideration of the impact that the requested data would have on the labeling of this drug for its potential for reproductive toxicity, no further data should be requested from the sponsor.

New Data Submitted Unrelated to Approvable or Phase 4 Issues

Although not requested by the Agency, the sponsor submitted reports of the following ADME /PK studies to Amendment #30 (dated 09-February-07).

- Study # CAM/05: Quantitative Whole Body Autoradiography and Excretion Balance Following a Single Oral Administration of [¹⁴C]-Tetrabenazine to Partially Pigmented Rats.
- Study # CAM/08: Quantitative Whole Body Autoradiography and Excretion Balance Following a Single Oral Administration of [¹⁴C]-Tetrabenazine to Male Mice.

5

The two in vivo tissue distribution studies, conducted to support mass balance studies in humans, were submitted after the human study was conducted.

3

The acute-dose tissue distribution study conducted with [¹⁴C]-TBZ in pigmented rat indicated that TBZ and/or its metabolites bind to melanin-containing tissues, suggesting that there could be accumulation in these tissues over time, possibility resulting in toxicity in these tissues after extended use. This was not

adequately evaluated in the chronic toxicity studies in rat and dog. The chronic toxicity study in rat was conducted in an albino strain, and neither ophthalmologic nor microscopic examination of eye was conducted in the chronic toxicity study in dog. Statements regarding this concern will be included in the product labeling.

— / / / / / / /

**APPEARS THIS WAY
ON ORIGINAL**

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

/s/

Andrea Powell
1/9/2008 06:16:45 PM
PHARMACOLOGIST

Lois Freed
1/9/2008 06:19:05 PM
PHARMACOLOGIST
Please see memo for comments.

MEMORANDUM

**DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration**

**Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research**

Date: January 7, 2007

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 21-894 (tetrabenazine, Xenazine[®]), Amendments 0029 (1/31/07; original response to Agency's approvable letter), 0031 (2/16/07), 0033 (4/10/07), 0039 (7/20/07), 0056 (10/19/07)

[Note: dates used in this memo are based on the sponsor's eCTD submission, not the "official application" which was "submitted in paper as stated on the form FDA 356h on September 26, 2005" (cover letter, Amendment 0029, 1/31/07)]

The nonclinical portions of the sponsor's response to the Agency's approvable (AE) letter (dated March 24, 2006) have been reviewed by Andrea M. Powell, Ph.D. (Pharmacology/Toxicology Review and Evaluation, NDA 21-894; draft of January 7, 2008). Based on review of the nonclinical data, Dr. Powell has concluded that "the NDA package...does not meeting the 'usual' standard for approval for a chronic-use drug. " It is Dr. Powell's opinion that the primary nonclinical deficiency is the lack of an adequate chronic toxicology study in rodent, due to "incomplete and unreliable reporting of the clinical signs in the chronic toxicity study in rat..." However, Dr. Powell concludes that the sponsor need not address this deficiency post-approval since it is unlikely that the additional nonclinical data would have any clinical impact. I concur that these data are not needed.

Dr. Powell further notes that the sponsor also has not adequately responded to the following nonclinical deficiencies to be addressed prior to approval, as stated in the March 24, 2006 AE letter:

1. There is a lack of adequate in vivo metabolism data in the animal species used in the definitive nonclinical studies. There is a similar lack of metabolism data in humans. You need to provide additional data identifying and quantitating the major circulating metabolites in animals and humans. These data are needed in order to determine the relevance (and adequacy) of the nonclinical studies to an assessment of human risk. In particular, there is concern that the potential toxicity of the major circulating drug-related material in humans (peak 16) may not have been adequately assessed in animals.

4. The published findings of Satou et al. (Satou T et al. *Exp Toxicol Pathol* 53(4):303-308, 2001) raise a concern that tetrabenazine may have neurotoxic effects. Therefore, it is particularly important to understand how extensively the brain was examined in the 26-week and 9-month oral toxicity studies in rats and dogs, respectively. The reports of these studies do not provide sufficient detail regarding the methodology used in the microscopic examination of brain. You need to document that the microscopic examination of brain in the chronic studies was conducted using techniques sensitive enough to have detected, if present, neuropathological findings similar to those reported by Satou et al (2001).
5. The equivocal finding in females in the in vivo micronucleus assay in rat needs to be further investigated, particularly considering the lack of carcinogenicity data on tetrabenazine. The in vivo micronucleus assay needs to be repeated exploring a range of doses. Although the equivocal finding was only in females, it is difficult to understand why females would be more sensitive than males based on the available plasma exposure data; therefore, we ask that you include both males and females in the repeat assay.

Since, as Dr. Powell notes, the medical team has determined that the clinical data justify approval of tetrabenazine with no additional nonclinical studies, Dr. Powell recommends that deficiencies # 1 and 4 should be addressed post-approval, and that #5 need not be addressed since the issue of genotoxicity can be adequately addressed in labeling without these data. I concur.

In addition to the “pre-approval” issues discussed, the following nonclinical Phase 4 recommendations were included in the March 24, 2006 AE letter:

1. Submission of final study reports for the 26-week p53 transgenic mouse assay and the 2-year carcinogenicity study in rats.
2. Conduct of a fertility and early embryonic development (to implantation) study. You should commit to a timeline for conduct of the study and submission of the final study report.
3. The following apparent discrepancies in the report of the pre- and post-natal development study need to be addressed:
 - a. the lack of corpora lutea and preimplantation loss data in F1 females. These data need to be submitted if collected.
 - b. the number of stillbirths versus early postnatal deaths. You need to specify which pups were determined to be stillborn due only to the lack of milk in the stomach versus those determined to be stillborn by the lack of lung floatation (with or without lack of milk in the stomach); the lack of milk in the stomach alone does not necessarily indicate a stillborn pup. In addition, you need to explain why the summary table (page 39) indicates a dose-related increase in stillbirths, whereas the individual line listings (page 204-207) fail to indicate a stillbirth in any litter.
 - c. apparent discrepancies in the data for individual dams, low-dose female B73509, mid-dose female B73526, and high-dose female B73557. You need to provide all data (including pregnancy, litter, and final disposition) for these dams.

According to Dr. Powell’s review, the sponsor has submitted the results of the 26-week study in p53 transgenic mouse, but has not committed to a timeline for submission of final study reports for the 2-year carcinogenicity or the reproductive toxicology studies in rats. The sponsor has also not addressed issue #3; however, Dr. Powell has concluded

that a response is not needed since accurate labeling can be written without these data. I concur.

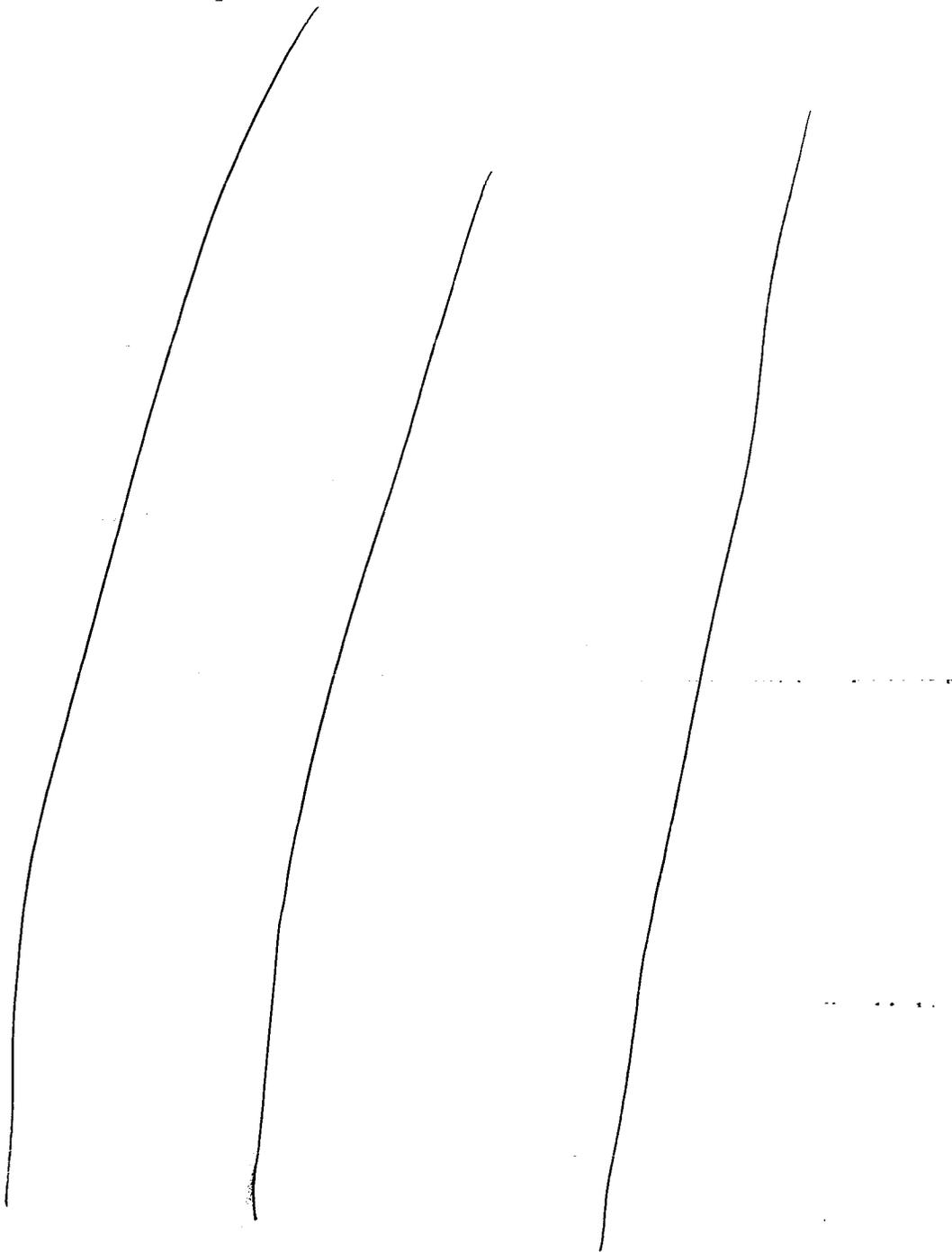
The following wording should be relayed to the sponsor:

1. The carcinogenic potential of tetrabenazine has not been adequately assessed. We acknowledge that you have submitted a final report for a 26-week oral carcinogenicity in P53N5-T heterozygous mice in Amendment 0056 (10/19/07). We also acknowledge that a 2-year carcinogenicity study is ongoing in male rats and a separate 2-year carcinogenicity study in female rats is planned. The 2-year studies may be completed post approval; however, you need to commit to dates for submission of final study reports for these studies.
2. You have not conducted a study of fertility and early embryonic development (to implantation) for tetrabenazine. This study may be conducted post approval; however, you need to commit to a date for submission of the final study report for this study.
3. You have not adequately responded to our request for in vivo metabolism data in the animal species used in the nonclinical studies of tetrabenazine (most importantly, the reproductive toxicology and carcinogenicity studies). We acknowledge that you have submitted a draft report for a study that may provide the necessary data _____ study no. CAM/35; Amendment 0056, 10/19/07); however, submission of a draft report at this stage of development is unacceptable. Although the final report may be submitted post approval, you need to commit to a date for submission of this report.
4. We acknowledge that you have conducted additional histopathology assessments for the 26-week oral toxicity study in rat (Amendment 0031, 2/16/07; Amendment 0031, 4/10/07) and the 9-month oral toxicity study in dog (Amendment 0039, 7/20/07) in order to address concerns regarding the potential for tetrabenazine to produce neurotoxicity, as reported by Satou T et al. *Exp Toxicol Pathol* 53(4):303-308, 2001. Although you report no additional neuropathology findings in either species, the methodology used in these assessment do not appear to have been sufficiently sensitive to rule out potential neurotoxic effects.

Based on our review and further internal discussions, we have concluded that a neurotoxicity study of tetrabenazine using methodology and a multiple dose regimen similar to that used by Satou et al. (2001) would provide the best evaluation. Consideration should be given to including a group in which tetrabenazine is administered i.p. as in Satou et al. (2001) in order to facilitate comparisons between studies. Ideally, tetrabenazine should be tested at several dose levels with the high dose being a maximum tolerated dose.

This study may be conducted post approval; however, you need to commit to a date for submission of the final study report. We would suggest that you submit a study protocol for review prior to initiation of the study.

Recommended labeling as of December 21, 2007 [note: the labeling below has been modified based on further internal discussion and on discussion with the sponsor and, therefore, is not exactly the same as that communicated to the sponsor in the Approvable letter dated 12/26/07]:



2 Page(s) Withheld

 Trade Secret / Confidential

 Draft Labeling

 Deliberative Process

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

/s/

Lois Freed
1/7/2008 05:12:34 PM
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: 21-894
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 9/26/05
PRODUCT: Xenaxine® (tetrabenazine)
INTENDED CLINICAL POPULATION: Huntington's disease
SPONSOR: Prestwick Pharmaceuticals, Inc.
DOCUMENTS REVIEWED: Submissions (arranged by Agency receipt date):

- 9/26/05 (#000) (original submission)
- 12/15/05 (response to request for information)
- 12/19/05 (#004)
- 12/23/05 (#005, #006, #007 and #008)
- 2/21/06 (response to request for information)

REVIEW DIVISION: Division of Neurology Products (HFD-120)
PHARM/TOX REVIEWER: Andrea M. Powell, Ph.D.
PHARM/TOX SUPERVISOR: Lois, M. Freed, Ph.D.
DIVISION DIRECTOR: Russell Katz, MD
PROJECT MANAGER: Teresa Wheelous, R.Ph.

Date of review submission to Division File System (DFS): 3/30/06

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	10
2.6.1 INTRODUCTION AND DRUG HISTORY.....	10
2.6.2 PHARMACOLOGY.....	12
2.6.2.1 Brief summary	12
2.6.2.2 Primary pharmacodynamics.....	13
2.6.2.3 Secondary pharmacodynamics.....	13
2.6.2.4 Safety pharmacology	13
2.6.2.5 Pharmacodynamic drug interactions.....	21
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	21
2.6.4 PHARMACOKINETICS/TOXICOKINETICS	21
2.6.4.1 Brief summary	21
2.6.4.2 Methods of Analysis	21
2.6.4.3 Absorption	21
2.6.4.4 Distribution	22
2.6.4.5 Metabolism	24
2.6.4.6 Excretion.....	28
2.6.4.7 Pharmacokinetic drug interactions.....	28
2.6.4.8 Other Pharmacokinetic Studies	28
2.6.4.9 Discussion and Conclusions	29
2.6.4.10 Tables and figures to include comparative TK summary	30
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	33
2.6.6 TOXICOLOGY	34
2.6.6.1 Overall toxicology summary.....	34
2.6.6.2 Single-dose toxicity	36
2.6.6.3 Repeat-dose toxicity	37
2.6.6.4 Genetic toxicology.....	65
2.6.6.6 Reproductive and developmental toxicology.....	103
2.6.6.7 Local tolerance.....	143
2.6.6.8 Special toxicology studies	143
2.6.6.9 Discussion and Conclusions	144
2.6.6.10 Tables and Figures.....	160
2.6.7 TOXICOLOGY TABULATED SUMMARY	160
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	161
APPENDIX/ATTACHMENTS	167

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From a pharmacology/toxicology standpoint, the current package does not support approval for the following reasons (for a more detailed discussion and delineation, see the general conclusion and recommendation section at the end of the review):

- We cannot ensure that the pivotal nonclinical studies adequately characterize the toxicity of the major drug-related circulating products in humans after oral administration of tetrabenazine (TBZ). The sponsor must provide data that demonstrate that the major drug-related circulating products in humans have been adequately tested in the pivotal nonclinical studies including the pivotal test species/strains, and, when relevant, the metabolic activation systems used in the pivotal *in vitro* studies (e.g., *in vitro* genetic toxicology studies).
- The report of the 26-week toxicity study in rat with a 13-week interim kill (Report # 20730) is inadequate to fully describe the results of the study. A request for further information regarding the conduct and reporting of the clinical observations was sent to the sponsor on 24-Jan-06, and to date, a response has not been submitted. Until the requested information has been submitted and reviewed, there is no assurance that the sponsor has submitted a full and accurate data set for each animal. In addition, the study report did not contain a separate pathology report, and the integrated summary report does not contain the signature of the study pathologist. Therefore, there is no assurance that the discussion of the pathology findings accurately reflects the views of the pathologist. With regard to the pathology assessment, the sponsor should provide (1) a copy of the pathologist's report, (2) a discussion of the parasite infection and implications (if any) on the validity of the study, and (3) establish no-effect dose levels for multifocal accumulations of alveolar macrophages and physiological mammary hyperplasia. The sponsor attributes the treatment-related increase in physiological mammary hyperplasia to a treatment-related increase in serum prolactin or a change in the pattern of prolactin release. The sponsor has not provided data to support this contention. Although serum prolactin was not assessed in the 4-week or 13/26 week toxicity studies, it was supposed to have been evaluated in Study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats). The prolactin data from this study do not appear to have been submitted to the NDA, and should be submitted. There appears to be a notable lack of vigilance in the conduct and reporting of this study that should be further investigated.
- In the study report for the chronic toxicity study in rat a reference was cited describing TBZ-induced neurotoxicity and neuropathology in rat with repeat dosing (Satou *et al.* Repetitive administration of tetrabenazine induces irreversible changes in locomotion and morphology of the substantia nigra in rats, *Exp Toxic Pathol* 2001: 53: 303-308). Based on the findings of Satou *et al.*, the CNS histopathology in the chronic toxicity studies in rat and dog was expanded to include an examination of the pons in rats and the substantia nigra in dog. The extent of the histopathological examination of the brain was not specified, nor the inclusion of any special techniques (if any) in the examination of the brain. Without further information, it is not possible to preclude treatment-induced neuropathology. The sponsor should provide a detailed description of the histopathologic examination of the brains from the chronic toxicity studies in rat and dog, with an emphasis on the techniques used, and the extent of the examination, especially with regard to the substantia nigra.

- The sponsor has proposed to conduct carcinogenicity studies as a phase 4 commitment (discussed further under phase 4 commitments). In order to accept this commitment, the sponsor should have adequately characterized the genotoxic potential of the test compound and the relevant major circulating drug-related compounds (in humans) in the standard genetic toxicology battery. The sponsor has conducted Ames tests and *in vitro* chromosome aberrations assays for TBZ and the stereoisomeric metabolites α - and β -dihydrotrabenazine (α -HTBZ and β -HTBZ) in the presence and absence of metabolic activation (using rat S9). The results of the Ames tests were negative for all three test articles, and the results of the *in vitro* chromosome aberrations tests were reproducibly positive for TBZ (in the presence of metabolic activation) and for both α -HTBZ and β -HTBZ (in the presence and absence of metabolic activation). The sponsor conducted two *in vivo* assessments of chromosome damage in rodent hematopoietic cells with TBZ. In the initial study, an *in vivo* micronucleus assay in rat, TBZ was negative for males and produced equivocal results in females. The second test was *in vivo* micronucleus assay conducted only in male mice. The results of this assay were negative; however, based on information available about the drug at the time of its conduct, the study should have been conducted in both males and females. The sponsor should resolve the equivocal finding in females in the *in vivo* micronucleus assay in rat by conducting an additional assay in female rats, using multiple doses of TBZ including, 100 mg/kg, the dose that produced the equivocal response in the original assay. This should be conducted prior to approval.

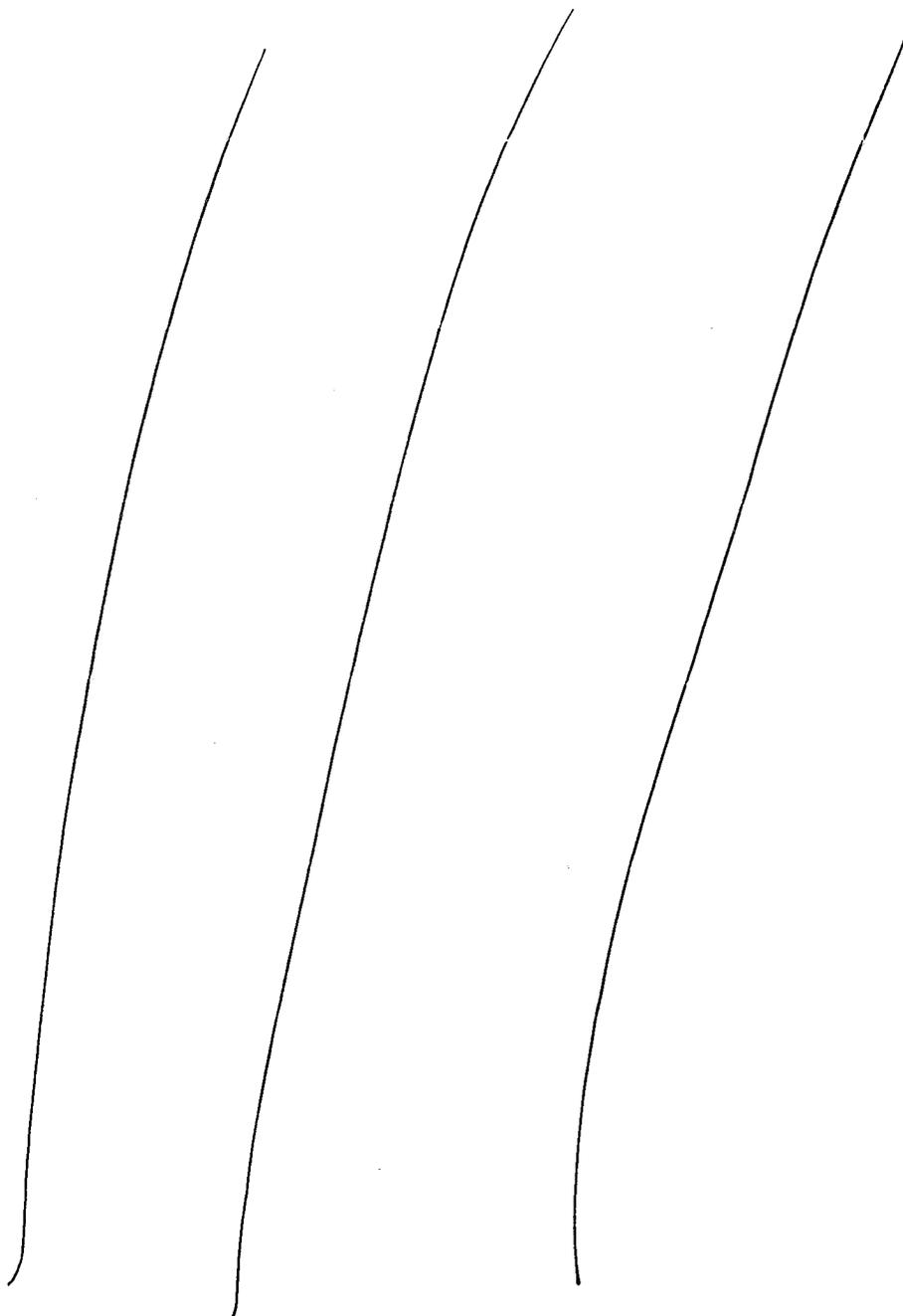
In addition the following issues should be made phase 4 commitments:

- The sponsor has not submitted carcinogenicity assessments as part of the NDA, and "...commits to conduct and report the findings of two rodent carcinogenicity studies upon NDA approval." According to the sponsor, the symptoms of Huntington's disease generally manifest between the ages of 35 and 40 years and death usually occurs after approximately 15 years from the onset of symptoms. The sponsor is seeking approval for chorea associated with Huntington's disease, and not for an alteration of the course of the disease. If TBZ is shown to have a clinically meaningful effect on the lives of Huntington's disease patients, there would be a rationale for deferring carcinogenicity assessment until phase 4; however, the sponsor should commit to starting the carcinogenicity studies at or near the time of approval (i.e., the protocols are completed and the contract laboratories reserved, with an agreement in place for the starting dates for the studies at the time of approval).
- The sponsor should commit to conducting nonclinical assessments of fertility and early embryonic development with agreements in place for a starting date(s) at the time of approval.
- The adequacy of the segment III reproductive toxicity study in rat (Oral Developmental Toxicity Study and Pre- and Postnatal Development Study with Tetrabenazine, in the Rat (Segment III Study), Study # 7425-106) cannot be determined until the discrepancies, delineated in the final recommendation section, are resolved. It should be noted that the study is not a negative study (there was treatment-related perinatal pup death and developmental delays in pups); however, the requested information may provide more accurate labeling. In addition, there appears to be a notable lack of vigilance in the conduct and reporting of this study that should be further investigated.

B. Recommendation for nonclinical studies

The reviewer's recommendations for studies were discussed in context of the deficiencies and the phase 4 commitments mentioned above. Based on review of the requested data, additional studies may need to be conducted.

C. Recommendations on labeling



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

First, it should be stated that we cannot ensure that the pivotal nonclinical studies adequately characterize the toxicity of the major drug-related circulating products in humans after oral administration of tetrabenazine (TBZ). The sponsor must provide data that demonstrate that the major drug-related circulating products in humans have been adequately tested in the pivotal nonclinical studies including the pivotal test species/strains, and, when relevant, the metabolic activation systems used in the pivotal *in vitro* studies (e.g., *in vitro* genetic toxicology studies).

General toxicology:

The sponsor conducted a 26-week toxicity study with a 13-week interim kill in — CD®(SD) IGS BR treated twice daily, via oral gavage, with TBZ at doses of: 0, 2.5, 7.5 and 15 mg/kg, bid (or 0, 5, 15, and 30 mg/kg/day). Additional information is necessary to determine the acceptability of this study to characterize the toxicity of TBZ given chronically to rats. This information includes: (1) a response to the request for information sent to the sponsor on 24-Jan-06, including a full delineation of clinical observations and clarification of the observation schedule, (2) a copy of the pathologist's report, (3) a discussion of the parasite infection and implications (if any) on the validity of the study, (4) a detailed description of the histopathologic examination of the brain, with an emphasis on the techniques used, and the extent of the examination, especially with regard to the substantia nigra, and (5) the results of the serum prolactin assessment conducted in — Study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats). It should be noted that without further information, it can be stated that no-effect doses for treatment related lethargy and aggressiveness were not established in this study and that potentially treatment-related convulsions and deaths occurred at the HD (approximately 2.9 times the maximum recommended daily dose in humans (MRHD) on a mg/m² basis) (although, pending further information, the effect level for convulsions may decrease). No-effect levels (NOELs) were not established for treatment-related physiological hyperplasia of the mammary gland, multifocal accumulations of alveolar macrophages and chronic dermatitis. There was also a treatment-related induction of proestrus based on vaginal pathology in HDF. It should be noted that there appears to be a notable lack of vigilance in the conduct and reporting of this study that should be further investigated.

The sponsor conducted a 9-month toxicity study in beagle dogs (4/sex/group) treated once daily, via oral gelatin capsule, with TBZ at doses of 0, 1, 3, or 10 mg/kg/day. This study is the only chronic toxicity study conducted in non-rodent and it had notable design flaws (ophthalmologic examination, ECG and urinalysis were not conducted, and microscopic examination of the full tissue battery was not conducted [notable exclusions were: eyes/optic nerves, vagina, cervix, lachrymal gland, larynx, pharynx, skin, tongue, nasal cavity and bone marrow smear]). While these inadequacies are notable, they do not invalidate the assay. The major treatment-related toxicity identified in this study was dose-related abnormal clinical observations (including: hypoactivity, tremors, repetitive behaviors, hunched posture, recumbency, rigidity of limbs, sensitivity to touch, ataxia, reddened gums, skin and conjunctiva, abnormal respiration, excessive salivation, squinting eyes, and clear discharge from eyes). The NOEL for abnormal clinical observations was 1 mg/kg/day (LD) which is 0.32 times (on a mg/m² basis) the MRHD. The highest dose tested in the chronic study was 3.2 times (on a mg/m² basis) the MRHD. In the 2-week dose range finding study, a dose of 40 mg/kg/day (administered as 20 mg/kg bid) resulted in the unscheduled sacrifice of all four treated animal after 4 to 11 days of dosing due to treatment related clinical signs in all four dogs and possibly liver/gallbladder toxicity in one dog (based on clinical pathology findings; histopathology was not conducted).

The sponsor has also submitted the reports for a 90-day oral toxicity study in CD-1 mice and the 14-day dose range finding study used for the selection of doses in the 90 study. These studies have not been reviewed as part of the NDA review.

Neurotoxicity: In the study report for the chronic toxicity study in rat, a reference was cited describing TBZ-induced neurotoxicity and neuropathology in rat with repeat dosing (Satou *et al.* 2001. Repetitive administration of tetrabenazine induces irreversible changes in locomotion and morphology of the substantia nigra in rats. *Exp Toxic Pathol* 53: 303-308). In this study male Wistar rats were administered TBZ by intraperitoneal injection (1 mg/kg) either as a single injection or as daily injections for seven consecutive days. The results of the multiple dose portion of the study demonstrated (1) a statistically significant treatment-related neuronal cell loss in the substantia nigra/pars compacta (SNpc), (2) a decrease in SNpc neuron area, and (3) a decrease in cell size. These findings progressed with increasing survival time (estimated [based on graphs] to be up to approximately 50% neuronal cell loss and approximately 30% decrease in area) at 15 days post dose (the last time point studied). An increase in staining for GFAP indicating glial proliferation was also noted in the SNpc. In addition these animals demonstrated a treatment-related decrease in locomotion that was not completely reversible, even after a 15 day recovery period.

Based on the findings of Satou *et al.* (2001), the CNS histopathology in the chronic toxicity studies in rat and dog was expanded to include an examination of the pons in rats and the substantia nigra in dog. The extent of the histopathological examination of the brain was not specified, nor the inclusion of any special techniques (if any) in the examination of the brain. Without further information, it is not possible to preclude treatment-induced neuropathology. It should be noted that the sponsor states that TBZ "has been reported to cause parkinsonism" due to depletion of striatal dopamine. The sponsor also states that in clinical study TBZ 103,004, three (of 54) patients were noted with parkinsonism as a dose-limiting adverse event. The sponsor further states, "In these patients, dose adjustment resulted in maintained efficacy with complete reversal of the AE in 2 patients, and a partial resolution in one patient."

Embryo-fetal development in rabbit: The study was conducted in predated female Hra: (NZW)SPF rabbits treated once daily, via oral gavage, from gestation day (GD) 7-20 with TBZ at doses of 0, 10, 30 or 60 mg/kg/day. The NOEL for maternal toxicity was 10 mg/kg/day (approximately 1.9 times the MRHD on a mg/m² basis), based on treatment-related clinical observations (constricted pupils, squinted/closed eyes, rapid respiration, few or no feces, and recumbency) in MD and HD and changes in body weigh and food consumption seen at the HD. The NOEL for embryo fetal development was 60 mg/kg/day (the highest dose tested) (approximately 11.6 times the MRHD on a mg/m² basis).

Embryo-fetal development in rat: Premated female — CD@ (SD) IGS BR rats were treated once daily, via oral gavage, from gestation day (GD) 6-17 with TBZ doses of 0, 5, 15 or 30 mg/kg. The NOEL for maternal toxicity is 5 mg/kg/day (approximately 0.48 times the MRHD on a mg/m² basis), based on the occurrence of treatment-related clinical signs (hypoactivity and squinted or closed eyes) in MD and HD. The NOEL for embryo-fetal viability is a 15 mg/kg/day (approximately 1.5 times the MRHD on a mg/m² basis), based on an increase in post implantation loss and a slight increase in early resorptions in the HD group.

Prenatal and post natal development: Premated female — CD@ (SD) IGS BR rats were treated once daily, via oral gavage, from gestation day (GD) 6 – lactation day (LD) 20 with TBZ doses of 0, 5, 15 or 30 mg/kg. This study report has several discrepancies in the data that must be resolved before definitive conclusions can be made about the acceptability of the study, and the effects of

treatment of the F₀ generation on the subsequent (F₁) generation and the resulting offspring of that generation (F₂). Without further information, it can be stated that a NOEL for findings in the F₀ dams may not have been achieved, based on an effect on pup retrieval data at the lowest dose tested (despite lack of clinical observation in the dams). It should be noted that it is not possible to ascribe the effect to dam or pup, since it is very difficult to distinguish between dam and pup effects. The NOEL for F₁ perinatal pup survival was 5 mg/kg/day (approximately 0.48 times the MRHD on a mg/m² basis). There were treatment-related delays in the pinna unfolding (HD), hair growth (all doses), eye opening (HD), vaginal opening (all doses) and preputial separation (MD and HD). Therefore, a NOEL for development was not established. Conclusions about an effect of treatment (of the F₀ dams) on the reproductive function in the F₁ generation cannot be made until the sponsor resolves the discrepancies in the data and provides, if available, corpora lutea counts and an evaluation of preimplantation loss for the F₁ females.

Genetic toxicology: The sponsor has conducted Ames tests and *in vitro* chromosome aberrations assays for TBZ, and the stereoisomeric metabolites α -HTBZ and β -HTBZ in the presence and absence of metabolic activation (using rat S9). The results of the Ames tests were negative (for all three test articles) and the results of the *in vitro* chromosome aberrations tests were reproducibly positive for TBZ (in the presence of metabolic activation) and for both α -HTBZ and β -HTBZ (in the presence and absence of metabolic activation). The sponsor conducted two *in vivo* assessments of chromosome damage (using rodent hematopoietic cells) with TBZ. The initial study was an *in vivo* micronucleus assay in rat, and TBZ was negative for males and produced equivocal results in females. The second test was *in vivo* micronucleus assay conducted only in male mice. The results of this assay were negative; however, based on information available about the drug at the time of its conduct, the study should have been conducted in both males and females.

The sponsor should resolve the equivocal finding in females in the *in vivo* micronucleus assay for TBZ in rat by conducting an additional assay in female rats, using multiple doses of TBZ including, 100 mg/kg, the dose that produced the equivocal response in the original assay. This should be conducted prior to approval.

Carcinogenicity assessments and an assessment of fertility and early embryonic development were not conducted.

B. Pharmacologic activity

The exact mechanism(s) of action for tetrabenazine in the treatment of chorea associated with Huntington's disease is unknown. Tetrabenazine reversibly inhibits human vesicular monoamine transporter type 2 (VMAT2) (K_i \approx 100 nM), resulting in decreased uptake of monoamines (such as dopamine, norepinephrine, serotonin and histamine) into synaptic vesicles and thus depletion of monoamine stores. Human VMAT2 is also inhibited by dihydrotetrabenazine (a mixture of α -dihydrotetrabenazine and β -dihydrotetrabenazine), and a major circulating metabolite of tetrabenazine in humans. α -dihydrotetrabenazine demonstrated a 2000 fold greater *in vitro* binding affinity than β -dihydrotetrabenazine to rat VMAT2 (similar studies with do not appear to have been conducted for human VMAT2). Tetrabenazine has demonstrated weak *in vitro* binding affinity at the dopamine D2 receptor (K_i = 2.1 μ M). In addition, the stereoisomeric metabolite, β -HTBZ, exhibits activity at the haloperidol-sensitive sigma receptor (K_i = 128 nM).

C. Nonclinical safety issues relevant to clinical use

The following issues are listed with the caveat that it has not yet been established that the pivotal nonclinical studies adequately characterize the toxicity of the major drug-related

circulating products in humans after oral administration of tetrabenazine (the sponsor must provide data that demonstrate that the major drug-related circulating products in humans have been adequately tested in the pivotal nonclinical studies including the pivotal test species/strains, and, when relevant, the metabolic activation systems used in the pivotal *in vitro* studies):

- Convulsions and death noted in the chronic rat study at 2.9 times MRHD (on a mg/m^2 basis).
- Treatment-related histopathology demonstrated in the chronic toxicity rat study (mammary gland, lung, uterus and skin).
- Clinical observations (such as, hypoactivity, tremors, repetitive behaviors, hunched posture, recumbency, rigidity of limbs, sensitivity to touch, ataxia, reddened gums, skin and conjunctiva, abnormal respiration, excessive salivation, squinting eyes, and clear discharge from eyes) based on the chronic toxicity study in dog.
- Lack of ophthalmologic assessment and ocular histopathology assessment in dog.
- Potential for treatment related neuropathology.
- Reproducibly positive genetic toxicology findings and the lack of carcinogenicity studies.
- Lack of fertility and early embryonic development studies.
- Increase in post-implantation loss, slight increase in early resorptions in the embryofetal development study in rat
- Increased perinatal pup deaths and treatment-related developmental delays in offspring in the pre-and peri-natal development study in rat.

**APPEARS THIS WAY
ON ORIGINAL**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-894

Review number: 1

Sequence number/date/type of submission: #000/correspondence date 23-Sept-05/original submission/and additional relevant subsequent submissions to the NDA through 21-Feb-06.

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: Prestwick Pharmaceuticals, Inc.

Manufacturer for drug substance: _____

Reviewer name: Andrea M. Powell, Ph.D.

Division name: Division of Neurology Products

HFD #: 120

Review completion date: March 30, 2006

Drug:

Trade name: Xenaxine®

Generic name: tetrabenazine

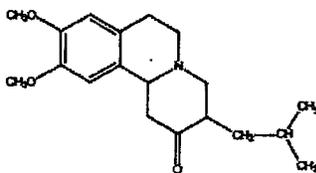
Code name: TBZ

Chemical name: *cis rac* 1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo[a]quinolizin-2-one

CAS registry number: 58-46-8

Molecular formula/molecular weight: C₁₉H₂₇NO₃ MW = 317.4

Structure:



Relevant INDs/NDAs/DMFs: IND 63,909 (sponsor's corresponding IND, opened 06-May-03)

Drug class: benzoquinolizine derivative, hexahydro-dimethoxy-benzoquinolizine derivative, vesicular monoamine transport 2 (VMAT2) inhibitor, monoamine depletor

Intended clinical population: Huntington's disease patients

Clinical formulation: oral tablet at strengths of 12.5 and 25 mg. The tablets contain tetrabenazine and the following excipients: lactose, maize starch, talc, magnesium stearate, and for the 25 mg dose, a coloring agent (iron oxide yellow _____)

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 21-894 are owned by Prestwick Pharmaceuticals, Inc., or are data for which Prestwick Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 21-894 that Prestwick Pharmaceuticals Inc. 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 Prestwick Pharmaceuticals Inc., 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 21-894.

Studies reviewed within this submission:

Pharmacology		
4.2.1.1.2	receptor binding screening profile	original submission
4.2.1.1.3	dihydratetabenazine IC50 at the sigma receptor	original submission
Safety Pharmacology		
4.2.1.3.1	tetabenazine binding to hERG	original submission
4.2.1.3.2	2418/002 – Prestwick rat respiratory system study	original submission
4.2.1.3.3	2418/001 – Prestwick dog cardiovascular system study	original submission
Pharmacokinetics		
4.2.2.3.1	Study # AG-A04001 – Prestwick in vitro protein binding study	original submission
4.2.2.4.4	Study # 1627 – Tetabenazine metabolite investigation. A study of the metabolites in human, dog, rabbit and mouse plasma from studies 1471/1 and 1546	original submission
Toxicology		
4.2.3.2.2.1	7425-114 – 14-day oral gavage study with tetabenazine to assess toxicokinetics and prolactin levels in rats Revised TK report submitted 23-Dec-05	original submission & revised in amendment #0005
4.2.3.2.2.2	Study # 19371 – Cambridge rat 4-week oral repeat dose study	original submission
4.2.3.2.2.3	Study # 20730 – Cambridge rat 26-week oral repeat dose study with 13 week interim kill Revised report submitted 19-Dec-05	original submission & revised report in amendment #0004.
4.2.3.2.3.1	7425-100 – Prestwick dog 15-day oral dose ranging study	original submission
4.2.3.2.3.2	7425-101 – Prestwick dog 9-month oral repeat-dose study	original submission
Genetic toxicology		
4.2.3.3.1.1	study # 19082 – Cambridge tetabenazine testing for mutagenic activity with salmonella typhimurium TA1535, TA1537, TA98 and TA100 and Escherichia coli WP2uvrA	original submission
4.2.3.3.1.2	Study # BCR/001 – α -Dihydratetabenazine bacterial reverse mutation test 29631A	original submission
4.2.3.3.1.3	Study # BCR/003 – β -Dihydratetabenazine bacterial reverse mutation test 29632A	original submission
4.2.3.3.1.4	study # 19406 - Cambridge tetabenazine chromosomal aberration assay with Chinese Hamster ovary cells in vitro	original submission
4.2.3.3.1.5	Study # BCR/002 - α -Dihydratetabenazine in vitro mammalian chromosome aberration test in CHL cells 29631C	original submission
4.2.3.3.1.6	Study # BCR/004 - β -Dihydratetabenazine in vitro mammalian chromosome aberration test in CHL cell 29632C	original submission

4.2.3.3.2.1	—	study # 19434 – tetrabenazine micronucleus test in bone marrow of rats (0h + 24h dosing and 48h sampling)	original submission
-	—	study # 7425-116 – In vivo mouse micronucleus assay	Amendment #0007
Reproductive toxicology			
4.2.3.5.2.1	—	7425-103 – Prestwick rabbit pilot dose-ranging segment 2 (prenatal/embryofetal) toxicology study	original submission
4.2.3.5.2.2	—	7425-104 – Prestwick rabbit segment 2 (prenatal/embryofetal) toxicology study	original submission
4.2.3.5.2.3	—	7425-106II – Prestwick rat segment 2/3 (Prenatal/embryofetal and peri/postnatal) toxicology study	original submission
4.2.3.5.3.1	—	7425-106III – Prestwick rat segment 2/3 (prenatal/embryofetal and perinatal/postnatal) toxicology study	original submission
Carcinogenicity			
-	—	7425-109 – DRAFT 4-week dose range finding study and toxicokinetic study with tetrabenazine in C57BL/6 mice Reviewed under IND 63,909	Amendment #0008

Studies not reviewed within this submission:

Toxicology studies			
4.2.3.2.1.1	—	7425-105 – Prestwick Mouse 14-day oral repeat dose toxicity study	original submission
4.2.3.2.1.2	—	7425-102 – Prestwick mouse 90-day oral repeat-dose toxicity study	original submission
-		Revised TK report # TK7425-102 for 90 day mouse Submitted 23-Dec-05	Amendment #0006
Methods validations for TK			

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

In this section and throughout the review, TBZ will be used as the designation for tetrabenazine. DHTZ will be used as the designation for dihydrotetrabenazine, the unresolved mixture of α -dihydrotetrabenazine (α -HTBZ) and β -dihydrotetrabenazine (β -HTBZ), and a major circulating metabolite of TBZ in animals and humans.

The sponsor did not conduct mechanistic studies to characterize the primary pharmacology of tetrabenazine (TBZ). They have provided literature references in support of the proposed mechanism of action and additional pharmacologic characterization. The sponsor conducted a minimal receptor screening assay for TBZ, α -HTBZ and β -HTBZ, in which the three agents were tested at a single concentration, 1.0×10^{-7} M, for the ability to inhibit binding of standard ligands to a variety of receptors including: neurotransmitters, steroids, ion channels, secondary messengers, prostaglandins, growth factors and hormones, brain and gut peptides, as well as testing in several enzyme systems. Data were presented only as percent inhibition of binding at a concentration of 10^{-7} M. In this system, only one receptor subtype was identified for further investigation, the haloperidol sensitive-sigma receptor (β -HTBZ displaced haloperidol from the sigma non-selective receptor ($IC_{50} = 1.43 \times 10^{-7}$ M and $K_i = 1.28 \times 10^{-7}$ M).

The mechanism(s) of action for tetrabenazine in the treatment of chorea associated with Huntington’s disease is unknown. Tetrabenazine reversibly inhibits human vesicular monoamine transporter type 2 (VMAT2) ($K_i \approx 100$ nm), resulting in decreased uptake of monoamines (such as dopamine, norepinephrine, serotonin and histamine) into synaptic vesicles and thus a reversible depletion of

monoamine stores. Human VMAT2 is also inhibited by dihydrotetrabenazine (a mixture of α -dihydrotetrabenazine and β -dihydrotetrabenazine), and a major circulating metabolite of tetrabenazine in humans. α -dihydrotetrabenazine demonstrated a 2000 fold greater *in vitro* binding affinity than β -dihydrotetrabenazine to rat VMAT2 (similar studies with do not appear to have been conducted for human VMAT2). Tetrabenazine has demonstrated weak *in vitro* binding affinity at the dopamine D2 receptor ($K_i = 2.1 \mu\text{M}$). In addition, the stereoisomeric metabolite, β -HTBZ, exhibits activity at the haloperidol-sensitive sigma receptor ($K_i = 128 \text{ nM}$).

With regard to the secondary pharmacodynamics of TBZ, the sponsor relied mostly on literature citations, which were not reviewed for the NDA. The sponsor did conduct a cardiovascular safety pharmacology study in conscious, instrumented dogs (demonstrating a dose-related increases in heart rate and dp/dt_{max} , and a slight treatment- but not dose-related increase in QT_{cf}), a respiratory safety pharmacology study in rats (demonstrating a treatment-related sustained increase in tidal volume, a transient increase in minute volume and a transient increase in respiration rate that was followed by a sustained decrease) and a hERG binding assay (not the standard hERG I_{K_r} current assay).

2.6.2.2 Primary pharmacodynamics

Mechanism of action: The exact mechanism(s) of action for tetrabenazine in the treatment of chorea associated with Huntington's disease is unknown. Tetrabenazine reversibly inhibits human vesicular monoamine transporter type 2 (VMAT2) ($K_i \approx 100 \text{ nM}$), resulting in decreased uptake of monoamines (such as dopamine, norepinephrine, serotonin and histamine) into synaptic vesicles and thus a reversible depletion of monoamine stores. Human VMAT2 is also inhibited by dihydrotetrabenazine (the unresolved mixture of α -dihydrotetrabenazine and β -dihydrotetrabenazine), and a major circulating metabolite of tetrabenazine in humans. α -dihydrotetrabenazine demonstrated a 2000 fold greater *in vitro* binding affinity than β -dihydrotetrabenazine to rat VMAT2 (similar studies with do not appear to have been conducted for human VMAT2). Tetrabenazine has demonstrated weak *in vitro* binding affinity at the dopamine D2 receptor ($K_i = 2.1 \mu\text{M}$). In addition, the stereoisomeric metabolite, β -HTBZ, exhibits activity at the haloperidol-sensitive sigma receptor ($K_i = 128 \text{ nM}$).

Drug activity related to proposed indication: TBZ, the metabolite HTBZ (the unresolved mixture of α -HTBZ and β -HTBZ), and α -HTBZ bind reversible to VMAT2, resulting in decreased uptake of monoamines into synaptic vesicles and thus a reversible depletion of monoamine stores.

2.6.2.3 Secondary pharmacodynamics

No studies were conducted by the sponsor.

2.6.2.4 Safety pharmacology

Neurological effects: No studies were conducted by the sponsor.

Cardiovascular effects: The sponsor conducted a hERG binding assay and an *in vivo* assessment in conscious instrumented male dog.

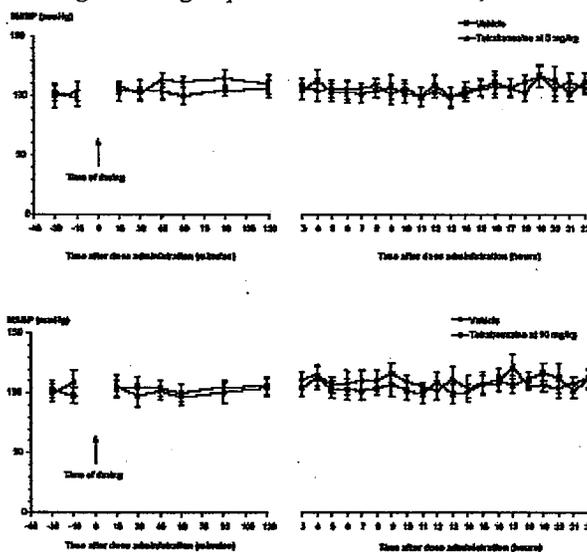
hERG Binding: Potassium Channel I_{K_r} (hERG) Binding Assay was conducted by

_____ This was not a standard hERG assay (measuring I_{K_r} currents), it was a binding assay measuring the displacement of [^3H]Astemizole (3.0 Ci/mmol, 40 nM) from Chinese hamster ovary cells expressing hERG. TBZ, α -HTBZ and β -HTBZ were each tested to a final concentration of 10^{-7} M . At this concentration, none of the test compounds displaced [^3H]Astemizole from its binding site, however, according to the sponsor, terfenidine (100 μM) did (details not provided).

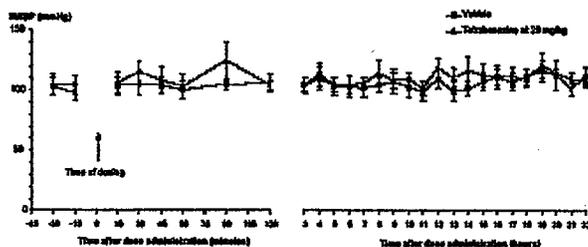
Tetrabenazine: cardiovascular effects in the conscious dog by radiotelemetry — study # 2418/001 (GLP, QAed) conducted at _____ The TBZ used in this study was from batch # 105481, stated purity 99.3%. In this study, four male beagle dogs (2-3 years of age, weighing 11.8 – 15 kg) were dosed by oral capsule with 0 (empty gelatin capsule), 5, 10, or 20 mg/kg, QD of TBZ in a cross over design with at least 7 days between dosing periods. The animals were not naïve dogs; they had been used in other similar studies, with a washout period of approximately 4 wks prior to dosing with TBZ. The animals were instrumented and the following parameters were assessed: blood pressure (systolic, diastolic and mean arterial), heart rate, left ventricular pressure, maximum rate of change in LVP (dp/dt_{max}) ECGs (RR, QRS, PR, QT and QT_{cf} intervals and height of the R-wave and visual inspection), change in activity above baseline. QT_{cf} is QT_c corrected using the Fridericia correction. Parameters were assessed at two intervals 15 minutes apart prior to dosing (nominally, 30 and 15 min prior to dosing), then at time 0, 15, 30, 45, 60 and 90 min post dose and hourly up to 22 hrs post dose.

Clinical signs: Treatment-related clinical observations were confined to one high dose treated animal (#2), which according to the sponsor was noted as “subdued, exhibited staggering gait, tremors and emesis” at approximately 3 h post-dose, showed “some improvement” at approximately 5 hrs post dose and by 12 hrs post dose was only noted with slightly subdued behavior.

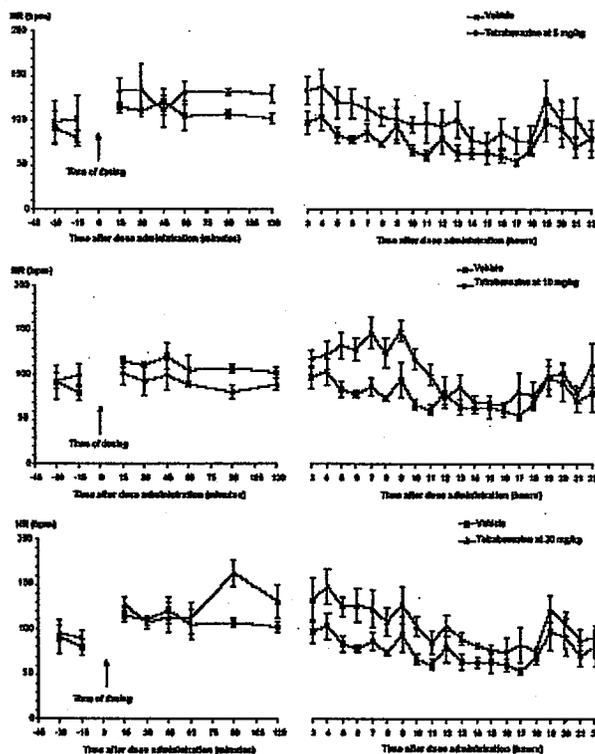
Blood pressure: According to the sponsor, the only treatment-related effect was a small transient increase in the HD (20 mg/kg) at 90 min post dose. The sponsor attributed this to the effects in animal #4 and stated that it was associated with a large increase in motor activity. Examination of the data also reveals a transient increase in MAP for the LD (5 mg/kg) at 45-90 min post dose and for the HD (20 mg/kg) at the later time points of 11-14 hrs post dose. The following sponsor-supplied figures summarize the changes in the group mean MAP for the LD, MD and HD, respectively.



Best Possible Copy

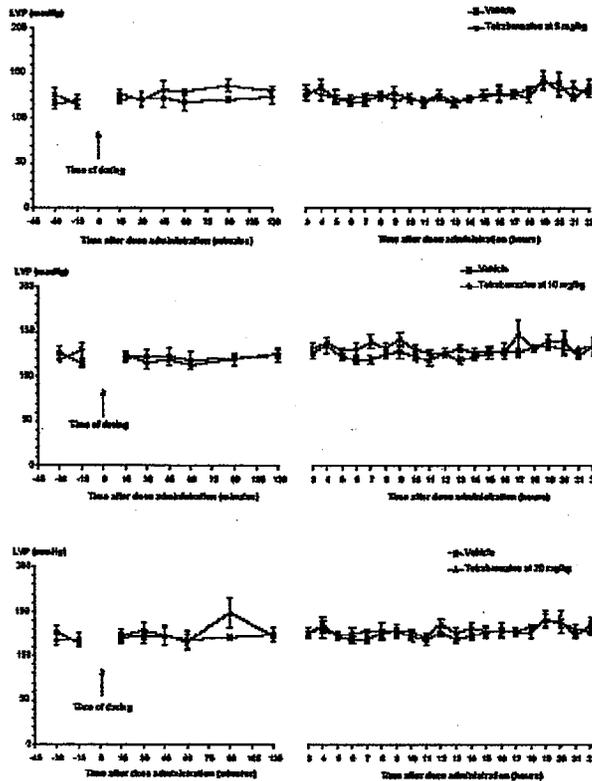


Heart rate: There was a dose-related increase in heart rate starting with a slight increase at the LD. At the mid dose the increase in heart rate had an onset of 3 hrs post dose and increased through 9 hrs post dose and returned to baseline at 11 hrs post dose. In the HD the increased heart rate (described as tachycardia by the sponsor) was noted at 90 min – 4 hrs post dose, and returning to baseline by 10 hrs post dose. According to the sponsor, none of these changes were statistically significant. The following sponsor-supplied figures summarize the changes in the group mean HR for the LD, MD and HD, respectively.



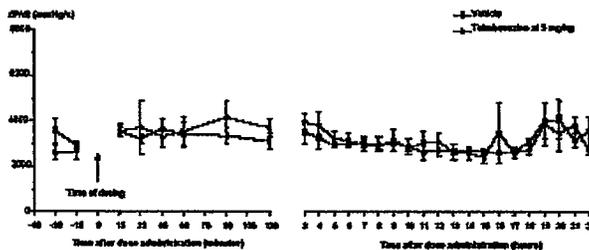
Left ventricular pressure: According to the sponsor, the only treatment-related effect was a small transient increase in the HD (20 mg/kg) at 90 min post dose. The sponsor attributed this to the effects in animal #4 and stated that it was associated with a large increase in motor activity. In addition, examination of the data reveals an increase in the LD at 90 min post dose, in the MD group at 6-7 hrs and 17 hrs post dose. The following sponsor-supplied figures summarize the changes in the group mean LVP for the LD, MD and HD, respectively.

Best Possible Copy

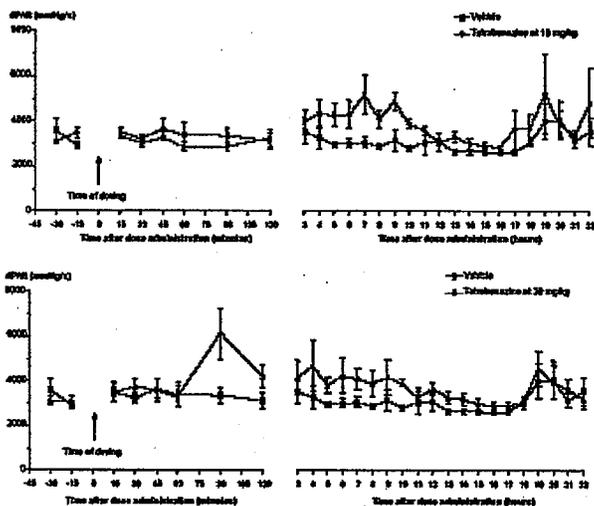


dP/dt_{max} : According to the sponsor, there were no “noticeable change” compared to vehicle in the LD (5 mg/kg). The MD (10 mg/kg) produced an increase (not statistically significant) in dP/dt_{max} (beginning at 4 hrs, peaking at 7 hrs, remaining increased through 9-10 hrs post dose, and returning to near baseline by 11 hrs post dose). The sponsor notes an additional transient increase at 19 and 22 hrs post dose, which they attribute to animals #4 and #1 (associated with increased motor activity in these animals). The HD (20 mg/kg) produced an increase in dP/dt_{max} (peaking at 90 min, remaining increased though not at peak levels through 10 hrs post dose). The sponsor attributes the degree of increase to one animal (#4); however, notes more modest increases in the others. In addition, examination of the data reveals increases in the LD group at 90 min post dose and 16 hrs post dose. The following sponsor-supplied figures summarize the changes in the group mean dP/dt_{max} for the LD, MD and HD, respectively.

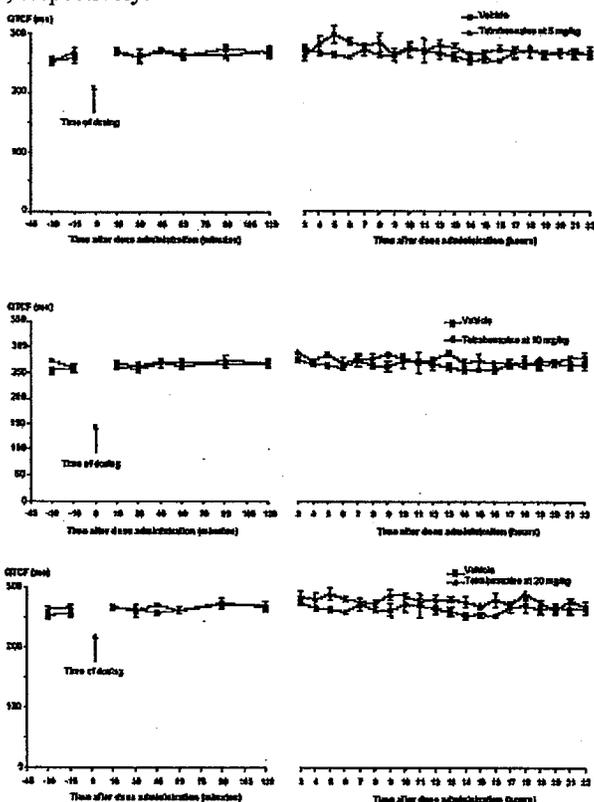
Best Possible Copy



Best Possible Copy



ECG Waveform: According to the sponsor, “Expected changes in the RR interval (corresponding to changes in heart rate) were observed. There were no noticeable changes in the corrected QT interval (QT_{CF}) between pre-dose and post-dose values on any dosing occasion.” Examination of the data suggests a treatment, although not dose-related increase in QT_{CF} when compared to the vehicle control. The following sponsor-supplied figures summarize the changes in the group mean QT_{CF} for the LD, MD and HD, respectively.



Best Possible Copy

Visual Inspection of the ECG wave form: a qualitative examination of the ECG waveform was conducted for each animal at each timepoint that a cardiovascular assessment was determined. According to the sponsor, "No morphological changes – pre or post dose were observed." The report does not state that assessments were made by a veterinary cardiologist.

The sponsor concluded that treatment-related positive chronotropic (increase in hear rate) and inotropic (increase in dP/dt max) effects seen at MD and HD may be due to sympathetic stimulation.

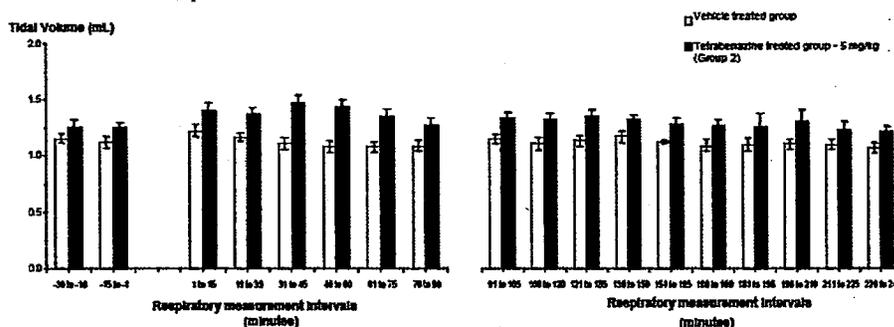
Pulmonary effects:

Effects of tetrabenazine on respiratory parameters in the freely moving conscious rat using whole body plethysmography: (study # 2418/2004) (GLP/QAed) conducted at [redacted] The TBZ used in this study was from batch # 105481, stated purity 99.3%. The dosing formulation was prepared in 0.1% (w/v) Tween-80® and 0.5% carboxymethylcellulose (CMC) (this also served as the vehicle control). The results of the concentration verification analysis demonstrated that all of the TBZ solutions were less than their nominal concentration; LD (64.8 – 68.7%), MD (77.6 – 83.4%), and HD (87.3 – 94.2%, mean = 90.9%).

Male Sprague Dawley (CD (SD) IGS BR) rats (6/group) were dosed by oral gavage (5 ml/kg) at TBZ doses of 0, 5, 15, and 30 mg/kg. At the time of treatment, the animals were approximately 6-7 weeks old and weighed 190-256g. The animals were placed in the plethysmography box for acclimation for 30 minutes prior to two 15 minute intervals of baseline readings. Changes in tidal volume, respiratory rate and minute volume were recorded and averaged over 15 minute intervals for 4 hrs post dose. The data from one animal were not recorded due to signal disturbance; therefore for C, LD and HHD, n= 6 and for the MD n= 5.

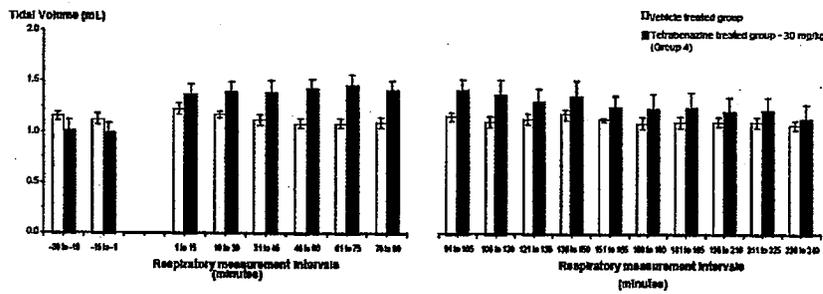
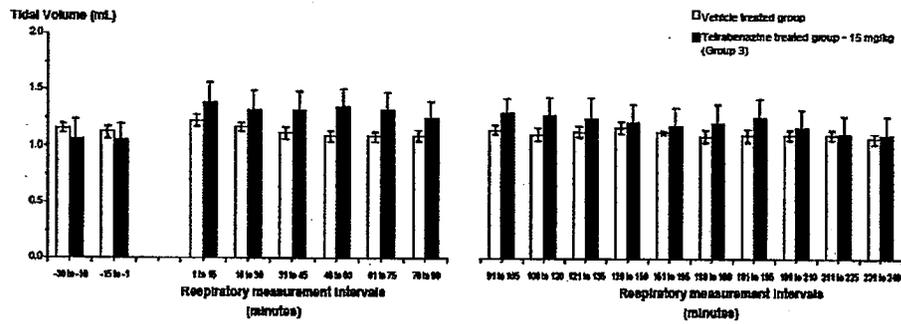
The sponsor notes that in all groups there was a transient small increase in the respiratory parameters during the first 15 minutes post dose, which was considered to be related to the dosing procedure.

Tidal Volume: The following three sponsor-supplied figures depict a treatment-related increase in tidal volume at all doses tested. In the HD it the tidal volume had not returned to baseline at the end of the 4 hr observation period.

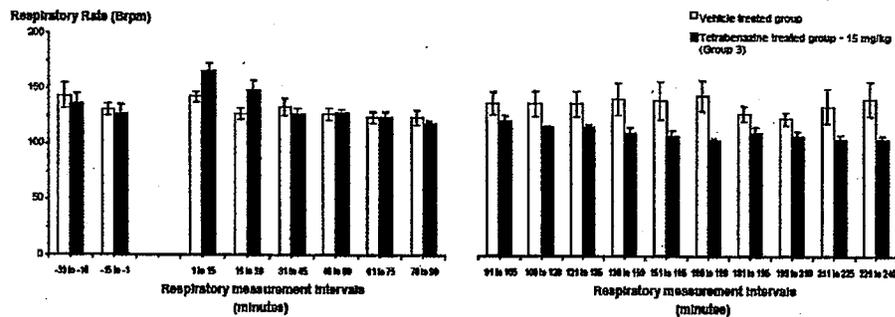
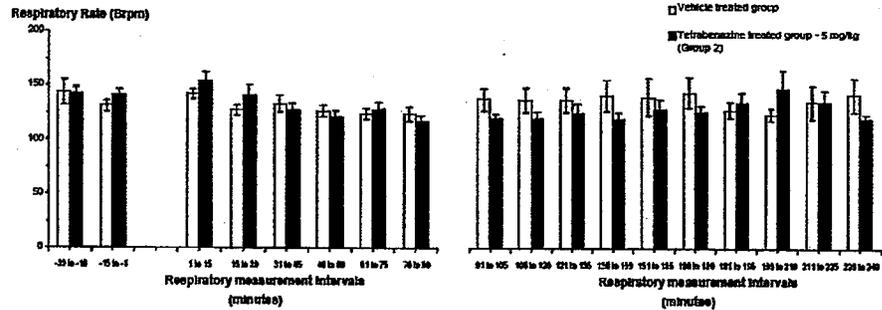


Best Possible Copy

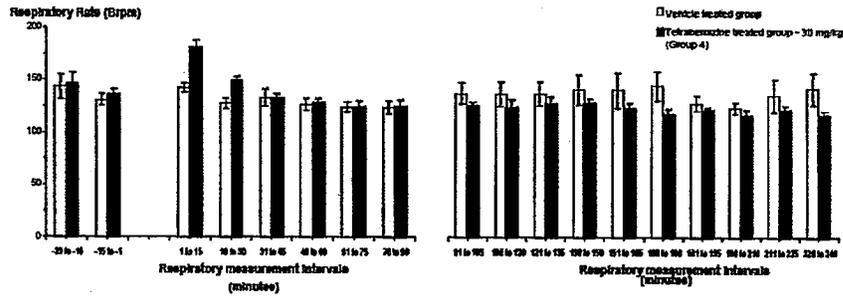
Best Possible Copy



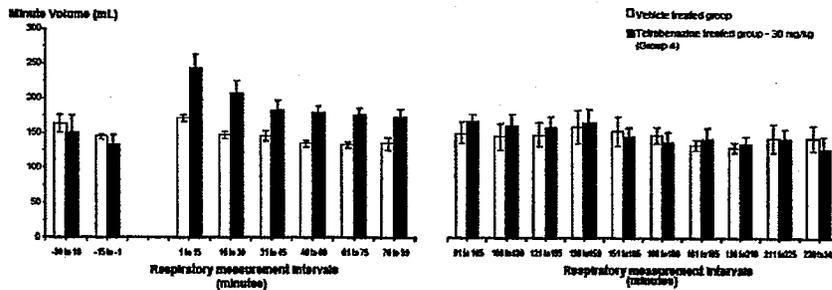
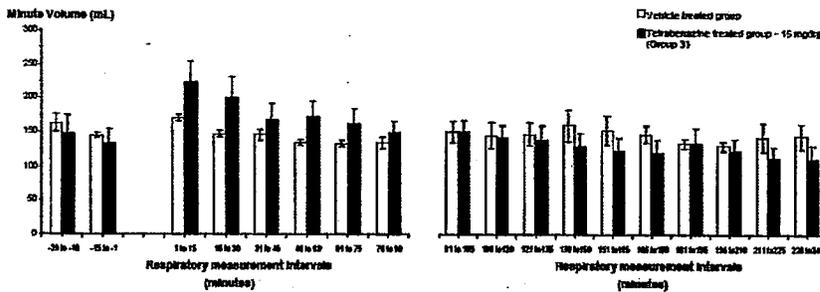
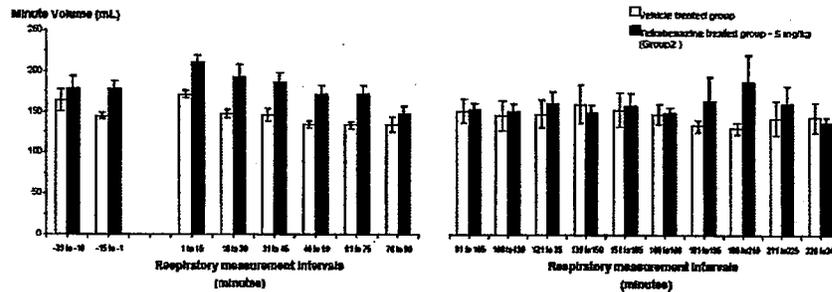
Respiratory rate: The following three sponsor-supplied figures depict a dose-related initial increase in respiratory rate followed by a sustained decrease. The sustained decrease was of greatest magnitude in the MD group. The sustained decreases had not resolved by the end of the 4 hr recording period.



Best Possible Copy



Minute Volume: following three sponsor-supplied figures depict a dose-related initial increase in minute volume which returned to baseline or slightly below (the secondary decrease was greater in the LD and MD than the HD).



The sponsor conducted a statistical analysis of the data and stated that only the increases in tidal volume from the MD and HD are statistically different from control.

Renal effects: No studies were conducted by the sponsor.

Best Possible Copy

Gastrointestinal effects: No studies were conducted by the sponsor.

Abuse liability: No studies were conducted by the sponsor

2.6.2.5 Pharmacodynamic drug interactions

No studies were conducted by the sponsor.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

n/a

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The sponsor did not conduct the standard nonclinical ADME studies. Nonclinical data describing the general tissue distribution and excretion of the TBZ are based on literature references from the 1950 and 1960s. The sponsor has provided more recent references for brain distribution studies for TBZ and/or metabolites in mouse, rat, monkey and human. The data available on the *in vivo* metabolic profiles in humans and animals are inadequate to determine whether the pivotal nonclinical studies adequately characterize the toxicity of the major circulating drug-related components in humans after oral administration.

2.6.4.2 Methods of Analysis

The methods used to quantitate TBZ, α -HTBZ and β -HTBZ were not reviewed.

2.6.4.3 Absorption

The sponsor did not conduct any nonclinical single- or repeat-dose pharmacokinetic studies. The sponsor states that there are no studies characterizing the nonclinical pharmacokinetics after repeat dose administration to animals; however toxicokinetic analyses were conducted for the pivotal repeat-dose toxicity studies.

Mehvar *et al.* (1987b) investigated the PK of TBZ or HTBZ in male SD rats after a single oral dose (0.5, 1.0, 2.5, 5.0 and 10 mg/kg) or i.v. dose (1 mg/kg) of TBZ or a single i.v. dose (1 mg/kg) of HTBZ. (Mehvar, *et al.* 1987b. Pharmacokinetics of tetrabenazine and its major metabolite in man and rat. Drug Metab Dispos 15(2): 250-255). According to the authors, the oral bioavailability (AUC_{oral}/AUC_{iv}) of TBZ was 17%, and based on an estimate of the ratio AUC_{oral} to AUC_{iv} of HTBZ after oral and iv administration of TBZ, the authors determined that the low oral bioavailability of the TBZ was due to presystemic metabolism rather than poor absorption. A copy of the authors figure depicting plasma TBZ and HTBZ levels following oral and i.v. administration of TBZ follows.

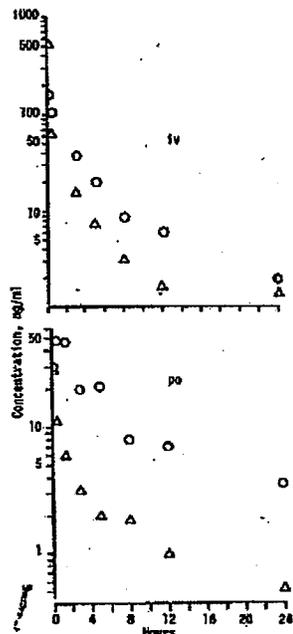


FIG. 3. Individual plasma TBZ (Δ) and HTBZ (\circ) concentration-time curves following iv and po administration of 1 mg/kg doses of TBZ to rats.

2.6.4.4 Distribution

The sponsor did not conduct any tissue distribution studies for TBZ or its metabolites.

General Tissue Distribution: The sponsor refers to Pletcher *et al.* (1962) for a discussion of tissue distribution in animals. (Pletcher *et al.* 1962. Benzoquinolizine derivatives: a new class of monoamine decreasing drugs with psychotropic action. *International Review of Neurobiology* 4: 275-306).

According to the authors:

- “In animals tetrabenazine has a relatively short biological half-life in plasma and organs; it is between 1½ and 3¼ hours in guinea pigs after intraperitoneal injection and is even shorter in rabbits after intravenous administration (Quinn *et al.*, 1959; Schwartz *et al.*, 1960). The compound has special affinity for body fat, where its concentration is up to 40 times higher than in plasma. Studies with tritium labeled tetrabenazine show no preferential affinity for special brain structures in mice (Stumpf *et al.*, 1961). Because of the short half-life time no cumulation of tetrabenazine occurs in the organism. After repeated administration only small amounts of the drug are present in plasma and tissues of rabbit 24 hours after the last dose. Some discrepancies in the concentration of tetrabenazine in the organs as found by various authors may be due to differences in animal species and analytical methods (Quinn *et al.*, 1959; Schwartz and Rieder, 1961). In the urine of rabbits less than 2% of intravenously administered tetrabenazine is excreted unchanged within the first 24 hours (Quinn *et al.*, 1959). These experiments indicate that almost all of the drug undergoes metabolic transformation.”

In addition, Quinn GP, *et al.* (1959) examined tissue distribution of TBZ (Ro1-9569) in male NZW rabbits administered single i.v. doses of TBZ (50 mg/kg over 10 minutes). (Quinn *et al.* 1959. Biochemical and pharmacological studies of RO 1-9569 (tetrabenazine), a non-indole tranquilizing agent with reserpine-like effects. *J Pharmacol Exp Ther* 127: 103-109). The tissue distribution of TBZ is provided in the authors' summary table that is reproduced below. The authors concluded that “the rapid disappearance of the drug from plasma and brain results from its rapid biotransformation rather than extensive localization in tissues.”

TABLE 2

*Ro 1-9569 concentration of rabbit tissues
at various time intervals*

Animals were killed at various times after drug
was administered intravenously (50 mg/kg).

Tissue	Concentration of Ro 1-9569 (µg/g)				
	10 min	20 min	60 min	6 hrs	24 hrs
Plasma	20	9.7	3.6	0.8	0.0
Brain	45	29	17	1.3	0.0
Heart	74	24	7.7	2.0	0.0
Small intestine	53	35	22	4.8	1.5
Liver	11	4.6	2.9	0.8	0.0
Kidney	76	39	24	4.9	0.0
Lung	57	50	22	10	1.5
Muscle	40	27	7.0	1.4	0.0
Spleen	46	32	7.6	—	0.0
Fat (perirenal)	11	38	70	14	2.2

Brain distribution

- DaSilva and Kibourn (1988) have shown that in female CD-1 mice, intravenously administered [¹¹C]-tetrabenazine was rapidly taken up into the brain and the rank order of radiolabel uptake at 10 min post dose was “striatum > hypothalamus > hippocampus > cortex = cerebellum”. (DaSilva JN and Kibourn MR. 1992. *In vivo* binding of [¹¹C]tetrabenazine to vesicular monoamine transporters in mouse brain. *Life Sci* 51(8): 593-600). The authors state that washout of the radiolabel was slowest from the striatum and the hypothalamus; however, in general only 0.21% of the dose was present in the brain at 60 min post dose.
- DaSilva *et al.* (1993) have shown that in female pigtail monkeys, intravenously administered [¹¹C]-tetrabenazine demonstrates rapid uptake and clearance from the brain. (DaSilva *et al.* 1993. *In vivo* imaging of monoaminergic nerve terminals in normal and MPTP-lesioned primate brain using positron emission tomography (PET) and [¹¹C]tetrabenazine. *Synapse* 14(2): 128-131).
- Mehvar and Jamali (1987a) have shown that in male SD rat, TBZ and HTBZ cross the blood brain barrier when administered by the i.p. route, and that the time course for TBZ and HTBZ concentrations in the brain parallel those in serum. (Mehvar R and Jamali F. 1987a. Concentration-effect relationships of tetrabenazine and dihydrotetrabenazine in the rat. *J Pharm Sci* 76(6): 461-465).
- According to the sponsor, PET scan studies conducted in humans using [¹¹C]-tetrabenazine or [¹¹C]- α -HTBZ “confirm that both compounds cross the blood-brain barrier and preferably label the striatum.”

Protein binding: In study # AG-A04001, the sponsor assessed the *in vitro* plasma protein binding for TBZ, α -HTBZ and β -HTBZ in plasma samples from human, Beagle dog, rabbit (strain not specified), rat (strain not specified), and mouse (strain not specified) using equilibrium dialysis. Within each species plasma was from a mixed gender pool. The results are summarized in the following reviewer-generated summary table (the sponsor-supplied summary table from the integrated summary was incorrect). In all species but rat, TBZ, α -HTBZ and β -HTBZ were not highly plasma protein bound. In rat, the plasma protein binding of α -HTBZ and β -HTBZ increases with increasing concentration to a mean of 97% bound.

Species	Nominal Conc (ng/ml)	% bound (mean)
Human	TBZ: 50, 100, 200	82, 84, 85
	α -HTBZ: 50, 100, 200	68, 60, 64
	β -HTBZ: 50, 100, 200	61, 59, 63
Beagle dog	TBZ: 50, 100, 200	69, 66, 72
	α -HTBZ: 50, 100, 200	63, 51, 47
	β -HTBZ: 50, 100, 200	73, 71, 72
Rabbit (strain not specified)	TBZ: 50, 100, 200	78, 79, 79
	α -HTBZ: 50, 100, 200	34, 41, 54
	β -HTBZ: 50, 100, 200	14, 36, 33
Rat (strain not specified)	TBZ: 50, 100, 200	85, 85, 82
	α -HTBZ: 50, 100, 200	73, 74, 97
	β -HTBZ: 50, 100, 200	67, 64, 97
Mouse (strain not specified)	TBZ: 50, 100, 200	82, 80, 85
	α -HTBZ: 50, 100, 200	50, 45, 49
	β -HTBZ: 50, 100, 200	30, 44, 48
<p>"Results from buffer spiked with each test compound and dialyzed against blank buffer for 24 hours indicate that equilibrium was attained for α- and β-dihydrotrabenazine, but not for tetrabenazine. Consequently the values reported for the % bound of tetrabenazine to plasma proteins may be slightly overestimated."</p>		

2.6.4.5 Metabolism

In 1966 Schwartz *et al.* published a putative metabolic pathway for TBZ based the metabolic analysis of urine from animals (rabbit [male, strain not specified] and dog [female, strain not specified]), each treated with a single i.p. injection of TBZ, and human (sex not specified) treated with a single s.c. injection of TBZ. (Schwartz *et al.* 1966. Metabolic studies of tetrabenazine, a psychotropic drug in animals and man. *Biochem Pharmacol* 15(5), 645-655). The authors reported nine components in the urine, five unconjugated and four conjugated with glucuronic acid. In all species, the glucuronide conjugates were the most prevalent urinary component.

The sponsor conducted a limited investigation of the *in vivo* metabolic profiles in animals and humans (Study # — 1627, Tetrabenazine Metabolite Investigation — a Study of the Metabolites in Human, Dog, Rabbit and Mouse Plasma from Studies — 1471/1 and — 15460). The sponsor used a technique that involves a precursor scanning for metabolite detection. According to the sponsor, "Precursor scanning for metabolite detection is more specific and sensitive than scanning over an entire mass range." Plasma samples were obtained from humans and animals as stated in the table below:

**APPEARS THIS WAY
ON ORIGINAL**

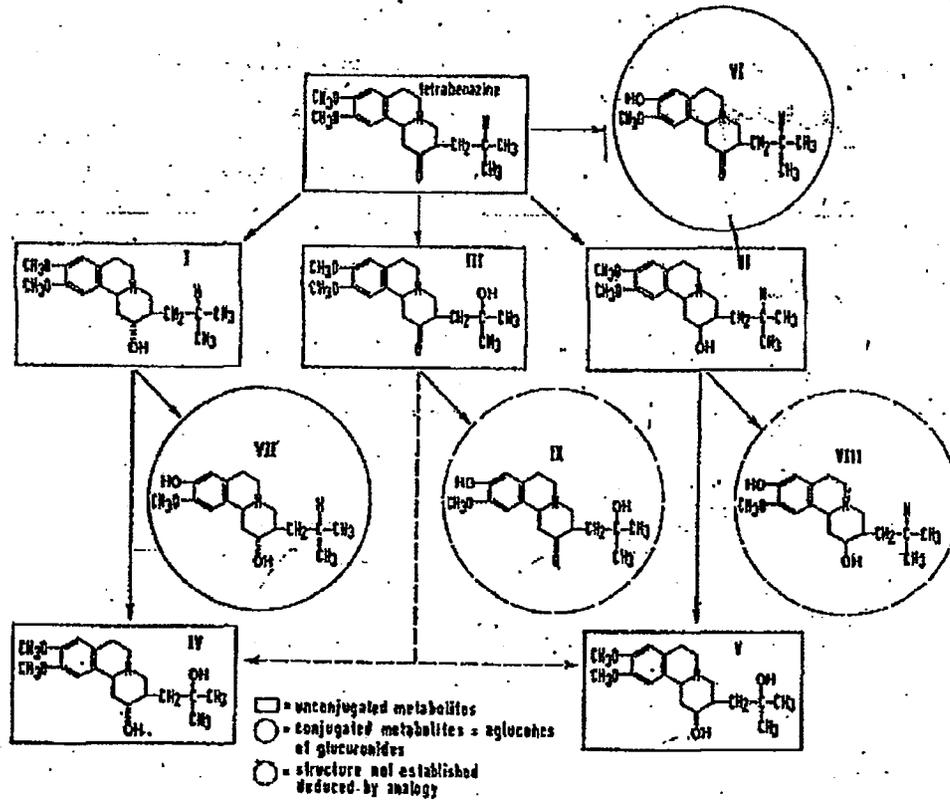
Plasma sampling for the <i>in vivo</i> metabolic profile			
Species	Study	Dose	Condition
Human	normal volunteers (#TBZ 103,003)	25 mg tablet, n=3	(Day 1, predose and 2, 4, 6, 8 and 16 hrs post dose)
	H. D. patients (# TBZ103,004)	100 mg/day, n = 2	Wk 12, time of sampling relative to dose not stated
dog (beagle)	9-month oral toxicity study (#7425-101)	1 mg/kg/day, n= 1 (#637) 10 mg/kg/day, n=2 (#643, 662)	Day 1, predose and 1, 2, 4 and 12 hrs post dose
Rabbit (Hra: (NZW) SPF)	Oral gavage DRF study for developmental toxicity study (#7425-103)	2.5 mg/kg/day, n=1 (#F61112) 15 mg/kg/day, n=2, (n=2, # F61119 & # F61122)	Day 7, predose and 0.25, 0.5, 2 and 4 hrs post dose
Mouse CD-1@(ICR)BR)	90 Day oral gavage toxicity study (#7425-102)	30 mg/kg/day, (n=1/time point)	Day 1 predose (#A74200 6M) 0.25 hr (#A74203 6M) 0.5 hr (#A7431 6F) 1 hr (#A74209 6M) 2 hrs (#A74437 6F) 4 hrs (#A74215 6M) 6 hrs (#A74218 6M)
Rat	not evaluated	not evaluated	-

The sponsor stated that "Using this technique several peaks were detected in the chromatograms of plasma extracts that have the potential to be metabolites of tetrabenazine." The sponsor presented a putative assignment of metabolites based on the metabolic scheme established by Schwartz *et al.* (1966) and the data are presented in the following sponsor provided table in terms of relative abundance of each putative metabolite in plasma. The metabolic pathway proposed by Schwartz follows the table. In this scheme, metabolites I and II appear to be the stereoisomeric metabolites, α -dihydrotetrabenazine (α -HTBZ) and β -dihydrotetrabenazine (β -HTBZ). Metabolites VII and VIII appear to be formed from the subsequent metabolism of α -HTBZ and β -HTBZ. Note that it appears that CD1@(ICR)BR mice do not have circulating levels of α -HTBZ and β -HTBZ or the subsequent metabolites.

Schwartz Assignment*	Molecular Formula	Metabolite	Theoretical Transition	Relative abundance			
				HUMAN	DOG	RABBIT	MOUSE
I & II	C19H29NO3	HYDROGENATION (2H-TBZ)	320→185/220	14% / 100%	100% / 7%	100% / 84%	
III	C19H27N04	OXIDATION	334→185/220	1%	7%	20%	
IV & V	C19H29N04	OXIDATION & HYDROGENATION	338→185/220	9%	19%		
VI	C18H25N03	DEMETHYLATION	304→151/206	1%	2%		43%
VII & VIII	C18H27N03	DEMETHYLATION & HYDROGENATION	306→151/206	15%	9%	24%	
IX	C18H25N04	DEMETHYLATION & OXIDATION	320→151/206	4% / 8%	2% / 4%		100% / 11%

APPEARS THIS WAY
ON ORIGINAL

Metabolic Pathway proposed by Schwartz *et al.*



No further evaluation of metabolism in animals has been conducted.

In response to an Agency request, the sponsor recently conducted and *in vivo* mass balance study in normal healthy male volunteers administered a single 25 mg dose of ¹⁴C-TBZ (study # RD204/24124) and the following comments on the pharmacokinetics and metabolism of TBZ in humans is based on the Clinical Pharmacology and Biopharmaceutics Review of NDA 21-8949 by Dr. Sally Usdin Yasuda, dated 06-Mar-06.

The results of this study (submitted to the NDA on 09-Dec-05) demonstrate that in humans TBZ is well absorbed after oral administration, extensively metabolized (with at least 19 metabolites identified), and the metabolites are excreted predominantly by renal elimination. Unchanged TBZ is not found in the urine and does not circulate to a significant extent (concentration of approximately 10 ng/ml). The mass balance study identified four major circulating components (defined here as circulating at levels greater than 15% of total circulating radioactivity); (1 & 2) the sulfate conjugates of O-dealkylated HTBZ (components P11 and P13), (3) α-HTBZ (component P18) and, (4) the most abundant component (P16) that has yet to be resolved (see the table below taken from Dr. Yasuda's review for details). In hepatic impairment, the exposure to TBZ increases greater than 70 fold over normal healthy volunteers and the half-lives of α-HTBZ and β-HTBZ increase.

Best Possible Copy

Component	0.25 - 1.5 hours	2 - 8 hours	4 - 8 hours	Mean
P1	ND	ND	ND	NC
P2	ND	ND	ND	NC
P3	ND	ND	ND	NC
P4	ND	ND	ND	NC
P5	ND	ND	ND	NC
P6	ND	ND	4.70 (4.88)	NC
P7	ND	ND	ND	NC
P8	ND	ND	8.23 (8.44)	NC
P9	ND	ND	ND	NC
P10	ND	ND	ND	NC
P11	28.44 (18.87)	28.52 (20.41)	15.23 (18.80)	22.73 (17.88)
P12	ND	ND	ND	NC
P13	28.73 (18.04)	41.77 (28.68)	24.22 (28.88)	30.91 (24.82)
P14	ND	ND	ND	NC
P15	ND	ND	ND	NC
P16	92.54 (25.24)	50.11 (24.66)	19.77 (21.85)	30.81 (28.81)
P17	13.86 (9.71)	6.54 (4.52)	4.48 (4.37)	8.22 (8.40)
P18	25.73 (18.30)	18.70 (14.55)	14.08 (15.80)	18.83 (15.15)
Other	1.52 (1.88)	ND	ND	NC

Results expressed as ng equivalents (Values in parentheses indicate % sample radioactivity)
 ND Component not detected NC Not calculable

These components represent:

P1-9: mono-hydroxy-HTBZ and glucuronides of O-dealkylated HTBZ

P10: monohydroxy HTBZ

P11 and P13: sulfate conjugates of O-dealkylated HTBZ

P15 and P17: O-dealkylated HTBZ and/or β-HTBZ (tentative)

P18: α-HTBZ

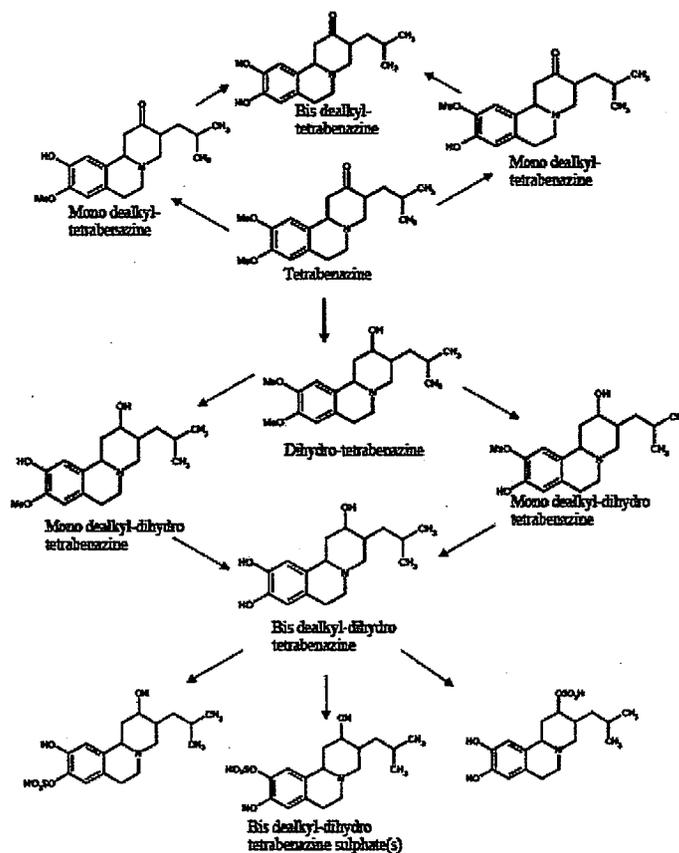
P16, the largest component of the plasma, was not identified.

Best Possible Copy

On 26-Jan-06 Dr. Yasuda requested that the sponsor "... reconcile the data from the original "mass balance study," the "Schwartz assignments," and the recent mass balance to account for the metabolites and the metabolic scheme." The sponsor has recently submitted a response on 21-Feb-06, which has not yet been reviewed.

Dr. Yasuda presented the following metabolic scheme in her NDA review (page 18).

**APPEARS THIS WAY
ON ORIGINAL**



Further evaluation and comparison across species of the *in vivo* metabolic profile for orally administered TBZ will be deferred until the review of the 21-Feb-06 submission and further elucidated the metabolic profile in humans.

2.6.4.6 Excretion

The sponsor has not conducted any nonclinical studies assessing the excretion of TBZ or its metabolites into urine, bile, feces or milk. The only literature reference that the sponsor cites regarding this topic is Schwartz *et al.*, which was discussed in the metabolism section above. The recently conducted mass balance study in normal human volunteers indicates that after oral administration of radiolabeled TBZ, approximately 75% of radiolabel is excreted in the urine, as metabolites. Unchanged parent is not detected in the urine.

2.6.4.7 Pharmacokinetic drug interactions

n/a

2.6.4.8 Other Pharmacokinetic Studies

No studies were conducted by the sponsor.

2.6.4.9 Discussion and Conclusions

The nonclinical assessment of PK and ADME provided in this NDA was minimal; however, toxicokinetic assessments were conducted in the repeat-dose toxicity studies. The sponsor did conduct an assessment of plasma protein binding in humans, beagle dog, and unspecified strains of rat, mouse and rabbit. In all species but rat, TBZ, α -HTBZ and β -HTBZ were not highly plasma protein bound. In rat, the plasma protein binding of α -HTBZ and β -HTBZ increases with increasing concentration to a mean of 97% bound.

Other information for pharmacokinetics, tissue distribution and excretion were based on literature references. There is very limited information available on tissue distribution or excretion of TBZ or its metabolites. The sponsor has cited literature references that demonstrate that TBZ and/or its metabolites cross the blood brain barrier and are taken up by the brain in mice, monkeys, rats and humans. Oral bioavailability was assessed in rats and found to be 17%, and the low oral bioavailability (of a 1 mg/kg dose) was demonstrated to be due to metabolism rather than poor absorption.

The characterization of the *in vivo* metabolic profile in animals has been very limited. The sponsor conducted Study # — 1627, Tetrabenazine Metabolite Investigation – a Study of the Metabolites in Human, Dog, Rabbit and Mouse Plasma from Studies — 1471/1 and — 1546. In this study the sponsor used a technique that involves a precursor scanning for metabolite detection. According to the sponsor, “Precursor scanning for metabolite detection is more specific and sensitive than scanning over an entire mass range.” Plasma samples obtained from the following species were assessed: (1) mice [from the 90-day oral gavage toxicity study in — :CD-1@(ICR)BR mice], (2) dog [from the 9-month oral toxicity study in beagle dogs], (3) rabbit [from the oral gavage DRF study in Hra: (NZW) SPF rabbits], (4) humans [normal volunteers and Huntington disease patients]. It should be noted that plasma from rats has not been assessed. The sponsor stated that “Using this technique several peaks were detected in the chromatograms of plasma extracts that have the potential to be metabolites of tetrabenazine.” The sponsor presented a putative assignment of metabolites based on the metabolic scheme established by Schwartz *et al.* (1966). Schwartz *et al.* had established a urinary metabolic profile based on analysis of urine after intraperitoneal administration of TBZ to rabbit (male, strain not specified) and dog (female, strain not specified), and by the subcutaneous administration to human (sex not specified). Schwartz identified nine urinary components (five unconjugated, and four conjugated with glucuronic acid), with the glucuronide conjugates noted as the prevalent components in all species.

The clinical and nonclinical development of TBZ was based on the belief that the two major circulating metabolites in humans after oral administration of TBZ were α -dihydro-tetrabenazine (α -HTBZ) and β -dihydro-tetrabenazine (β -HTBZ). Therefore, the TK assessments in the pivotal nonclinical toxicology studies were based on monitoring plasma levels of TBZ and the stereoisomeric metabolites α -HTBZ and β -HTBZ (measured in chiral assays), or dihydro-tetrabenazine (HTBZ) (measured in a non-chiral assay in the earlier studies). The sponsor recently conducted an *in vivo* mass balance study in normal healthy male volunteers. The results of this study demonstrated that in humans TBZ is well absorbed after oral administration, extensively metabolized, and the metabolites are excreted predominantly by renal elimination. Unchanged TBZ is not found in the urine, and does not circulate to a significant extent. In this study four major circulating components were identified (defined here as circulating at levels greater than 15% of total circulating radioactivity). The most abundant component (P16) has yet to be resolved.

Conclusion: The *in vivo* metabolic profile of orally administered TBZ in animals and humans has not been adequately characterized, especially with regard to the unresolved component P16, which is the major circulating component in humans. Characterization of *in vivo* the metabolic profile in humans is necessary to ensure that the pivotal nonclinical studies adequately characterize the toxicity of TBZ and the major circulating human metabolites.

2.6.4.10 Tables and figures to include comparative TK summary

TK from the 15-Day Capsule Dosing Toxicity Study in Dogs, study # 7425-100: The following two reviewer-generated tables summarize the TK from this study. There is no separate review for this non-GLP toxicity study (conducted in 2/sex/groups); however, the findings are discussed as part of the evaluation of the chronic toxicity study in dogs.

Tetrabenazine TK Parameters - Group Mean (Sexes Combined) and Range					
Day 1		5 mg/kg, QD	10 mg/kg, QD	20 mg/kg, QD	40 mg/kg, QD
	C_{max} obs (ng/ml)		57.6 ± 33.6 (15.68 – 97.77)	179 ± 312 (12.64 – 646.60)	428 ± 349 (7.71 – 753.60)
T_{max} (hr) (median)		0.50 (0.50 – 1.00)	1.50 (0.50 – 1.50)	0.75 (0.50 – 1.00)	1.00 (1.00 – 2.00)
AUC _{0-t} (hr.ng/ml)		106 ± 30.0 (77.09 – 134.01)	311 ± 406 (32.25 – 902.22)	801 ± 544 (72.84 – 1,293.92)	1,497 ± 1,201 (117.55 – 2,647.41)
AUC _∞ (hr.ng/ml)		113 ± 36.7 (91.15 – 155.86) (\$)	-	1,104 ± 312 (765.46 – 1,380.04) (\$)	2,078 ± 1,004 (921.55 – 2,726.11)
$t_{1/2}$ (hr)		6.46 ± 1.67 (5.02 – 8.28)	-	6.12 ± 2.51 (3.31 – 8.17)	7.99 ± 1.85 (6.85-10.13) (\$)
Day 14		2.5 mg/kg, BID	5 mg/kg, BID	10 mg/kg, BID	20 mg/kg, BID
	C_{max} obs (ng/ml)	25.6 ± 39.5 (2.38 – 84.57)	571 ± 393 (20.23 – 865.00)	478 ± 319 (44.04 – 811.60)	
T_{max} (hr)	1.50 (1.00 – 1.50)	0.75 (0.50-1.00)	1.25 (0.50 – 2.00)		
AUC ₀₋₈ (hr.ng/ml)	77.1 ± 110 (13.11 – 241.92)	651 ± 463 (76.80 – 1,124.50)	764 ± 453 (208.58 – 1,195.18)		
$t_{1/2}$ (hr)	4.57 ± 3.47 (2.11 – 7.02) (^)	4.00 ± 0.09 (3.93-4.10) (\$)	3.37 ± 0.61 (2.80-4.02) (\$)		
(-) could not be calculated, (+) based on 1, (^) based on 2, (\$) based on 3					

APPEARS THIS WAY ON ORIGINAL

APPEARS THIS WAY ON ORIGINAL

Dihydrotrabenazine (Non-Chiral) TK Parameters - Group Mean (Sexes Combined) and Range					
	5 mg/kg, QD	10 mg/kg, QD	20 mg/kg, QD	40 mg/kg, QD	
Day 1	C _{max} obs (ng/ml)	148 ± 61.5 (103.10 – 236.70)	265 ± 280 (61.54 – 662.70)	700 ± 738 (86.30 – 1,766.00)	977 ± 647 (64.37 – 1,460.00)
	T _{max} (hr) (median)	1.00 (1.00 – 2.50)	1.75 (0.50 – 2.50)	1.00 (1.00)	1.50 (1.00 – 2.50)
	AUC _{0-t} (hr.ng/ml)	488 ± 353 (188.05 – 969.52)	1,255 ± 1,059 (296.77 – 2,385.29)	3,701 ± 3,404 (790.89 – 8,626.13)	4,682 ± 3,385 (333.82 – 7,979.78)
	AUC _∞ (hr.ng/ml)	376 ± 258 (194.12 – 558.38) (^)	- (2,490.45) (+)	4,803 ± 3,481 (2,744.86 – 8,821.17) (\$)	6,310 ± 2,288 (3,843.43 – 8,363.18) (\$)
	t _{1/2} (hr)	3.08 ± 1.26 (2.19 – 3.97) (^)	- (5.98) (+)	4.99 ± 0.66 (4.61 – 5.76) (\$)	4.80 ± 1.08 (4.17 – 6.05)
	2.5 mg/kg, BID	5 mg/kg, BID	10 mg/kg, BID	20 mg/kg, BID	
Day 14	C _{max} obs (ng/ml)	179 ± 263 (10.71 – 570.70)	1,387 ± 1,029 (173.00 – 2,685.00)	932 ± 567 (172.60 – 1,510.00)	
	T _{max} (hr) (median)	2.00 (1.00 – 3.00)	2.25 (0.50 – 3.00)	2.00 (1.00 – 2.00)	
	AUC ₀₋₈ (hr.ng/ml)	973 ± 1,607 (47.69 – 3,377.43)	5,430 ± 4,602 (618.63 – 7,828.10)	3,768 ± 2,922 (705.73 – 7,627.40)	
	t _{1/2} (hr)	4.65 ± 1.87 (3.00 – 6.67) (\$)	3.62 ± 1.66 (1.73 – 4.80) (\$)	3.76 ± 1.31 (2.53 – 5.35)	

(-) could not be calculated, (+) based on 1, (^) based on 2, (\$) based on 3

14-Day Oral Gavage with Trabenazine to Assess Toxicokinetics and Prolactin Levels in Rats, study # 7425-114: A draft report of the in-life portion of this study was submitted to the NDA (module 4, volume 11), and revised TK report was submitted in NDA amendment #0005. This study was conducted to provide a TK assessment in rats for the stereoisomeric metabolites of TBZ (i.e., α-HTBZ and β-HTBZ). The TK assessment in the chronic toxicity studying rats evaluated TBZ and HTBZ (using a non-chiral assay). This additional TK study was conducted in CD@ (SD) IGS BR rats that were administered TBZ at daily doses of 15 mg/kg, bid, with daily doses administered approximately 12 hrs apart (except on Days 1 and 14, when only the morning dose was administered). According to the revised TK report, "Interfering peaks were found to be present in the trabenazine chromatograms ... and trabenazine plasma concentrations were reported for information only. Consequently, the trabenazine concentrations ... should be considered as information not relevant to the primary objective of the assessment of the toxicokinetics of α-HTBZ and β-HTBZ." At this dose (15 mg/kg), exposure to α-HTBZ was greater in males and exposure to β-HTBZ was greater in females. The ratio of α-HTBZ to β-HTBZ was approximately four times greater in males than females. Since the assessment of the TK of TBZ was considered unreliable, only the data for exposure to α-HTBZ and β-HTBZ will be provided based on sponsor-provided figures and tables.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

α -HTBZ

Figure 2: Plasma concentrations of α -HTBZ in composite rats on Days 1 and 14 during oral gavage administration of tetrabenzazine to male and female rats for 14 days (15 mg/kg on Days 1 and 14 and 15 mg/kg/b.i.d on Days 2-13).

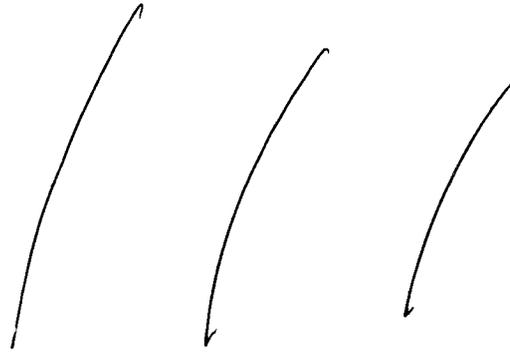


Table 2: Summary of toxicokinetic parameters for α -HTBZ during oral gavage administration of tetrabenzazine to male and female rats for 14 days (15 mg/kg on Days 1 and 14 and 15 mg/kg/b.i.d on Days 2-13)

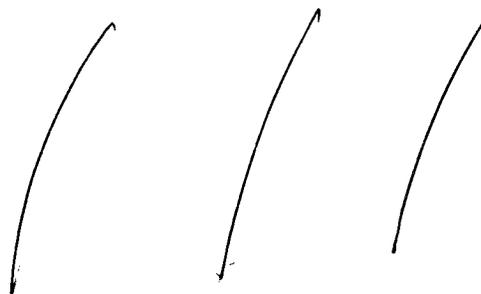
Parameter	Day 1		Day 14	
	Female	Male	Female	Male
C _{max} (ng/mL)	137	392	40	614
T _{max} (h)	0.50	1.60	0.30	1.00
AUC _{0-∞} (h•ng/mL)	381	962	192	1,788

Best Possible Copy

β -HTBZ

Figure 3: Plasma concentrations of β -HTBZ in composite rats on Days 1 and 14 during oral gavage administration of tetrabenzazine to male and female rats for 14 days (15 mg/kg on Days 1 and 14 and 15 mg/kg/b.i.d on Days 2-13).

15 r



Time (h)

Table 3: Summary of toxicokinetic parameters for β -HTBZ during oral gavage administration of tetrabenazine to male and female rats for 14 days (15 mg/kg on Days 1 and 14 and 15 mg/kg/b.i.d on Days 2-13).

Parameter	Day 1		Day 14	
	Female	Male	Female	Male
C _{max} (ng/mL)	10.1	4.5	5.2	11.7
T _{max} (h)	0.50	1.00	0.50	1.00
AUC ₀₋₂₄ (h•ng/mL)	9.36	4.91	7.91	20.3

α -HTBZ to β -HTBZ Ratio

Table 4: Ratios of α -HTBZ-to- β -HTBZ AUC during oral gavage administration of tetrabenazine to male and female rats for 14 days (15 mg/kg on Days 1 and 14 and 15 mg/kg/b.i.d on Days 2-13).

AUC ₀₋₂₄ Ratio	Day 1		Day 14	
	Female	Male	Female	Male
n/b	40.7	195.8	25.5	87.3

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

In addition to the information provided in the reviewer-generated table below, the available data are discussed within the relevant reviews.

TK Assessments in Pivotal Toxicity Studies						
Species	Study	Doses	TK Assessments			
			TBZ	DHTBZ	α -HTBZ	β -HTBZ
Rat	13/26 week oral toxicity study	2.5, 7.5 and 15 mg/kg, BID	X	X	-	-
	4-wk oral toxicity study	2.5, 7.5 and 15 mg/kg, BID	X	X	-	-
	14-day TK study	15 mg/kg bid	X (*)	-	X	X
	Embryofetal development study	5, 15 and 30 mg/kg, QD	-	-	-	-
	Pre & post natal development study	5, 15, and 30 mg/kg, QD	-	-	-	-
	In vivo micronucleus assay	25, 50 and 100 mg/kg, QD	-	-	-	-
Dog	9-month oral toxicity study	1, 3, and 10 mg/kg, QD	X	-	X	X
	15-day DRF study	5, 10, 20 and 40 mg/kg, QD	X	X	-	-
Rabbit	Embryofetal development study	10, 30 and 60 mg/kg/day, QD	-	-	-	-
	DRF for embryofetal development	1.2, 2.5, 7.5 and 15 mg/kg, QD	X	-	X	X
Mouse	90 oral gavage toxicity	10, 30, and 60 mg/kg, QD	X (*)	-	X	X
	14-day DRF study	5, 10, 30 and 100 mg/kg, QD	-	-	-	-
	In vivo micronucleus assay	10, 20, 40 and 80 mg/kg, QD	-	-	-	-
C57BL/6 Mouse	DRAFT 4-wk oral DRF study	7.5, 15, 30 and 60 mg/kg/day	X (*)	-	X	X

(*) – plasma TBZ could not be assessed due to technical difficulties

Best Possible Copy

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

First, it should be stated that we cannot ensure that the pivotal nonclinical studies adequately characterize the toxicity of the major drug-related circulating products in humans after oral administration of tetrabenazine (TBZ). The sponsor must provide data that demonstrate that the major drug-related circulating products in humans have been adequately tested in the pivotal nonclinical studies including the pivotal test species/strains, and, when relevant, the metabolic activation systems used in the pivotal *in vitro* studies (e.g., *in vitro* genetic toxicology studies).

General toxicology: The sponsor conducted a 26-week toxicity study with a 13-week interim kill in CD@SD IGS BR treated twice daily, via oral gavage, with TBZ at doses of: 0, 2.5, 7.5 and 15 mg/kg, bid (or 0, 5, 15, and 30 mg/kg/day). Additional information is necessary to determine the acceptability of this study to characterize the toxicity of TBZ given chronically to rats. This information includes: (1) a response to the request for information sent to the sponsor on 24-Jan-06, including a full delineation of clinical observations and clarification of the observation schedule, (2) a copy of the pathologist's report, (3) a discussion of the parasite infection and implications (if any) on the validity of the study, (4) a detailed description of the histopathologic examination of the brain, with an emphasis on the techniques used, and the extent of the examination, especially with regard to the substantia nigra, (5) and the results of the serum prolactin assessment conducted in Study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats). It should be noted that without further information, it can be stated that no-effect doses for treatment related lethargy and aggressiveness were not established in this study and that potentially treatment-related convulsions and deaths occurred at the HD (approximately 2.9 times the maximum recommended daily dose in humans (MRHD) on a mg/m² basis) (although, pending further information, the effect level for convulsions may decrease). No-effect levels were not established for treatment-related physiological hyperplasia of the mammary gland, multifocal accumulations of alveolar macrophages and chronic dermatitis. There was also a treatment-related induction of proestrus based on vaginal pathology in HDF. It should be noted that there appears to be a notable lack of vigilance in the conduct and reporting of this study that should be further investigated.

The sponsor conducted a 9-month toxicity study in beagle dogs (4/sex/group) treated once daily, via oral gelatin capsule, with TBZ at doses of 0, 1, 3, or 10 mg/kg/day. This study is the only chronic toxicity study conducted in non-rodent and it had notable design flaws (ophthalmologic examination, ECG and urinalysis were not conducted, and microscopic examination of the full tissue battery was not conducted [notable exclusions were: eyes/optic nerves, vagina, cervix, lachrymal gland, larynx, pharynx, skin, tongue, nasal cavity and bone marrow smear]). While these inadequacies are notable, they do not invalidate the assay. The major treatment-related toxicity identified in this study was dose-related abnormal clinical observations (including: hypoactivity, tremors, repetitive behaviors, hunched posture, recumbency, rigidity of limbs, sensitivity to touch, ataxia, reddened gums, skin and conjunctiva, abnormal respiration, excessive salivation, squinting eyes, and clear discharge from eyes). The NOEL for abnormal clinical observations was 1 mg/kg/day (LD) which is 0.32 times (on a mg/m² basis) the MRHD. The highest dose tested in the chronic study was 3.2 times (on a mg/m² basis) the MRHD. In the 2-week DRF study, a dose of 40 mg/kg/day (administered as 20 mg/kg bid) resulted in the unscheduled sacrifice of all four treated animal after 4 to 11 days of dosing due to treatment related clinical signs in all four dogs and possibly liver/gallbladder toxicity in one dog (based on clinical pathology findings; histopathology was not conducted).

The sponsor has also submitted the reports for a 90-day oral toxicity study in CD-1 mice and the 14-day dose range finding study used for the selection of doses in the 90 study. These studies have not been reviewed as part of the NDA review.

Neurotoxicity: In the study report for the chronic toxicity study in rat, a reference was cited describing TBZ-induced neurotoxicity and neuropathology in rat with repeat dosing (Satou *et al.* 2001. Repetitive administration of tetrabenazine induces irreversible changes in locomotion and morphology of the substantia nigra in rats, *Exp Toxic Pathol* 53: 303-308). In this study male Wistar rats were administered TBZ by intraperitoneal injection (1 mg/kg) either as a single injection or as daily injections for seven consecutive days. The results of the multiple dose portion of the study demonstrated (1) a statistically significant treatment-related neuronal cell loss in the substantia nigra/pars compacta (SNpc), (2) a decrease in SNpc neuron area, and (3) a decrease in cell size. These findings progressed with increasing survival time (estimated [based on graphs] to be up to approximately 50% neuronal cell loss and approximately 30% decrease in area) at 15 days post dose (the last time point studied). An increase in staining for GFAP indicating glial proliferation was also noted in the SNpc. In addition these animals demonstrated a treatment-related decrease in locomotion that was not completely reversible, even after a 15 day recovery period.

Based on the findings of Satou *et al.*, the CNS histopathology in the chronic toxicity studies in rat and dog was expanded to include an examination of the pons in rats and the substantia nigra in dog. The extent of the histopathological examination of the brain was not specified, nor the inclusion of any special techniques (if any) in the examination of the brain. Without further information, it is not possible to preclude treatment-induced neuropathology. It should be noted that the sponsor states that TBZ "has been reported to cause parkinsonism" due to depletion of striatal dopamine. The sponsor also states that in clinical study TBZ 103,004, three (of 54) patients were noted with parkinsonism as a dose-limiting adverse event. The sponsor further states, "In these patients, dose adjustment resulted in maintained efficacy with complete reversal of the AE in 2 patients, and a partial resolution in one patient."

Genetic toxicology: The sponsor has conducted Ames tests and *in vitro* chromosome aberrations assays for TBZ, and the stereoisomeric metabolites α -HTBZ and β -HTBZ in the presence and absence of metabolic activation (using rat S9). The results of the Ames tests were negative (for all three test articles) and the results of the *in vitro* chromosome aberrations tests were reproducibly positive for TBZ (in the presence of metabolic activation) and for both α -HTBZ and β -HTBZ (in the presence and absence of metabolic activation). The sponsor conducted two *in vivo* assessments of chromosome damage (using rodent hematopoietic cells) with TBZ. The initial study was an *in vivo* micronucleus assay in rat, and TBZ was negative for males and produced equivocal results in females. The second test was *in vivo* micronucleus assay conducted only in male mice. The results of this assay were negative; however, based on information available about the drug at the time of its conduct, the study should have been conducted in both males and females.

The sponsor should resolve the equivocal finding in females in the *in vivo* micronucleus assay for TBZ in rat by conducting an additional assay in female rats, using multiple doses of TBZ including, 100 mg/kg, the dose that produced the equivocal response in the original assay. This should be conducted prior to approval.

Carcinogenicity: The sponsor has not submitted carcinogenicity assessments as part of the NDA. The sponsor notes that in the preNDA meeting which took place on 01-Feb-05, the Division stated that the lack of carcinogenicity studies would not be a basis for a "refusal to file" for the NDA (this is documented in the meeting minutes). Furthermore, in the NDA the sponsor, "...commits to conduct and report the findings of two rodent carcinogenicity studies upon NDA approval."

It should be noted that on 12-Sept-05, the sponsor submitted a request (IND 63,909, serial # 62) for concurrence on the conduct of a 26-wk transgenic mouse (p53) assay and the doses for the proposed assay. The information was discussed at the executive CAC meeting of 25-Oct-05 and minutes of the deliberation, and the recommendations and conclusions of the executive CAC were sent to the sponsor on 27-Oct-05.

Reproductive toxicology:

Fertility and early embryonic development: In the NDA package the sponsor notes that a fertility study is planned but has not yet been initiated. At the end-of-phase 2 meeting that took place on 30-Jun-04, it was agreed that this study could be provided as a Phase 4 commitment.

Embryo-fetal development in rabbit: The study was conducted in predated female Hra: (NZW)SPF rabbits treated once daily, via oral gavage, from gestation day (GD) 7-20 with TBZ at doses of 0, 10, 30 or 60 mg/kg/day. The NOEL for maternal toxicity was 10 mg/kg/day (approximately 1.9 times the MRHD on a mg/m² basis), based on treatment-related clinical observations (constricted pupils, squinted/closed eyes, rapid respiration, few or no feces, and recumbency) in MD and HD and changes in body weight and food consumption seen at the HD. The NOEL for embryo fetal development was 60 mg/kg/day (the highest dose tested) (approximately 11.6 times the MRHD on a mg/m² basis).

Embryo-fetal development in rat: Premated female — CD@ (SD)IGS BR rats were treated once daily, via oral gavage, from gestation day (GD) 6-17 with TBZ doses of 0, 5, 15 or 30 mg/kg. The NOEL for maternal toxicity is 5 mg/kg/day (approximately 0.48 x the MRHD on a mg/m² basis), based on the occurrence of treatment-related clinical signs (hypoactivity and squinted or closed eyes) in MD and HD. The NOEL for embryo-fetal viability is a 15 mg/kg/day (approximately 1.5 x the MRHD on a mg/m² basis), based on an increase in post implantation loss and a slight increase in early resorptions in the HD group.

Prenatal and post natal development: Premated female — CD@ (SD)IGS BR rats were treated once daily, via oral gavage, from gestation day (GD) 6 – lactation day (LD) 20 with TBZ doses of 0, 5, 15 or 30 mg/kg. This study report has several discrepancies in the data that must be resolved before definitive conclusions can be made about the acceptability of the study, and the effects of treatment of the F₀ generation on the subsequent (F₁) generation and the resulting offspring of that generation (F₂). Without further information, it can be stated that a NOEL for findings in the F₀ dams may not have been achieved, based on an effect on pup retrieval data at the lowest dose tested (despite lack of clinical observation in the dams). It should be noted that it is not possible to ascribe the effect to dam or pup, since it is very difficult to distinguish between dam and pup effects. The NOEL for F₁ perinatal pup survival was 5 mg/kg/day (approximately 0.48 x the MRHD on a mg/m² basis). There were treatment-related delays in the pinna unfolding (HD), hair growth (all doses), eye opening (HD), vaginal opening (all doses) and preputial separation (MD and HD). Therefore, a NOEL for development was not established. Conclusions about an effect of treatment (of the F₀ dams) on the reproductive function in the F₁ generation cannot be made until the sponsor resolves the discrepancies in the data and provides, if available, corpora lutea counts and an evaluation of preimplantation loss for the F₁ females.

Special toxicology: No studies were conducted by the sponsor.

2.6.6.2 Single-dose toxicity

No studies were conducted by the sponsor.

2.6.6.3 Repeat-dose toxicity

Study title: Tetrabenazine – 26 Week Toxicity Study in Rats with Twice Daily Dosing, Administered by Gavage and 13 Week Interim Kill

Key study findings: Further information required before final interpretation

Study no.: — Report # 20730

Volume # original () revised study report in volumes 9.1-9.2

Conducting laboratory and location: _____

Date of study initiation: 03-Aug-01

GLP compliance: yes - OECD

QA report: yes (x) no ()

Drug, lot #, and % purity: lots/batches 99501 and 100730 (chromatographic purity by HPLC – 99.9%) (stored at ambient temperature, in the dark).

Dosing formulations: prepared weekly in 0.5% carboxymethylcellulose. Dosing formulations were sampled (in triplicate) immediately after preparation on Day 1, weeks 6, 13, 18 and 26 of study (the 2.5 mg/kg, bid solution from Day 1 was reanalyzed; however, results not reported) additional sampling of the dosing formulation for the 2.5 mg/kg, bid group, were obtained during weeks 14, 15 and 18, due to low values at previous sampling. The results of the formulation analyses are summarized in the following table. According to the report, the formulations for all dose levels were homogenous. According to the sponsor, "This work included validation of formulation procedures and establishment of the necessary stability of the formulations."

Analysis of Dosing Formulations							
	Day 1	Wk 6	Wk 13	Wk 14	Wk 15	Wk 18	Wk 26
0 mg/ml	none found	none found	none found	-	-	none found	none found
5 mg/ml	79% → (\$)	81%	62.8%	65.2%	84.8%	37.4% → 91.6% (*)	85.2%
7.5 mg/ml	104%	103.3	92%	-	-	108.7%	92.7%
15 mg/ml	99%	94.3%	93.3%	-	-	97.3%	91.7%

(\$) reanalyzed during Wk 2, results not reported; however, according to the sponsor, "confirmed original values."
 (*) reanalyzed from a fresh formulation

Methods

Doses: 0, 2.5, 7.5 and 15 mg/kg, bid (or 0, 5, 15, and 30 mg/kg/day)

Species/strain: rat/Sprague-Dawley — CD@ (SD) IGS BR from _____

Number/sex/group or time point (main study): 10/sex/gr for 13 wks and 20/sex/gr for 26 wks

Route, formulation, volume, and infusion rate: twice daily oral gavage up to and including the morning of necropsy. The daily doses were separated by approximately 12 hrs, except on days designated for TK sampling (when single doses were administered).

Satellite groups used for toxicokinetics or recovery: none

Age: at arrival approximately 4 weeks old. The acclimation period was 15 days.

Weight: at arrival the weight of a subset of animals was 74-80g for males and 65-76g for females

Sampling times: after 13 and 26 weeks of treatment (after 13 wks of treatment, the 10 lowest numbered surviving animals in each group were sacrificed).

Housing: animals (same sex, same treatment) were group housed (5/cage)

Unique study design or methodology (if any): -

Justification of doses: "Dose levels were agreed with the Sponsor after evaluation of preliminary studies carried out by the Sponsor or under a separate protocol and contract at _____ Project No. 455298, 4 Week Toxicity Study in Rats with Administration by Gavage (2 times daily)). Major findings in the 4-week study included mild/marked lethargy during the post dose phase and reduced body weight

gain in animals dosed at 15 mg.kg⁻¹.bid. Dose levels took into account the maximum tolerated dose in the test model and other factors such as anticipated human exposure.”

Results

Mortality: Viability was assessed twice daily (early morning and late afternoon). In the original submission the sponsor did not provide a summary of unscheduled deaths. The following table was compiled with data sent (15-Dec-05) in response to a reviewer request for information. The study was designed such that at week 14, within each group and sex, the ten animals with the lowest identification number were sacrificed (interim sacrifice group). Therefore, the sample size at the 14 wk sacrifice is 10/sex/group and the sample size for the 26 wk sacrifice was ≤ 20 /sex/group. Unscheduled deaths (moribund sacrifices or animals found dead) occurred in all treatment groups. However, within each treatment group the number surviving until the wk 26 terminal sacrifice was adequate for evaluation.

Viability after 14 and 26 Weeks of Treatment					
		# sacrificed after 14 weeks of treatment (interim sacrifice)		# sacrificed after 26 weeks of treatment (terminal sacrifice)	
		males	Females	males	females
control	0 mg/kg, bid	10	10	19	17
Low dose	2.5 mg/kg, bid	10	10	20	19
Mid dose	7.5 mg/kg, bid	10	10	15	18
High dose	15 mg/kg, bid	10	10	16	19

Unscheduled deaths and moribund sacrifices are listed in the following reviewer-generated table. The unscheduled deaths or sacrifices in the C and LD animals, MDF and the one MD male, noted as suspected gavage accident, do not appear to be related to treatment; however, the sacrifice of the control female that became pregnant during the course of the study, suggests a lack of vigilance during the conduct of the study. The moribund sacrifices of the remaining four MDM were possibly indirectly related to treatment; due to wounds/lesions on the neck, thorax and abdomen. The animals were group housed, and TBZ administration was noted to induce hyperactivity and aggressive behavior in a dose-related fashion. The mortality/morbidity of the three moribund sacrificed HDM and single HDM and single HDF found dead are assumed to be treatment-related. Upon review of information on unscheduled sacrifices and deaths, the adequacy of the report to fully describe the results of the study came into question. For example, HDM #110 was sacrificed in moribund condition during week 23 of the study, and the sponsor listed the cause of moribund condition as chronic dermatitis (the dermatitis was listed as moderate in severity and appeared to be confined to the muzzle). In the description of this animal’s condition there was no mention of convulsions; however, the line listings for this animal noted convulsions on days 133, 142 and 154 (and the animal was sacrificed during week 23).

Summary of Unscheduled Deaths (comments in italics were taken directly from sponsor’s submission of 15-Dec-05)			
group	animal #	Date	Data
C	23-M	wk 15 – sacrificed	<i>“Irregular respiration, subdued, hunched body, swollen neck, stained fur on the head, pale/discolored skin.”</i> COD: <i>“Lymphocytic-lymphoma”</i> No further comment needed.
C	125-F	wk 8 – sacrificed	<i>“Sacrificed due to pregnancy (swollen ventral abdomen)”</i> No further comment needed.
C	133-F	wk 14 – sacrificed	<i>“Damaged eye.”</i> COD: <i>“Retrolbulbar hemorrhage with eye protrusion.”</i> <i>According to the sponsor, secondary to orbital blood sampling.</i> No further comment needed.
C	141-F	wk 20 – sacrificed	<i>“Body hunched, scabs on head, ulcerative mass.”</i> COD: <i>“Ulcerative abscess ventral thorax”</i> No further comment needed.
LD	154-F	wk 13 – sacrificed	<i>“Swollen Hindlimb.”</i> COD: <i>“Hindlimb lesion.”</i> Tissue from gross lesion lost during processing, no further comment needed.

Summary of Unscheduled Deaths (comments in italics were taken directly from sponsor's submission of 15-Dec-05)			
group	animal #	Date	Data
MD	76-M	wk 24 – sacrificed	<p><i>“Lesion ventral abdomen, sparse hair, At necropsy, wound found to contain plant material within dermis.”</i> COD: <i>“Foreign body reaction – ulcerated wound ventral abdomen.”</i></p> <p>No further comment needed. (cage mate of 77)</p>
MD	77-M	wk 24 – sacrificed	<p><i>“Lesion dorsal surface and lesion of the foot.”</i> COD: <i>“Ulcerated wound dorsal thorax and hyperkeratotic dermatitis of foot.”</i></p> <p>No further comment needed. (cage mate of 76)</p>
MD	83-M	wk 24 – sacrificed	<p><i>“Increased activity, lesion dorsal neck, ear swollen.”</i> COD: <i>“Ulcerated wound dorsal neck.”</i></p> <p>No further comment needed. (cage mate of 84)</p>
MD	84-M	wk 10 – sacrificed	<p><i>“Open lesion dorsal neck, fast respiration, agitated.”</i> COD: <i>“Ulcer dorsal neck.”</i></p> <p>No further comment needed. (cage mate of 83)</p>
MD	90-M	wk 27 – sacrificed	<p><i>“Wheezing respiration, subdued, tremors, hunched body, weight loss, piloerection, skin cold to touch. At necropsy, tear on esophagus.”</i> COD: <i>“Suspected gavage error.”</i></p> <p>No further comment needed.</p>
MD	198-F	wk 14 – sacrificed	<p><i>“Damaged eye, stained fur on head.”</i> COD: <i>“Retrolbar hemorrhage with eye protrusion.”</i> According to the sponsor, secondary to orbital blood sampling.</p> <p>No further comment needed.</p>
MD	199-F	wk 21 – sacrificed	<p><i>“Irregular respiration, dragging hind limbs, discolored skin.”</i> COD: <i>“Lymphocytic leukemia.”</i></p> <p>According to the narrative the sponsor refers to this as lymphocytic lymphoma; however, the necropsy listing is malignant lymphocytic leukemia. No further comment needed.</p>
HD	93-M	wk 9 – sacrificed	<p><i>“Animal subdued, tremors, hunched body, weight loss, stained fur.”</i> COD: <i>“Undetermined.”</i></p> <p>Further information/comment needed: need reliable information on date of sacrifice and clinical signs.</p> <p>According to the data in hand: Day 58 immediately after the morning dose – markedly subdued, tremors in hand, intermittently, body hunched, weight loss, both eyes partially closed, staining on fur. Body weight during wk 8 was unremarkable, but terminal body weight not provided.</p>
HD	104-M	wk 22 – sacrificed	<p><i>“Animal subdued, intermittent tremors, hunched body, weight loss, stained fur, piloerection.”</i> COD: <i>“Undetermined.”</i></p> <p>Further information/comment needed: need reliable information on date of sacrifice and clinical signs.</p> <p>According to the data in hand: Day 147, immediately after the morning dose - irregular respiration, subdued, intermittent tremors (in cage), walking on tip toes, hunched, marked weight loss, piloerections. There was a decrease in body weight from wk 20 → 21 (455 → 428g).</p>
HD	110-M	wk 23 – sacrificed	<p>According to the sponsor, <i>“Scabs and swollen muzzle, piloerection, unkempt coat.”</i> COD: <i>“Chronic dermatitis.”</i></p> <p>Further information/comment needed: need reliable information on date of sacrifice and clinical signs.</p> <p>According to the data in hand: the last day 159 of observations (immediately after the morning dose): convulsions, body hunched, swollen muzzle, piloerection, two bald areas, unkempt coat, scabs/lesions on muzzle, swollen muzzle. Convulsions were also noted on days 133, 142, and 154 (with hind limb dragging).</p> <p>Necropsy page does not list a cause of death. The animal was noted with two encrusted scabs on its muzzle. Related histopathological findings on the muzzle are ulcer, and chronic moderate dermatitis (muzzle). Last body weight provided (wk 22) was unremarkable.</p>

Summary of Unscheduled Deaths (comments in italics were taken directly from sponsor's submission of 15-Dec-05)			
group	animal #	Date	Data
HD	116-M	wk 20 – found dead	<i>“Increased activity. Diagnosis not possible due to extensive autolysis.” COD: “Undetermined.”</i> Further information/comment needed: need reliable information on date of sacrifice and clinical signs. Only noteworthy clinical sign listed from day 113-134 (last entry) was increased activity. Last body weight provided (wk 19) was unremarkable.
HD	228-F	wk-14 – found dead	<i>“No clinical finding immediately prior to death. At histopathological examination, minimal inflammatory cell foci of the liver, mild hyperplasia of the mammary gland.” COD: “Undetermined.”</i> Further information/comment needed: need reliable information on date of sacrifice and clinical signs. Last body weight provided (wk 13) was unremarkable, body weight gain wk 0-13 was unremarkable. Death occurred sometime between day 92-98 and the last clinical observations were day 28 (lethargy) and day 43 (“abnormal colour, discoloured skin on, muzzle”). Histopathology unremarkable, except mild physiologic mammary hyperplasia and vaginal classification of proestrus (with vaginal mucification and epithelial thinning).

Information obtained from sponsor in submission dated, 15-Dec-05.

Clinical signs: According to the protocol, frequent daily observations were made. Weekly detailed clinical examinations were conducted (“including appearance, movement and behavior patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta”). From week 13 onwards, animals were palpated on a weekly basis. The sponsor’s summary of treatment-related clinical observations focused on lethargy, hyperactive behavior, and aggressive behavior. The sponsor’s summary table presents only “selected” post dose clinical signs and is labeled as such. There was no mention of treatment-related convulsions in the summary discussion or summary table; however, based on limited review of the individual animal data, at least two HD animals were reported with convulsions (see reviewer-generated table below for details).

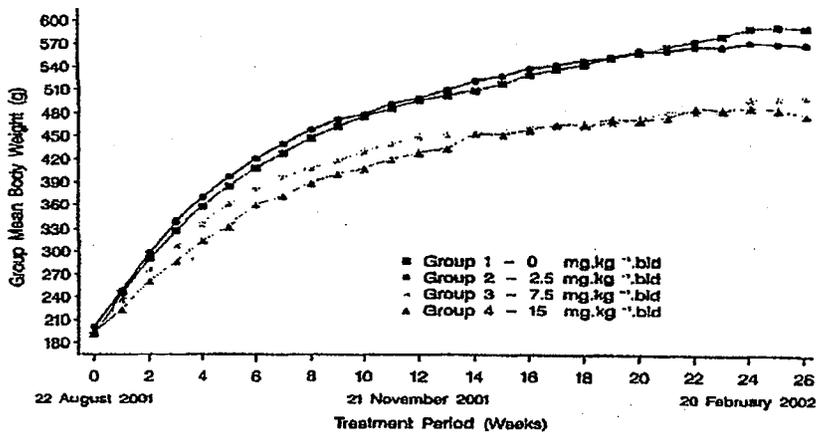
Incidence of Convulsions			
group	Animal	Day	Notation
High dose	110-M	133 - before am dose	• “convulsions, once., 15 sec”
		142 – immediately after pm dose	• “convulsions, once., an took fit lasting approx 1min”
		154 – before pm dose	• “convulsions, continuously., approx 1-2mins” • “dragging hind limbs, continuously, during convulsion”
		159 – before am dose, and immediately after am dose	• “Convulsions once., Before dosing”
High dose	111-M	172 – before am dose	• “convulsions, once., lasted approx 10 seconds”
		176 – time not stated	• “convulsions, continuously., After handling approx 10secs”

Further examination of the individual animal data suggested that the data sets for each animal may not be complete and additional information was requested from the sponsor (see discussion section for details). Further interpretation of a treatment-related effect on behavior and potential signs leading to mortality or morbidity should be deferred until the submission and review of the requested data.

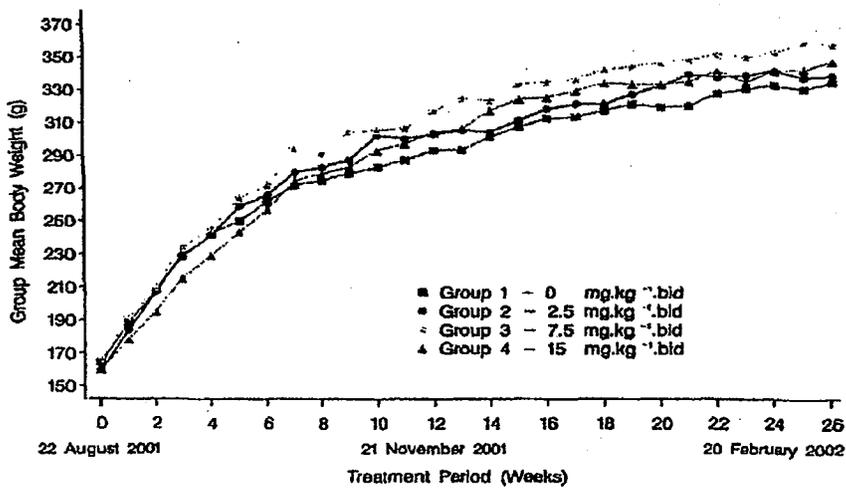
Body weights: Body weights were obtained once prior to the initiation of treatment and daily for the first 13 wks of treatment body weights; however, they were reported only weekly. From wk 13 onward, weights were obtained and recorded weekly. More frequent body weights were obtained for animals as needed based on physical condition. Copies of the sponsor-supplied summary figures follow.

There were statistically significant decreases in group mean body weight gain in MDM and HDM throughout the study (except MDM, wk 1). There was also a slight decrease in mean body weight for the LDM from wk 21 which was not statistically significant. At wk 26, the decreases in mean body weight were 4%, 15% and 19% from the control for LDM, MDM and HDM respectively. For HDF there was an initial period of statistically significant decreased mean body weight gain (wks 1-4) and an additional 2-wk period (wks 5-6) which did not attain significance. From week 7 onward HDF demonstrated a slight increase in mean body weight gain occasionally attaining statistical significance. The LDF and MDF demonstrated increases in mean body weight gain throughout the study (statistically significant for 18 of the 26 wks for the MD and occasionally statistically significant for the LD). At wk 26, the increases in mean body weight were 1%, 7% and 4% for LDF, MDF, and HD, respectively.

Mean Body Weight in Males



Mean Body Weight in Females



Food consumption: Food consumption was assessed by cage (5 animals/cage) on a weekly basis beginning one week prior to the initiation of dosing. Conclusions about this parameter are limited based on group housing. For MDM and HDM mean food consumption was decreased (statistically significant) at wks 1-3. In general, from wk 4 onward, LDM, MDM and HDM had greater mean food consumption

than controls, occasionally attaining statistical significance. The mean food consumption was decreased (statistically significant) in HDF at wk 1. From wk 3 onward, mean food consumption was generally increased (often significantly) in MDF and HDF. The mean food consumption in LDF followed a more variable pattern.

Water consumption: Water consumption was monitored by visual inspection (by cage [5/cage]) on a weekly basis. No data or results were reported.

Ophthalmoscopy: Ophthalmic examinations (indirect, 1% tropicamide) were conducted in all animals prior to the initiation of treatment, then in the control and high dose animals only at week 13 and 26. According to the sponsor, "There were no significant or treatment related findings observed in any animal of either sex." Examination of the data did not reveal evidence of a treatment related effect.

EKG: not assessed

Hematology: samples were obtained from 10/sex/group (non-fasted animals) at wks 14 and 26 via the orbital sinus (under isoflurane anesthesia). Analysis was not conducted on interim kill animals. The following parameters were assessed: hemoglobin, red blood cell count, hematocrit, white blood cell count and differential, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets, and differential white blood cell counts. The same sampling period was used for evaluation of the coagulation parameters, prothrombin time and activated partial thromboplastin time. However, according to the report, the amount of blood placed in tubes was approximately 33% greater than called for in the protocol (≈ 1 ml rather than 0.75 ml) so that the final citrate ratio is lower than defined. The sponsor stated, "This deviation from protocol is not considered to have affected the outcome or integrity of the study."

The sponsor noted the following as possibly treatment related: decreases in eosinophils in MDM and HDM at week 14 and 26, and decreases in basophils, lymphocytes, monocytes and WBCs in MDM and HDM at wk 26. According to the sponsor, the increases in MCH and MCV noted in MDM and HDM and HDF (MCV only) at wk 26 were not considered treatment related due to "the absence of histological evidence, and the magnitude of the changes." The sponsor also noted that the decrease in HCT on HDF at wk 14 was not considered to be related to treatment due to its small magnitude, and lack of occurrence in males.

A summary of the data are provided in the following reviewer-generated table. Possible treatment-related changes include: decreased RBCs in HDM and HDF (wks 14 and 26), increased MCH in MDM (wk 26) and HDM (wks 14 and 26), increased MCV in MDM, HDM, and HDF (wk 26), decreased WBC in MDM (wk 26) and HDM (wk 14 and 26) due to a decrease in lymphocytes, monocytes, eosinophils and basophils. Monocytes were also decreased in LDF, MDF and HDF (wk 26). There were decreases in LUC (large unclassified cells) at wk 26 in MDM, HDM and HDF. Platelets were decreased in LDM, MDM and HDM (wks 14 and 26) and in MDF and HDF (wk 26). Peripheral smears were obtained only from unscheduled sacrifices; however, it is not clear that they were assessed. Bone marrow smears were obtained but not evaluated.

Hematology (with reference to concurrent control)							
		LDM	MDM	HDM	LDF	MDF	HDF
Hb	wk 14	-	1% ↑	-	2% ↑	1% ↓	3% ↓
	wk 26	-	3% ↑	3% ↑	1% ↑	-	1% ↓
RBC	wk 14	-	-	4% ↓	-	1% ↓	3% ↓
	wk 26	-	-	4% ↓	-	-	4% ↓
Hct	wk 14	-	2% ↑	2% ↓	-	2% ↓	3% ↓ *
	wk 26	1% ↑	2% ↑	-	-	1% ↓	1% ↓
MCH	wk 14	-	-	3% ↑	1% ↑	1% ↓	-
	wk 26	-	3% ↑ *	6% ↑ ***	2% ↑	-	3% ↑
MCV	wk 14	-	1% ↑	2% ↑	-	-	-
	wk 26	1% ↑	3% ↑ *	5% ↑ **	-	2% ↓	3% ↑ *
MCHC	wk 14	-	-	1% ↑	-	-	-
	wk 26	-	-	1% ↑	2% ↑	2% ↑	-
WBC	wk 14	-	3% ↑	7% ↓	9% ↑	13% ↑	13% ↑
	wk 26	-	29% ↓ **	27% ↓ *	19% ↑	15% ↑	11% ↑
Neut	wk 14	22% ↑	56% ↑	20% ↑	6% ↓	-	5% ↑
	wk 26	8% ↑	8% ↑	1% ↑	6% ↑	10% ↑	6% ↑
Lymph	wk 14	2% ↓	3% ↓	9% ↓	12% ↑	16% ↑	14% ↑
	wk 26	1% ↓	34% ↓ **	31% ↓ **	22% ↑	18% ↑	14% ↑
Mono	wk 14	-	14% ↑	5% ↓	15% ↓	10% ↓	5% ↑
	wk 26	4% ↑	44% ↓ **	24% ↓	18% ↓	23% ↓	32% ↓
Eos	wk 14	20% ↓	40% ↓ **	45% ↓ **	46% ↑	15% ↑	15% ↑
	wk 26	11% ↑	28% ↓ *	28% ↓ *	38% ↑	-	15% ↑
Baso	wk 14	-	-	20% ↓	33% ↑	33% ↑	33% ↑
	wk 26	-	50% ↓ **	50% ↓ ***	-	-	-
LUC	wk 14	-	14% ↑	32% ↓	21% ↓	17% ↓	8% ↓
	wk 26	13% ↑	53% ↓ **	27% ↓ *	-	-	20% ↓
platelet	wk 14	7% ↓	7% ↓	15% ↓	5% ↑	2% ↑	16% ↑
	wk 26	5% ↓	15% ↓	14% ↓	-	3% ↓	4% ↓
PT	wk 14	10% ↓	10% ↓	10% ↓	-	25% ↑	6% ↑
	wk 26	-	6% ↑	6% ↑	6% ↓	6% ↓	6% ↓
APTT	wk 14	17% ↓	13% ↓	17% ↓	-	5% ↑	5% ↑
	wk 26	-	4% ↓	8% ↓	5% ↓	9% ↓	5% ↓

* p<0.05, **p<0.01, ***p<0.001
 (-) less than 1% change
 LUC = large unclassified cells

Clinical chemistry: samples were obtained from 10/sex/group (non-fasted animals) at wks 14 and 26 via the orbital sinus (under isoflurane anesthesia). Analysis was not conducted on interim kill animals. The following parameters were assessed: urea, glucose, aspartate aminotransferase, alanine aminotransferase, total bilirubin, sodium, potassium, chloride, creatinine, total protein, albumin, AG ratio, phosphate and calcium. According to the sponsor, the decreased calcium levels seen in MDM, HDM and HDF (wks 14 and 26) was not considered to be related to treatment due to the small magnitude of the change and the lack of correlated histological evidence. The sponsor used a similar explanation to dismiss the decreases in potassium levels in the MDM, HDM and HDF. The sponsor noted that the other statistically significant findings (i.e., changes in AST, ALT, total protein, glucose, sodium, albumin and phosphate) were not related to treatment due to the lack of consistent pattern, small magnitude of the changes, lack of relationship to dose, and lack of correlated histopathological findings.

Examination of the data revealed several potentially treatment-related changes in clinical chemistry parameters; (as demonstrated in the following reviewer-generated table); however, they were not correlated to treatment induced pathology and thus relevance to treatment would be questionable.

Clinical Chemistry (with reference to concurrent control) (subset)							
		LDM	MDM	HDM	LDF	MDF	HDF
urea	wk 14	-	2% ↓	10% ↓	4% ↓	6% ↑	2% ↑
	wk 26	9% ↓	4% ↓	13% ↓	2% ↓	-	4% ↑
glu	wk 14	4% ↑	4% ↑	-	4% ↑	1% ↓	2% ↓
	wk 26	5% ↑	4% ↓	14% ↓ **	7% ↑	6% ↑	10% ↑
AST	wk 14	19% ↑	36% ↑ **	59% ↑ ***	32% ↑	11% ↑	12% ↓
	wk 26	14% ↑	22% ↑	23% ↑	98% ↑ ***	60% ↑ *	34% ↑
ALT	wk 14	2% ↑	4% ↑	13% ↑	30% ↑	18% ↑	11% ↓
	wk 26	5% ↓	21% ↑ *	11% ↑	62% ↑ **	53% ↑ *	33% ↑
Na	wk 14	-	-	1% ↑	-	-	0.7% ↓ **
	wk 26	-	-	-	-	-	-
K	wk 14	2% ↓	5% ↓ *	10% ↓ **	7% ↓	2% ↓	12% ↓ ***
	wk 26	5% ↓	2% ↓	10% ↓ **	3% ↓	-	8% ↓
Cl	wk 14	-	-	2% ↑	1% ↑	-	-
	wk 26	-	1% ↑	1% ↑	-	1% ↑	1% ↑
TP	wk 14	-	3% ↓	4% ↓	4% ↓	3% ↓	7% ↓
	wk 26	3% ↓	6% ↓ **	6% ↓ ***	3% ↓	5% ↓	8% ↓ **
Alb	wk 14	-	2% ↓	2% ↓	2% ↓	-	8% ↓
	wk 26	3% ↓	5% ↓ *	8% ↓ ***	2% ↓	6% ↓	10% ↓ **
AG-R	wk 14	-	-	-	6% ↑	6% ↑	11% ↓
	wk 26	-	7% ↑	-	6% ↑	-	6% ↓
Crea	wk 14	4% ↑	9% ↑	9% ↑	-	2% ↓	2% ↓
	wk 26	4% ↑	4% ↑	2% ↑	-	2% ↓	2% ↑
Ca	wk 14	2% ↓	3% ↓ ***	4% ↓ ***	2% ↓	-	4% ↓ **
	wk 26	2% ↓ *	4% ↓ ***	5% ↓ ***	-	-	4% ↓
Phos	wk 14	7% ↑	8% ↑	-	7% ↓	5% ↓	1% ↑
	wk 26	7% ↑	3% ↑	17% ↑ **	14% ↓ **	9% ↓ *	8% ↓
T.Bi	wk 14	24% ↓	5% ↑	-	18% ↑	29% ↑	29% ↑
	wk 26	11% ↑	-	11% ↑	13% ↑	38% ↑	6% ↓

* p<0.05, **p<0.01, ***p<0.001
 (-) less than 1% change

Urinalysis: Samples were obtained in the same animals as used for hematology, coagulation and clinical chemistry. The samples were collected over a 4 hr period of food and water deprivation (via metabolism cages). The following parameters were assessed: pH, specific gravity, volume, protein, glucose, ketones, bilirubin, urobilinogen, blood pigments, and microscopy of spun deposits. Analyses were not conducted on interim kill animals.

According to the sponsor, the changes in specific gravity noted at wks 14 and 26 were not related to treatment due to the small magnitude of the change. The sponsor noted that changes in urinary volume at 26 wks in LDM, MDM, HDM and MDF and HDF. A summary of the data are provided in the following reviewer-generated tables. Examination of the individual animal data did not reveal an obvious signal for renal toxicity.

Urinalysis									
	Wk	CM	LDM	MDM	HDM	CF	LDF	MDF	HDF
Specific Gravity	wk 14	1.035 ± 0.017	1.029 ± 0.009	1.033 ± 0.015	1.023 ± 0.006	1.023 ± 0.010	1.039 ± 0.013 **	1.032 ± 0.016	1.051 ± 0.014 ***
	wk 26	1.052 ± 0.013	1.028 ± 0.007 ***	1.026 ± 0.007 ***	1.042 ± 0.016	1.034 ± 0.016	1.043 ± 0.020	1.033 ± 0.011	1.035 ± 0.014
Urinary volume	wk 14	4.0 ± 2.3	3.3 ± 1.0	3.0 ± 0.9	5.4 ± 2.1	2.8 ± 1.7	2.1 ± 1.0	2.3 ± 0.9	2.0 ± 0.8
	wk 26	1.9 ± 0.07	3.8 ± 0.9 **	4.7 ± 1.6 ***	3.7 ± 1.6 **	1.6 ± 0.9	1.6 ± 0.6	2.8 ± 1.2 **	2.5 ± 1.0 *
Urinary pH	wk 14	8.6 ± 0.2	8.6 ± 0.5	8.5 ± 0.8	8.7 ± 0.2	8.9 ± 0.3	8.0 ± 1.2	8.7 ± 0.9	8.1 ± 1.3
	wk 26	9.0 ± 0.0	8.9 ± 0.2	8.9 ± 0.2	8.9 ± 0.2	8.8 ± 0.4	8.4 ± 0.8	8.0 ± 1.0	8.3 ± 0.7

* p<0.05, **p<0.01, ***p<0.001

Gross pathology: Animals were sacrificed by carbon dioxide asphyxiation followed by exsanguinations and subjected to full necropsy. It should be noted that there was no separate pathology report, and the integrated summary report does not contain the signature of the study pathologist. According to the sponsor, there were no gross pathology findings related to treatment. There was no evidence of treatment-related findings at the 14-wk interim sacrifice. Examination of the data from the 26-wk terminal sacrifice (plus unscheduled sacrifice) data revealed the following potentially treatment-related findings: (1) pale focus in the lungs (all lobes, many) in 2/20 HD, (2) damaged/swollen or abnormally colored ears, open sores and hair loss predominantly in MDM and HDMs (this cluster of findings may be due, in part, to treatment-related aggressive behavior and group housing of the animals), (3) low incidence finding of flaccid testes in MDM (1/20) and HDM (2/20) (additional low incidence findings in male reproductive tract include reddened testes, small testes, and small epididymis), (4) decrease in the number of HDF noted with dilated uterus (one/both horns) (30% in CF and 5% in HDF, possible reflection of the hormonal state). (See the following reviewer-generated table for details).

Potentially Treatment-Related Gross Pathology from Terminal Sacrificed and Unscheduled Sacrifice Animals									
		Males				Females			
		CM	LDM	MDM	HDM	CF	LDF	MDF	HDF
Lungs	pale focus, all lobes, many	0/20	0/20	0/20	2/20	0/20	0/20	0/20	0/20
Ear	damaged	0/20	0/20	2/20	0/20	0/20	0/20	0/20	0/20
Ear	swollen	0/20	1/20	6/20	4/20	0/20	0/20	0/20	0/20
Ear	abnormal color	0/20	0/20	1/20	1/20	0/20	0/20	1/20	0/20
skin/subcutis	open sores	0/20	0/20	3/20	0/20	0/20	0/20	0/20	0/20
skin/subcutis	hair loss	2/20	1/20	7/20	6/20	6/20	3/20	6/20	9/20
epididymis	small, both	0/20	0/20	0/20	1/20				
Testis	flaccid, one/both	0/20	0/20	1/20	2/20				
Testis	reddened, right	0/20	0/20	0/20	1/20				
Testis	small, one/both	1/20	0/20	0/20	2/20				
Uterus	dilated, one/both horns					6/20	0/20	0/20	1/20

Organ weights: The following organs were weighed: adrenals, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary, prostate, spleen, submaxillary (mandibular) salivary gland, testis, thymus, thyroid/parathyroid, and uterus. Organ weights relative to brain or body weight were not provided. The sponsor conducted a covariant analysis with body weight and based its analysis of the data on the covariant analysis. Examination of the data revealed the expected decreases in absolute organ weight for MDM and HDM in many tissues, based on the decreases in mean body weight, when compared to control (and the decreases in thymus weight in these groups were not associated with thymic atrophy). A finding that appears to be related to treatment is the decreases in absolute uterine weight in the LD, MD and HD females after 13 wks of treatment and at terminal sacrifice (+ unscheduled deaths), possibly related to treatment-related changes in endocrine status. The sponsor noted that the increase in adrenal weight in males was possibly related to treatment (however, it was not associated with abnormal histopathology). (See reviewer-generated table below for details). Also worth noting was the small statistically significant

increase in absolute brain weight in HDM at terminal sacrifice (plus unscheduled deaths); however, this change was not associated with abnormal histopathology and the relevance to treatment is questionable.

Changes in Organ Weights (relative to control) after 13-Wks or at Terminal Sacrifice (+ Unscheduled Deaths)							
		LDM	MDM	HDM	LDF	MDF	HDF
body weight	13-wk	-	6% ↓	18% ↓ ***	8% ↑ *	12% ↑ **	4% ↑
	TS/UD	5% ↓	17% ↓ ***	21% ↓ ***	1% ↑	5% ↑	2% ↑
Adrenals	13-wk	6% ↓	4% ↑	11% ↑	7% ↓	4% ↓	14% ↓
	TS/UD	11% ↑	20% ↑ *	37% ↑ ***	4% ↓	12% ↓	8% ↓
Brain	13-wk	-	2% ↑	2% ↑	3% ↑	1% ↑	3% ↑
	TS/UD	3% ↑	3% ↑	5% ↑ **	1% ↓	-	-
Thymus	13-wk	5% ↑	14% ↓	22% ↓	20% ↑	30% ↑	4% ↑
	TS/UD	10% ↓	31% ↓ **	39% ↓ ***	8% ↓	3% ↑	4% ↑
Uterus	13-wk				19% ↓	23% ↓ *	35% ↓ ***
	TS/UD				27% ↓	49% ↓ ***	45% ↓ ***

TS/UD = terminal sacrifice + unscheduled deaths
 * p<0.05, **p<0.01, ***p<0.001

Histopathology: It should be noted that there was no separate pathology report, and the integrated summary report does not contain the signature of the study pathologist (BSc BVSc MAnimSc FRIPHH MRCVS). The tissues listed in the appended table were preserved for all animals. The tissues were fixed in 10% neutral buffered formalin except for the eyes/optic nerve (Davidsons' fluid) and testes (Bouin's fluid). Only one eye and optic nerve per animal were examined histopathologically. Blood smears were obtained only from animals killed prematurely. Examination of tissues was confined to H&E stained sections from C and HD animals (interim sacrifice, terminal sacrifice) and unscheduled sacrifice animals from all groups. Mammary and vaginal tissues from all of the terminal sacrifice (plus unscheduled sacrifice) animals were also examined. CNS histopathologic examination consisted of sections from the forebrain, midbrain, cerebellum, pons and spinal cord (cervical, midthoracic, and lumbar). The pons was processed in all terminal sacrifice animals and any unscheduled deaths that occurred from wk 13-26; however, histopathologic examination was conducted only on the C and HD animals.

- Adequate Battery: yes (x), no () within reason (notable tissues missing from sampling and analysis include the lachrymal gland, larynx, nasal cavity, pharynx, hardierian gland and zymbal gland. Only one eye and optic nerve examined per animal.
- Peer review: yes (), no (x)

It should be noted that in this study at least 24 of 60 control animals and at least 17 of 60 HD treated animals were infected with pinworm parasites, this estimate was obtained based on the summary histopathology table and an examination of the individual animal data for the large intestine. Toxicology studies are supposed to be conducted in normal healthy animals, and clearly this was not the case for this study. This parasite infestation and implications (if any) on the validity of the study were not discussed in the study report. Pinworms are generally a local problem confined to the distal GI tract, and there were no background lesions in the intestines, other than parasites, and no treatment-related lesions in the GI tract.

Animals (by identification number) that are noted with pin worms in the intestinal tract		
	Males	Females
control	9, 10, 14, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30	122, 124, 246, 135, 136, 138, 139, 140, 141, 143
high dose	99, 107, 109, 110, 113, 114, 118	221, 222, 224, 225, 226, 227, 228, 229, 230, 237

With regard to the interim kill (animals sacrificed after 13 wks of treatment), there were no findings attributed to treatment. The sponsor's presentation of the histopathology summary data for terminal sacrifice animals (animals sacrificed after 26 weeks of treatment) also included data from any

unscheduled deaths in the C and HD groups only. It also included the histopathological findings in the vagina and mammary gland from MD animals. The histopathology summary table did not incorporate any findings from the single LD unscheduled sacrifice or seven MD unscheduled sacrifice animals (except for those in the mammary gland or vagina), nor was there a separate table summarizing this information.

With regard to the examination of the brain for signs of treatment-related neurotoxicity, the sponsor states that "no abnormalities were detected in brain sections from this study." Examination of the data did not reveal any effect of treatment. The sponsor elected to examine additional sections of the brain (i.e., the pons) based on the demonstration of tetrabenazine-induced neurotoxicity in the literature (Satou *et al.* 2001. Repetitive administration of tetrabenazine induces irreversible changes in locomotion and morphology of the substantia nigra in rats, Exp Toxic Pathol 53: 303-308). (See discussion section for further details).

Two reviewer-generated summary tables of potentially treatment-related histopathology findings follow. The data are presented in two separate tables for convenience of presentation since the number of treatment groups for which tissues were examined differed between the two tables. One table summarized the findings in mammary, uterine and vaginal tissue and the other table summarizes histopathology of the other organs. These tables were based on the sponsor's summary tables, and as such, do not include any findings from unscheduled sacrifice LD or MD animals (except mammary and vaginal tissues from the MD).

Terminal Sacrifice (Plus Unscheduled Sacrifice) - Potentially Treatment-Related Histopathology Findings						
			Males		Females	
			CM	HDM	CF	HDF
lung	alveolar macrophage accumulation	total	3/20	12/20	3/20	6/20
		minimal	2	8	3	6
		mild	1	4	0	0
pituitary gland	cyst, intermediate lobe	present	0/20	1/20	0/20	2/19
urinary bladder	calculus	total	1/20	3/20	0/20	0/20
liver	sinusoidal dilation, centrilobular	minimal	0/20	3/20	0/20	0/20
		total	0/20	3/20	0/20	0/20
skin/subcutis	dermatitis, chronic	total	0/20	3/20	0/20	0/20
		minimal		1		
		mild		1		
		moderate		1		

Terminal Sacrifice (Plus Unscheduled Deaths) - Histopathology of the Mammary Gland, Vagina and Uterus								
			Males			Females		
			CM	MDM	HDM	CF	MDF	HDF
mammary	focal interstitial fibrosis	minimal	0/19	0/19	0/16	1/20	0/20	0/20
mammary	physiological hyperplasia	total	0/19	1/19	1/16	0/20	4/20	17/20
		minimal		1	0		0	6
		mild			1		3	9
		moderate					1	2
mammary	pigment deposits, epithelial	total	0/19	4/19	1/16	0/20	4/20	0/20
		minimal		4	0		4	
		mild			1			
vagina	estrus cycle: diestrus	total				2/20	4/20	0/20
	estrus cycle: metestrus	total				7/20	9/20	0/20
	estrus cycle: estrus	total				7/20	0/20	0/20
	estrus cycle: proestrus	total				4/20	7/20	20/20
vagina	degeneration, epithelium	minimal				0/20	0/20	2/20
uterus	estrus dilation	total				8/19		4/20

#229 & 238 - minimal vaginal epithelial degeneration, both in proestrus
 The summary data from the sponsor combines the data generated in animals that were treated until terminal sacrifice and all unscheduled deaths.

APPEARS THIS WAY ON ORIGINAL

With regard to the C and HD animals sacrificed after 26 weeks of treatment and C and HD unscheduled deaths, plus specified tissues (mammary and vaginal tissues only) from the MD animals, the sponsor noted treatment-related findings only in the lung (multifocal accumulations of alveolar macrophages), vagina (mucification, epithelial thinning and degeneration, proestrus) and mammary gland (physiological hyperplasia). These findings as well as other potentially treatment-related findings suggested by examination of the data follow:

1. Minimal to mild multifocal accumulations of alveolar macrophages in HD (only C and HD assessed; tissues from the MD and possibly LD should have been assessed to establish a NOEL). According to the sponsor, "These accumulations tended to be at the bronchoalveolar junctions (centroacinar regions) and were not associated with an inflammatory response." The sponsor notes that the increase is statistically significant ($p < 0.01$). In the discussion/conclusion section, the sponsor states that, "The alveolar macrophage accumulation in the lungs, whilst showing statistical significance and an apparent relationship to treatment in the males, showed not clear relation to treatment in the females where there appeared to be a chance distribution and so could not be attribute to administration of Tetrabenazine." The validity of this argument is not clear, and a relationship to treatment in both males and females should not be excluded.
2. Increased incidence of "physiological" hyperplasia of the mammary gland in MD and HD males and females. According to the sponsor, "There was an increased grade (mild to moderate) and incidence of physiological hyperplasia of the mammary gland of 1/16 males and 11/20 females" in the HD group. The sponsor notes that this is statistically significant in females ($p < 0.001$) and states that the MD is the NOEL for this finding. Examination of the data demonstrates that a NOEL for physiological hyperplasia of the mammary tissue was not achieved, since it also occurred in the MD group (minimal-moderate). Tissues from the LD should have been assessed to establish a NOEL. The sponsor has not defined or described the term "physiological hyperplasia". In addition, minimal to mild pigment deposits were noted in mammary epithelium in 4/19-MDM, 4/20-MDF and 1/16-HDM. No explanation of this finding was provided (therefore, presumably the sponsor considered it a background finding). It should be noted that the mammary glands from the C and HD animals were unremarkable after 13 weeks of treatment; however, HDF #228 found dead wk-14 was noted with mild physiologic hyperplasia of the mammary gland.

The sponsor attributes the "physiological" hyperplasia to "an increase in circulatory prolactin levels or a change in the pattern of release of prolactin due to administration of the test item." The sponsor states that TBZ "directly blocks dopaminergic inhibition of prolactin secretion" (Login *et al.* (1982) Tetrabenazine has properties of a dopamine receptor antagonist. *Ann Neurol* 12(3):257-62). In the referenced study, female SD rats administered a single intraperitoneal injection of tetrabenazine (30 mg/kg) had significantly higher serum prolactin levels than a concurrent control at 1 hr (20-fold increased) and 16 hrs (4-fold increased) post dose and were indistinguishable from control by 24 hrs post dose. The sponsor has not provided data to support an increase of serum prolactin after oral administration of TBZ in rats (serum prolactin levels were not assessed in the 4-wk or the 13/26-wk study).

Prolactin levels were supposed to be determined as part of — Study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats (module 4, volume 11)). The results of the prolactin analysis were to be submitted separately from the study report and do not appear to have been submitted to the NDA. The in-life portion of this study was initiated on 24-Feb-05. A final study report for this study does not appear to have been submitted; the current report consists of an "in-life summary of draft data", study protocol and revisions, and a TK report. A revised TK report for this study was submitted to the NDA on 23-Dec-05 (amendment #005); however, the prolactin data were not included.

3. Vaginal cycle in proestrus in 100% of HDF and minimal vaginal epithelial degeneration in 2/20 HDF. According to the sponsor, "All Group 4 [HD] females showed vaginal mucification, with epithelial thinning and occasional epithelial degeneration, and appeared to be in proestrus." The sponsor notes that this is statistically significant ($p < 0.001$) and states that the MD is the NOEL for this finding. It should be noted that the vaginal tissues from the C and HD interim sacrifice animals were unremarkable after 13 weeks of treatment; however, HDF # 228, found dead wk 14 was noted with the vaginal cycle in proestrus (and with "vaginal mucification and epithelial degeneration). The histopathology finding of the vaginal tissue listed in the summary table and individual line listings were confined to the determination of stage of the estrus cycle and a finding of minimal vaginal epithelial degeneration in 2/20 HDF. No incidences of "vaginal mucification, with epithelial thinning" were reported. Without further information about the occurrence of vaginal mucification and epithelial thinning in all groups examined, it would not be possible to verify that a NOEL was achieved in the MD as stated by the sponsor. The sponsor states that TBZ "directly blocks dopaminergic inhibition of prolactin secretion" (Login *et al.* 1982). The sponsor references Batten and Ingelton (1987) stating that "There is an increase in prolactin levels during pro-oestrus in the normal rat oestrus cycle. This increase in circulating prolactin causes vaginal mucification in rats." (Batten, TFC and Ingelton, PM (1987) The hypothalamus and the pituitary gland. *In Fundamentals of Comparative Vertebrate Endocrinology*. Chester-Jones, I., Ingelton, PM, Phillips, JG (Eds). 1st Edition. Plenum Press, New York. Pgs 285-409). The sponsor did not supply this reference and it was unavailable through the FDA at the time of the review.

Vaginal mucification and epithelial thinning are not consistent with the description of rat vaginal histopathology during proestrus discussed in Greaves (Greaves P, 2000), "During prooestrus the number of cell layers increases and the granular layer (stratum granulosum) develop. As oestrus approaches, a horny stratum corneum also becomes prominent. During the latter part of oestrus and metaoestrus, the upper layers of the squamous epithelium become desquamated and there is a return of increasing numbers of leukocytes before the cycle is repeated. In pseudopregnancy and pregnancy the superficial cells of the rodent vaginal mucosa become cuboidal or cylindrical with vacuolation of immediate cell layers. In late pregnancy the superficial cells become mucus secreting." (Greaves, P. (2000) *Histopathology of Preclinical Toxicity Studies – Interpretation and Relevance in Drug Safety Evaluation*. 2nd Edition. Elsevier, Amsterdam pg 679).

Furthermore, the uterine histopathology (estrus dilatation in 4/20-HD versus 8/19-C) and the treatment-related decrease in uterine weight (27-45%) are not consistent with treatment-induced proestrus state in 100% of the HD animals. Greaves (Greaves P, 2000, pg 684) describes the uterus during proestrus as follows: "In prooestrus the uterine horns enlarge and fill with fluid. Epithelial cells lining the uterine cavity become more cuboidal. These cells meet a maximum during prooestrus, before the establishment of oestrus."

4. There was a slight increase in the incidence of cysts in the intermediate lobe of the pituitary gland in the HD (1/20 HDM and 2/19 HDF versus 0/40 controls). This is a low incidence finding may be associated with age, or with changes in prolactin secretion.
5. There was an increase in calculus in the urinary bladder in HDM (3/20 versus 1/20 in CM; only C and HD examined). This was not discussed by the sponsor; therefore, it is presumed that the sponsor considered this a background finding. There were no other abnormal histopathology of the urinary bladder in the HDM, and none of the 10-CM or 10-HDM sacrificed after 13 weeks of treatment had any abnormalities of the urinary bladder. In the recently conducted mass balance study in humans, the majority of the drug-related compounds are excreted renally, and there is

evidence of urinary excretion of metabolites in rat. The relevance of this low incidence finding is unknown.

6. There was an increase in incidence of minimal to moderate chronic dermatitis in HDM (3/20-HDM versus 0/20-CM). This was not discussed by the sponsor and presumably was considered by the sponsor to be a background finding.

Toxicokinetics: Blood samples were obtained via tail vein from 2/sex/gr at each of the following timepoints: predose, 0.5, 1.0, 1.5, 3, 6 and 11 hrs post dose on Days 1 and 34 and during Weeks 13 and 25. Plasma was separated and stored frozen for future analysis. Plasma samples from different time periods were stored under different conditions as follows:

- Day 1 @ -20°C
- Week 5 @ -20°C (for 5 months) → -80°C
- Week 13 @ -20°C (for 3 months) → -80°C
- Week 25 @ -80°C

According to the sponsor, "After review of the completed data, this storage error does not appear to have had any adverse affect on the outcome or integrity of the study." According to the sponsor, the concentration of TBZ and the metabolite, 2-hydroxytetrabenazine, were assessed in plasma samples using validated assays. It should be noted that 2-hydroxytetrabenazine is also known as dihydrotetrabenazine (HTBZ) and it is the unresolved mixture of the chiral metabolites α - and β -dihyrotetrabenazine (α -HTBZ and β -HTBZ). The following parameters were assessed for TBZ and HTBZ: C_{max} (observed), T max (observed), AUC (0-t), AUC (0- ∞), termination elimination half-life (T1/2el), terminal elimination rate constant (Kel), clearance (CL/F), and apparent volume of distribution (Vd/F). Copies of the sponsor's summary tables for TBZ and HTBZ follow. On the days of TK sampling, only one dose was administered (rather than BID dosing). It should be noted that half (7 out of 14) of the plasma samples from control males on wk 25 were noted to have quantifiable levels of HTBZ (from 1.6x – 9.6 x LOQ).

Examination of the data for TBZ indicates highly variable TK. In addition, for LDM, TBZ was not found in the plasma at any time point. Exposure to DHTBZ was much greater than exposure to TBZ in each group, suggesting extensive metabolism. In each dose group, the C_{max} (obs) and the AUC for HTBZ were greater in males than in females. Exposure to TBZ and HTBZ increased with increasing dose (except for TBZ in males at Wk 25).

TK Summary for TBZ

Pharmacokinetic Parameter Estimates (Mean, n=2) Day 1

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T1/2el (h)	Kel 1/h	CL/F ml.h ⁻¹ .kg ⁻¹	AUC (0-t) ng.h.ml ⁻¹	AUC (0- ∞) ng.h.ml ⁻¹	Vd/F ml.kg ⁻¹	Rsq
2.5	Male	ND	0	ND	ND	ND	0	0	ND	ND
	Female	ND	0	ND	ND	ND	0	0	ND	ND
7.5	Male	0.5	0.8108	NC	NC	NC	0.5308	NC	NC	NC
	Female	0.5	1.34	2.32	0.299	1782207	2.579	4.256	5892858	0.57
15	Male	0.5	9.1	0.64	1.087	1564612	9.186	9.847	1430313	0.92
	Female	0.5	6.005	0.64	1.083	1773216	7.996	8.459	1637370	1

ND = estimate is not determinable. No quantifiable concentrations were reported for the profile.
NC = not possible to calculate this estimate from the concentration data available.

Pharmacokinetic Parameter Estimates (Mean,n=2)
Week 5

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T½el h	Kel 1/h	AUC (0-t) ng.h.ml ⁻¹	AUC (0-∞) ng.h.ml ⁻¹	Rsq
2.5	Male	ND	0	ND	ND	0	0	ND
	Female	0.5	0.8108	NC	NC	0.5308	NC	NC
7.5	Male	ND	0	ND	ND	0	0	ND
	Female	1.5	2.81	0.85	0.8124	5.314	5.931	0.84
15	Male	0.5	82.22	5.2	0.1333	59.2	62.96	0.96
	Female	1	15.08	1.37	0.5059	29.88	31.44	1

ND = estimate is not determinable. No quantifiable concentrations were reported for the profile.
NC = not possible to calculate this estimate from the concentration data available.

Pharmacokinetic Parameter Estimates (Mean,n=2)
Week 13

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T½el h	Kel 1/h	AUC (0-t) ng.h.ml ⁻¹	AUC (0-∞) ng.h.ml ⁻¹	Rsq
2.5	Male	ND	0	ND	ND	0	0	ND
	Female	0.5	1.368	NC	NC	0.8083	NC	NC
7.5	Male	0.5	4.275	6.11	0.1135	6.248	10.87	0.91
	Female	0.5	5.140	18.94	0.0366	15.92	40.28	0.09
15	Male	1	8.77	3.19	0.2172	21.15	23.46	0.91
	Female	1	17.65	3.3	0.2097	68.88	72.93	0.58

ND = estimate is not determinable. No quantifiable concentrations were reported for the profile.
NC = not possible to calculate this estimate from the concentration data available.

Pharmacokinetic Parameter Estimates (Mean,n=2)
Week 25

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T½el h	Kel 1/h	AUC (0-t) ng.h.ml ⁻¹	AUC (0-∞) ng.h.ml ⁻¹	Rsq
2.5	Male	ND	0	ND	ND	0	0	ND
	Female	0.5	1.405	NC	NC	0.8279	NC	NC
7.5	Male	0.5	1.475	27.54	0.0252	4.433	24.36	0.04
	Female	0.5	6.41	1.66	0.418	11.75	12.95	0.98
15	Male	0.5	2.125	2.46	0.2816	6.618	8.399	0.95
	Female	0.5	24	3.04	0.2278	35.42	37.82	1

ND = estimate is not determinable. No quantifiable concentrations were reported for the profile.
NC = not possible to calculate this estimate from the concentration data available.

TK Summary for HTBZ:

Pharmacokinetic Parameter Estimates (Mean,n=2)
Day 1

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T½el h	Kel 1/h	AUC (0-t) ng.h.ml ⁻¹	AUC (0-∞) ng.h.ml ⁻¹	Rsq
2.5	Male	1.5	91.9	2.48	0.2791	259.6	269	1
	Female	1.5	38.15	2.12	0.3271	146.6	151.2	1
7.5	Male	1	127	2.15	0.3223	381	393.5	0.99
	Female	1.5	72.2	1.89	0.3659	297.3	304.1	0.99
15	Male	1	576	1.79	0.3889	1491	1514	0.99
	Female	1	278.5	1.97	0.3513	766.7	781.7	0.98

Pharmacokinetic Parameter Estimates (Mean,n=2)
Week 5

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T½el h	Kel 1/h	AUC (0-t) ng.h.ml ⁻¹	AUC (0-∞) ng.h.ml ⁻¹	Rsq
2.5	Male	1.5	95.35	1.4	0.4953	311.2	313.3	1
	Female	1.5	15.15	3.81	0.1818	43.93	48.64	0.96
7.5	Male	1.5	104.6	4.38	0.1583	850.3	1028	0.86
	Female	1	37.85	2.81	0.2464	163.7	176.5	1
15	Male	1.5	765	1.71	0.4059	2277	2310	0.99
	Female	1	302	2.68	0.2587	568.1	599.7	1

Pharmacokinetic Parameter Estimates (Mean,n=2)
Week 13

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T½el h	Kel 1/h	AUC (0-t) ng.h.ml ⁻¹	AUC (0-∞) ng.h.ml ⁻¹	Rsq
2.5	Male	0.5	98.85	2.1	0.3307	359.4	370.1	0.99
	Female	0.5	8.901	1.13	0.8158	22.05	22.86	0.99
7.5	Male	0.5	755.5	3.6	0.1925	1892	2138	0.99
	Female	1.5	43.75	3.8	0.1824	167.3	192.7	0.99
15	Male	1	1215	1.4	0.4943	3881	3908	1
	Female	1	232.5	2.34	0.2959	670.5	695.3	0.92

Pharmacokinetic Parameter Estimates (Mean,n=2)
Week 25

Dose Level (mg.kg ⁻¹ .bid)	Sex	Tmax (obs) (h)	Cmax (obs) (ng.ml ⁻¹)	T½el h	Kel 1/h	AUC (0-t) ng.h.ml ⁻¹	AUC (0-∞) ng.h.ml ⁻¹	Rsq
2.5	Male	1	179	2.31	0.3003	464.5	481.5	0.96
	Female	-	-	8.04	0.0862	26.68	42.29	0.13
7.5	Male	1.5	318	1.45	0.4793	1921	1936	0.92
	Female	0.5	79.85	2.67	0.2598	204.1	215.4	0.99
15	Male	3	440	1.98	0.3531	2886	2765	0.98
	Female	1.5	141.3	2.05	0.3387	492.2	502.3	0.92

- no value available

APPEARS THIS WAY
ON ORIGINAL

Study title: 9-Month Toxicity Study in Dogs**Key study findings:** drug-induced CNS-related clinical observations**Study no.:** — Study # 7425-101**Volume # 15, Module 4,****Conducting laboratory and location:** _____**Date of study initiation:** 22-May-03**GLP compliance:** yes, except: "The interpretation portion of the toxicokinetic analysis was not performed under strict Good Laboratory Practice Regulations." ("the computer systems used to generate the descriptive analyses (e.g., AUC)..." were not GLP compliant).**QA report:** yes (x) no ()**Drug, lot #, and % purity:** Tetrabenazine (TBZ), batch # 105481, with stated purity of 99.3%. Drug was administered in a gelatin capsules (neat). The capsules used for the vehicle control were empty.**Methods**

Doses: 0, 1, 3, and 10 mg/kg/day, QD, via oral capsule (A protocol deviation resulted in the administration of a lower than nominal dose to all groups during wk 25 and 1st day of wk 26 [estimates of decreases: 24-26%-LD, 16-21%-MD, and 18-21%-HD]. This deviation should not affect the integrity of the study.)

Species/strain: beagle dogs from _____ It should also be noted that MDM # H40641 was replaced on day 4 or 5 without explanation. In a submission dated 21-Feb-06, the sponsor provided a response to the Division's request for information about this animal. The sacrifice of this animal on day 4 was unrelated to treatment (humane sacrifice due to a prolapsed penis).

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: oral (gelatin) capsule

Satellite groups used for toxicokinetics or recovery: no satellite TK group and no recovery group

Age: at initiation of treatment, approximately 5-6 months

Weight: at initiation of treatment 5.9 – 9.4 kg for males and 5.2 – 7.7 kg for females

Sampling times: animals dosed daily for 39 weeks (terminal sacrifice was day 275)

Dose level justification: the following was taken directly from the sponsor's report:

In a pilot study, — 7425-100, tetrabenazine was given to dogs at doses of 2.5, 5, 10, or 20 mg/kg/dose per day, administered in two equally divided doses in the morning and in the afternoon, at least 6 hours between doses for up to 15 days. Twenty-mg/kg/dose (40 mg/kg/day) proved uniformly lethal on or before study Day 11. No tetrabenazine-related mortality occurred in the dogs dosed at 2.5, 5, and 10 mg/kg/dose. Tetrabenazine-related clinical signs included tremors and hypoactivity (2.5, 5, 10 mg/kg/dose); recumbency (male 2.5, 5, 10 mg/kg/dose); hunched posture (male 2.5, 10 mg/kg/dose); repetitive behavior (male 5, 10 mg/kg/dose); aggression (male 2.5, female 5, female 10 mg/kg/dose); pupillary constriction (2.5, 5, 10 mg/kg/dose); and reddened conjunctiva (10 mg/kg/dose), gums (female 2.5, 5, 10 mg/kg/dose), and ears (male 2.5, 5, 10 mg/kg/dose). In general, changes observed at more than one dose level occurred more frequently, and persisted longer as the dose level increased. There were no tetrabenazine-related changes in body temperature, blood pressure, body weight, hematology, coagulation, clinical chemistry, organ weight, or macroscopic data for these animals. Based on these results, dose levels of 1, 3, and 10 mg/kg/day were selected for the present 9-month study.

Results:

Mortality: One HDF (#H40661) was sacrificed in moribund condition during Wk 32 (Day 224). The sponsor's summary of this dog's condition follows:

"This dog exhibited tetrabenazine-related effects that included muzzle swelling, tremors, hypoactivity, recumbency, stereotypical behavior (notably head pressing and chewing), excessive salivation, panting and reddened tissues (notably muzzle, gums and inner ears). Clinical pathology test results were comparable to control values at the scheduled sample collection intervals. Macroscopic and microscopic tissue examination did not identify a specific anatomical cause of morbidity, however; based on the clinical signs, the morbidity is considered tetrabenazine-related."

The summary above conflicts with the separate veterinary report for this animal that describes recurrent problem with oral lesions (Days 108, 122, 168 and 224) requiring intervention (treatment with Augmentin® for the first three occasions and moribund sacrifice for the last occasion). The veterinary treatment entries for this animal note (1) on Day 108 necrotizing stomatitis with a guarded prognosis (animal treated), (2) on Day 122 notes that the size of the wound had improved; however, the "excessive stereotypical chewing" had caused the wound to bleed (animal treated), (3) on Day 168 small deep oral ulcer (animal treated), (4) on Day 224 "Severe, necrotic deep oral ulcer left buccal mucosa; ulceration right buccal mucosa. Dog has history of ulceration and has been treated previously. Dog head presses, vocalizes, etc. Hair loss on bridge of nose from head pressing, permanently swollen bridge of nose. Necrotic odor in mouth. Euthanized due to poor prognosis and for humane concerns." It should also be noted the sore in the oral cavity was noted at necropsy and histopathologically described as minimal multifocal edema, hemorrhage and acute inflammation. The TK parameters (TBZ, α -HTBZ and β -HTBZ) for this animal on Day 1 and Week 13 (the only data available) were unremarkable.

Clinical signs: Animals were observed twice per day (am and pm) for "mortality, abnormalities, and signs of pain and distress." Daily cageside observations were conducted approximately 60-90 minutes and 5-7 hrs post dose. Detailed observations were conducted prior to study initiation and weekly during the study. The summary table of clinical observations was of limited use because similar entries were generally split into several categories making it difficult to determine the overall incidence (e.g., red conjunctivae contained separate listings for "eye-right", "eye-left", and "eyes").

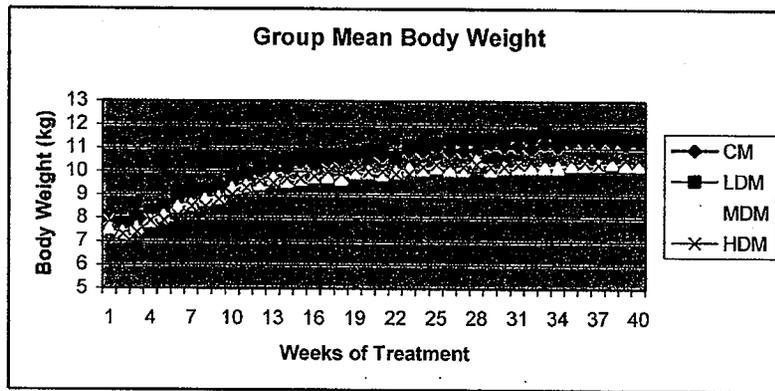
The sponsor describes treatment-related findings as follows:

- "Clinically, tetrabenazine-related changes included tremors (3 and 10 mg/kg/day dogs), hypoactivity (3 and 10 mg/kg/day dogs), recumbency (3 and 10 mg/kg/day dogs), stereotypical behavior (3 mg/kg/day females; 10 mg/kg/day dogs), excessive salivation (10 mg/kg/day dogs), red conjunctivae (3 and 10 mg/kg/day dogs), respiratory changes (labored/panting/rapid) (10 mg/kg/day dogs), reddened skin (notably muzzle, gums, ears) (3 and 10 mg/kg/day dogs). In general, clinical changes were present between 1 and 7 hours post dose and resolved before administration of the next dose."

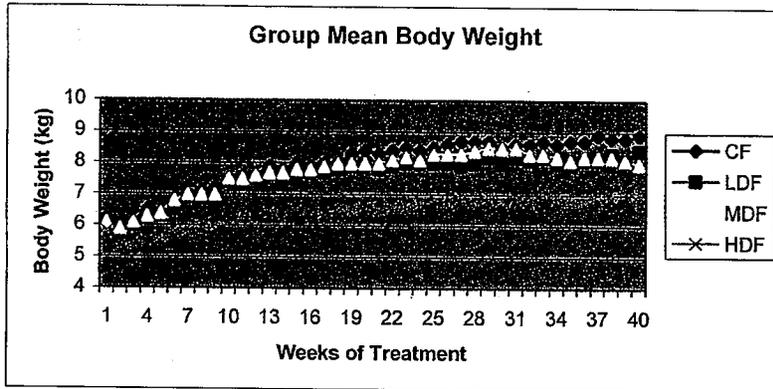
Examination of the data reveals that treatment-related findings were generally confined to the MD and HD groups (with only a single incident of hyperactivity in 1-LDF and a single incident of panting in a LDM) and are presented in the following reviewer-generated table:

Group/Dose	Treatment-Related Findings
Mid Dose 3 mg/kg/day	One male and one female dog were noted with frequent tremors and hypoactivity throughout the study, one male and one female with less frequent tremors and hypoactivity and the remainder with isolated or no occurrences of tremors and hypoactivity. In the most severely affected dogs there were isolated occurrences of <u>limb rigidity</u> (m), recumbency, excessive salivation (m), squinting, clear discharge from the eye, abnormal respiration (panting) (m). Several had infrequent or isolated incidences of red gums and skin (ear), red conjunctiva. Two females were noted with isolated incidences of head pressing. There were isolated incidences of thin appearance and a single incidence of hyperactivity. The notations for the signs were generally confined to the scheduled observation period ranging from 60-90 minutes post dose and 5-7 hrs post dose.
High Dose 10 mg/kg/day	Major treatment related clinical signs in males consisted of frequent hypoactivity and tremors (generally whole body) throughout the duration of the study and less frequent, or isolated incidences of stereotypic behavior (head pressing, barking, growling, biting chewing, digging) hunched posture, recumbency, <u>rigidity of limbs</u> and sensitivity to touch scattered throughout the study. Red gums, skin (particularly the ears) and conjunctiva occurred generally only early in the study. Treatment-related abnormal respiration (panting/labored breathing) and excessive salivation also occurred, but with a later onset in the study. The notations for the signs were generally confined to the scheduled observation period ranging from 60-90 minutes post dose and 5-7 hrs post dose. The signs in females were similar to those noted in males with the addition of <u>frequent squinting eyes, and clear discharge from eyes</u> , infrequent notations of thin appearance, swollen muzzle, isolated ataxia (single occurrence). Female moribund sacrifice was notable due to the frequency of stereotypic behavior (especially headpressing and chewing, vocalization), swollen muzzle, red skin (ears) and gums at additional later points in the study

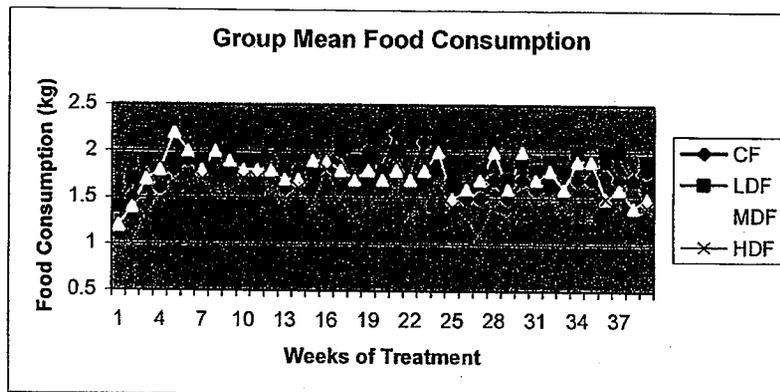
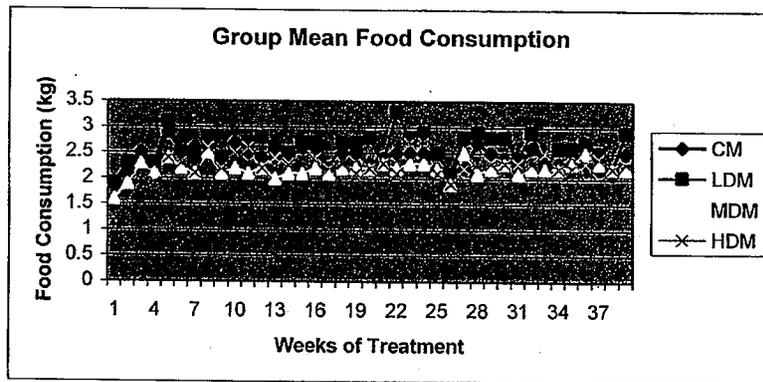
Body weights: Assessed prior to the initiation of treatment, Day 1 and then weekly until the end of the study. According to the sponsor, "While not statistically significant, mean body weights and body weight changes at 3 and 10 mg/kg/day were decreased compared to mean control values." Group mean body weights are depicted in the following reviewer generated figures. The most notable difference was in the HDF throughout the study (recall that one HDF (# 40661) was sacrificed in moribund condition during Wk 32). At wk 40, the mean decrease in body weight compared to the control was 9%-MDM, 7%-HDM, 6%-LDF, 10%-MDF and 20%-HDF (LDM were noted with a 0.9% increase).



Best Possible Copy



Food consumption: Assessed weekly throughout the study (animals had access to food for 6 hrs per day). According to the sponsor, there were “no adverse tetrabenazine-related changes” in food consumption. Examination of the data reveals that the mean weekly food consumption was generally decreased in HDF (see reviewer-generated figures below).



Ophthalmoscopy: not assessed

EKG: not assessed

Best Possible Copy

Blood Pressure Measurements: Assessed once prior to the first day of dosing, and days 1, 91, 179 and 268 of treatment, approximately 60-90 minutes post dose. The technique employed was not mentioned. According to the sponsor, there were “no adverse tetrabenazine-related changes” in blood pressure. A reviewer-generated summary table follows; however, the data seem unreliable due to technical problems. The shaded portions of the table denote groups in which 1, 2, 3 or 4 animals/group had values for the first reading per timepoint that were noted as “out of range” and only the second reading reported. The report does not state when the second reading was conducted (relative to the first reading, or relative to dosing).

Systolic Blood Pressure (mm Hg) (group mean ± SD)						
sex	Group	Day -11	Day 1	Week 13	Week 26	Week 39
Males	C	139 ± 15.4	118 ± 34.4	124 ± 32.6	136 ± 4.3	151 ± 19.9 [2]
	LD	138 ± 50.4 [1]	95 ± 48.1 [3]	134 ± 27.5	134 ± 8.5	133 ± 17.1
	MD	141 ± 20.1 [1]	124 ± 31.4 [2]	117 ± 42.2	157 ± 12.3 [2]	122 ± 34.4 [1]
	HD	150 ± 41.1 [1]	120 ± 39.1	145 ± 18.8	150 ± 12.7	151 ± 8.5
Females	C	109 ± 24.0 [1]	111 ± 28.6 [2]	139 ± 21.3	138 ± 26.1 [1]	144 ± 32.7 [1]
	LD	130 ± 21.1	139 ± 9.8	138 ± 50.5 [4]	163 ± 18.6 [2]	135 ± 56.6 [2]
	MD	115 ± 14.7 [1]	114 ± 20.6	139 ± 13.6 [2]	122 ± 30.1 [1]	126 ± 19.8
	HD	131 ± 15.8 [2]	137 ± 16.0	145 ± 17.8 [1]	132 ± 22.9	146 ± 22.5 (\$)
Diastolic Blood Pressure (mm Hg) (group mean ± SD)						
sex	Group	Day -11	Day 1	Week 13	Week 26	Week 39
Males	C	81 ± 27.5	78 ± 14.8	85 ± 25.5	91 ± 9.9	90 ± 19.9 [2]
	LD	108 ± 25.9 [1]	60 ± 35.1 [3]	65 ± 11.1	72 ± 15.7	81 ± 12.7
	MD	116 ± 19.8 [1]	89 ± 43.1 [2]	72 ± 23.4	97 ± 28.9 [2]	80 ± 14.7 [1]
	HD	84 ± 29.2 [1]	83 ± 29.6	109 ± 21.0	92 ± 18.7	112 ± 12.9
Females	C	65 ± 27.2 [1]	74 ± 29.1 [2]	78 ± 8.2	98 ± 24.8 [1]	85 ± 26.6 [1]
	LD	96 ± 23.5	102 ± 21.1	84 ± 38.2 [4]	76 ± 28.4 [2]	70 ± 28.3 [2]
	MD	85 ± 13.9 [1]	62 ± 21.6	80 ± 35.0 [2]	90 ± 36.8 [1]	98 ± 17.2
	HD	85 ± 33.3 [2]	89 ± 16.8	89 ± 10.6 [1]	89 ± 21.1	90 ± 50.1 (\$)
Mean Arterial Blood Pressure (mm Hg) (group mean ± SD)						
sex	Group	Day -11	Day 1	Week 13	Week 26	Week 39
Males	C	99 ± 20.7	92 ± 20.4	96 ± 27.5	108 ± 9.6	113 ± 19.7 [2]
	LD	116 ± 46.2 [1]	71 ± 38.8 [3]	89 ± 23.8	87 ± 21.0	94 ± 10.3
	MD	123 ± 21.0 [1]	100 ± 39.9 [2]	90 ± 29.2	116 ± 16.5 [2]	98 ± 24.9 [1]
	HD	97 ± 27.7 [1]	100 ± 33.0	120 ± 19.3	107 ± 14.3	125 ± 9.0
Females	C	82 ± 22.1 [1]	87 ± 27.9 [2]	91 ± 8.3	112 ± 20.4 [1]	96 ± 25.7 [1]
	LD	107 ± 22.8	112 ± 14.2	99 ± 41.5 [4]	113 ± 14.0 [2]	88 ± 36.8 [2]
	MD	95 ± 13.9 [1]	81 ± 21.1	100 ± 26.5 [2]	102 ± 33.9 [1]	108 ± 22.1
	HD	94 ± 39.2 [2]	102 ± 15.9	98 ± 10.3 [1]	110 ± 19.4	106 ± 45.3 (\$)

(^)- based on n=3, due to replacement of animal # 40641
 (\$)- based on n=3, due to moribund sacrifice of # 40661 during Wk 32.
 [1], [2], [3], [4] – first reading out of range for 1, 2, 3, or 4 animals/group, respectively; results of second reading reported

Body Temperature: Assessed once prior to the first day of dosing, and days 1, 91, 179 and 268 of treatment, approximately 60-90 minutes post dose. The technique employed was not described. According to the sponsor, there were “no adverse tetrabenazine-related changes” in body temperature. Examination of the data suggest a slight treatment-related increase in mean body temperature for HDM during Wk 26 and Wk 39 and for one HDF (#40659) during Wk 13 (BT for this animal decreased at subsequent time points). See the reviewer-generated table follows:

		Body Temperature (group mean \pm SD)				
sex	Group	Day -11	Day 1	Week 13	Week 26	Week 39
Males	C	38.5 \pm 0.38	38.4 \pm 0.27	38.2 \pm 0.44	38.0 \pm 0.57	38.2 \pm 0.21
	LD	37.2 \pm 2.49	38.4 \pm 0.06	38.6 \pm 0.31	38.0 \pm 1.24	38.6 \pm 0.14
	MD	38.4 \pm 0.28	38.3 \pm 0.35 (^)	38.5 \pm 0.31	38.3 \pm 0.91	38.3 \pm 0.36
	HD	38.6 \pm 0.33	38.6 \pm 0.32	38.5 \pm 0.50	38.6 \pm 0.61	38.9 \pm 0.38
Females	C	38.6 \pm 0.25	38.5 \pm 0.22	38.6 \pm 0.32	38.0 \pm 1.05	38.5 \pm 0.17
	LD	38.4 \pm 0.28	38.7 \pm 0.34	38.6 \pm 0.26	38.5 \pm 0.90	38.6 \pm 0.41
	MD	38.2 \pm 0.12	38.5 \pm 0.13	38.4 \pm 0.17	38.3 \pm 0.52	38.4 \pm 0.42
	HD	38.4 \pm 0.45	38.2 \pm 0.50	38.9 \pm 0.80	37.9 \pm 0.41	38.6 \pm 0.25 (\$)

(^)- based on n=3, due to replacement of animal # 40641
 (\$)- based on n=3, due to moribund sacrifice of # 40661 during Wk 32.

Hematology/Coagulation: Blood samples were taken prior to the initiation of treatment, then after at least 13 wks and 39 wks of treatment from overnight fasted animals (via jugular vein). The following parameters were assessed: erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count and differential, blood smear, reticulocyte smear (prepared but not evaluated), prothrombin time and activated partial thromboplastin time. According to the sponsor, "No toxicologically relevant test article-related changes occurred... at any interval. Statistically significant changes occurred in a few clinical pathology variables, but are considered spurious because of the small magnitude of change, the direction of change was not biologically relevant, they occurred at the pretreatment interval, or they were comparable to the pretreatment values for the same group." Examination of the data suggests that at Wk 40 there was a slight decrease in WBC for both males and females in the HD (total, lymphocytes, neutrophils, and eosinophils in males and females, and monocytes and basophils in females).

Clinical chemistry: Blood samples were taken prior to the initiation of treatment, then after at least 13 wks and 39 wks of treatment from overnight fasted animals (via jugular vein). The following parameters were assessed: glucose, urea nitrogen, creatinine, total protein, albumin, cholesterol, total bilirubin, alanine aminotransferase, gamma glutamyltransferase, aspartate aminotransferase, calcium, inorganic phosphorus, sodium, potassium, chloride. According to the sponsor, "No toxicologically relevant test article-related changes occurred... at any interval. Statistically significant changes occurred in a few clinical pathology variables, but are considered spurious because of the small magnitude of change, the direction of change was not biologically relevant, they occurred at the pretreatment interval, or they were comparable to the pretreatment values for the same group." Examination of the data revealed no clear effect of treatment. AST was increased (\approx 10x baseline) in one HDF (#40659) at wk 14 (returned to \approx baseline at wk 40, no related histopathology). ALT was increased at wk 40 in one LDM (#40638) (\approx 5x baseline, no related histopathology) and one HDM (#40646) (\approx 4x baseline, no related histopathology).

Urinalysis: not assessed

Gross pathology: Animals were fasted overnight, anesthetized, exsanguinated, and necropsied. According to the sponsor, there were "no adverse tetrabenazine-related changes" in macroscopic pathology. Thickening of the mammary gland, uterine wall and vaginal wall occurred only in tetrabenazine treated animals, but with no relationship to dose (see reviewer-generated table that follows for details). The thickening of the mammary gland and uterine wall were not associated with abnormal histopathology. The single incidence of thickening of the vaginal wall was associated with moderate, diffuse edema.

Gross Pathology of Uterus, Vagina and Mammary Gland									
group		Males				Females			
		CM	LDM	MDM	HDM	CF	LDF	MDF	HDF
dose (mg/kg/day)		0	1	3	10	0	1	3	10
mammary gland	thicken, moderate, all regions					0/4	2/4	1/4	1/3 + 1/1*
uterus	wall thickened, moderate, both horns					0/4	4/4	0/4	1/3 + 0/1*
vagina	wall thickened, moderate					0/4	1/4	0/4	0/3 + 0/1*

* - refers to the moribund sacrifice on Day 224

Organ weights: adrenals, brain, epididymis, heart, kidneys, liver with gallbladder (drained), ovaries, spleen, testes, thymus, thyroids and parathyroids. According to the sponsor, there were “no adverse tetrabenazine-related changes” in absolute or relative (to brain and body weight) organ weights. Examination of individual organ weights was precluded due to the lack of table listing (individual animal data were listed only on the necropsy report for each animal). Thus, interpretation of this parameter was based on group mean data. In the reviewer’s analysis (see table below for details), organ weight relative to brain weight was not considered, since there was an apparent, slight decrease in absolute brain weight in HDM and HDF (not associated with abnormal histopathology). In the HD there were slight decreases in the absolute and relative weight of the testes and epididymides that were not associated with abnormal pathology. Relative adrenal weight is increased for HDM and HDF; however, this was not accompanied by abnormal histopathology. In HDM the absolute and relative thyroid/parathyroid weight was increased; however, the only pathology noted in HDM and not also seen in CM was a single HDM with an ultimobranchial cyst (which is not related to treatment). It is worth noting that absolute and relative thymus weight were effected to a smaller degree than might be expected based on the severity of the treatment-related clinical signs observed throughout the study in the HD group (and to a lesser extent, the MD group), and effect on body weight in the HDF (20% decreased compared to control).

Notable Changes in Organ Weight									
group		Males				Females			
		CM	LDM	MDM	HDM	CF	LDF	MDF	HDF
n		4	4	4	4	4	4	4	3
body wgt	abs	11,275 ± 1,417	11,350 ± 1,150	10,300 ± 1,319	10,450 ± 988	8,850 ± 900	8,350 ± 1,085	8,000 ± 1,525	7,100 ± 781
brain	abs	78.5 ± 1.08	78.8 ± 3.93	75.8 ± 8.46	74.7 ± 2.58	74.1 ± 3.26	72.1 ± 7.52	72.7 ± 12.01	68.7 ± 10.29
	:BW	0.70 ± 0.090	0.70 ± 0.054	0.75 ± 0.150	0.72 ± 0.077	0.84 ± 0.097	0.88 ± 0.159	0.91 ± 0.063	0.97 ± 0.072
thyroid/parathyroid	abs	0.95 ± 0.224	0.97 ± 0.393	0.87 ± 0.210	1.24 ± 0.228	0.75 ± 0.123	0.76 ± 0.156	0.76 ± 0.199	0.73 ± 0.089
	:BW	0.008 ± 0.0019	0.009 ± 0.0039	0.008 ± 0.0016	0.012 ± 0.0021	0.008 ± 0.009	0.009 ± 0.0027	0.010 ± 0.0038	0.010 ± 0.0018
adrenal	abs	1.38 ± 0.021	1.26 ± 0.185	1.23 ± 0.144	1.46 ± 0.475	1.24 ± 0.237	1.19 ± 0.207	1.12 ± 0.250	1.18 ± 0.182
	:BW	0.012 ± 0.0015	0.011 ± 0.0013	0.012 ± 0.0011	0.014 ± 0.0039	0.014 ± 0.0030	0.014 ± 0.0034	0.014 ± 0.0024	0.017 ± 0.0016
thymus	abs	7.09 ± 1.540	4.53 ± 1.238	5.89 ± 0.802	6.05 ± 4.632	5.48 ± 1.442	4.58 ± 1.061	4.76 ± 3.499	4.73 ± 2.039
	:BW	0.063 ± 0.0102	0.040 ± 0.0104	0.057 ± 0.0044	0.057 ± 0.0391	0.061 ± 0.0121	0.055 ± 0.0139	0.057 ± 0.0369	0.067 ± 0.0305
testis	abs	15.9 ± 1.44	18.4 ± 2.67	15.5 ± 4.26	14.0 ± 2.44				
	:BW	0.14 ± 0.024	0.16 ± 0.018	0.15 ± 0.048	0.13 ± 0.014				
Epidid	abs	3.83 ± 0.243	3.83 ± 0.758	3.49 ± 0.159	3.43 ± 0.455				
	:BW	0.034 ± 0.0062	0.034 ± 0.0074	0.034 ± 0.0060	0.033 ± 0.0025				

abs = absolute weight

BW – weight relative to body weight

Histopathology: Although a separate pathologist’s report was not provided, the study pathologist’s signature is present on the general study report. The tissues listed in the appended table were preserved for all animals. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, stained with H&E and examined by light microscopy. Tissues were examined from all animals. CNS histopathologic examination consisted of the “brain (including substantia nigra)” and spinal cord (cervical, thoracic, and lumbar).

Adequate Battery: yes (), no (X). The sponsor did not preserve or examine the full list of tissues expected for a chronic toxicity study in non-rodents. Notable tissues missing

from sampling/examination include: bone marrow smear, eyes/optic nerves, vagina, cervix, lachrymal gland, larynx, nasal cavity, pharynx, skin, and tongue. (the fallopian tube was not sampled; however, that is less commonly studied).

Peer review: yes (), no (X)

Treatment-related thymic lymphoid depletion was noted in HDM (slight-moderate) and in one MDF (moderate/severe) (see table below for details), and there was a surprising lack of thymic atrophy in HDF, considering the frequency and severity of the clinical observations and the effect on body weight (20% decreased compared to control). Lymphohistiocytic infiltration of the prostate was noted in one LDM and one HDM, and the severity of this low incidence finding increased with increasing dose. Other findings (not listed in the table), such as focal or multifocal hemorrhage of the lung, alveolar macrophage infiltrate in lung, and proteinaceous cast in kidney were low incidence and of minimal severity. There were no treatment related findings in the brain.

Histopathology: Potentially Treatment-Related Findings									
		Males				Females			
Group		CM	LDM	MDM	HDM	CF	LDF	MDF	HDF
dose (mg/kg/day)		0	1	3	10	0	1	3	10
thymus – depletion, lymphocytic ^ - multifocal, \$-diffuse	total	0/4	0/4	0/4	3/4	0/4	0/4	1/4	0/3 + 0/1§
	minimal				0			0	
	slight				2^			0	
	moderate				1^			0	
	mod/severe							1\$	
prostate - infiltrate, lymphohistiocytic (focal)	total	0/4	1/4	0/4	1/4				
	minimal		0		0				
	slight		1		0				
	moderate				1				

§ - refers to the moribund sacrifice on Day 224
 The sponsor describes the severity scale as follows:

- minimal – the least amount of change observed with the light microscope
- slight – less than average amount of change, but readily discernable as abnormal
- moderate – the average amount of change expected for a lesion
- moderately severe (marked) – a marked amount of change with possible loss of function of the affected cells or organs
- severe – a great amount of change with a possible loss of function of the affected cells or organs and frequently involves large areas of the organ.

Toxicokinetics: Blood samples (via jugular vein) were obtained on Day 1, Week 13 and Week 39 at the following timepoints: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hrs post dose. Samples obtained from the control group were discarded without processing. Plasma was separated; the samples were frozen and shipped to _____ for assessment for tetrabenazine, and α -dihydro-tetrabenazine and β -dihydro-tetrabenazine. After the 24 hr sample, animals were administered Lactated Ringers solution (via sc route).

The sponsor describes the results of the TK analysis as follows (see the accompanying sponsor-generated summary tables):

- “The rate and extent of exposure to tetrabenazine and α - and β -HTBZ, as measured by C_{max} and AUC_{∞} and/or AUC_{0-24} , respectively, increased in a dose-related (β -HTBZ) or greater than dose-related (tetrabenazine, α -HTBZ) manner over doses ranging from 1 to 10 mg/kg/day for 39 weeks. Taking into consideration the small number of animals and the subsequent variability, there did not appear to be a difference between male and female dogs with respect to plasma

concentrations, C_{max} , or AUC on Day 1. In general, however, at Weeks 13 and/or 39 male dogs appeared to have higher plasma concentrations and mean C_{max} and AUC_{0-24} for tetrabenazine, α -HTBZ, and/or β -HTBZ than did female dogs. The ratios of α -HTBZ to tetrabenazine ranged from 0.66 to 2.21 and those for β -HTBZ to tetrabenazine from 1.09 to 6.14 and both were reasonably independent of the dose and duration of dosing. The exposure to β -HTBZ was greater than that for α -HTBZ, with α/β ratios ranging from 0.18 to 0.80.”

Review of the data reveals highly variable TK. Exposure increased with increasing dose, with no obvious indication of saturation of absorption or metabolism. In general, at wks 13 and 39, exposure (based on AUC) to the three quantified drug-related components was greatest for β -HTBZ, then α -HTBZ, and then TBZ (except for LDF at wk 13 for which the order was [β β -HTBZ > TBZ > α -HTBZ]).

Tetrabenazine

Parameter ¹	1 mg/kg/day		3 mg/kg/day		10 mg/kg/day	
	Female	Male	Female	Male	Female	Male
Day 1						
C_{max} (ng/mL)	73.5 ± 12.4	19.6 ± 15.1	144 ± 159	87.1 ± 51.2	375 ± 277	502 ± 328
T_{max} (h)	0.50	1.25	0.75	1.25	0.75	1.25
AUC_{0-24} (h*ng/mL)	89.8 ± 38.4	39.7 ± 26.7	265 ± 323	184 ± 50.0	707 ± 537	988 ± 325
AUC _r (h*ng/mL)	95.9 ± 49.9	33.0 ± 11.8	429 ± 500	195 ± 56.7	735 ± 555	1,020 ± 341
$t_{1/2}$ (h)	3.17 ± 1.01	3.99 ± 2.43	8.19 ± 0.25	5.37 ± 1.95	6.49 ± 0.38	5.31 ± 0.98
Week 13						
C_{max} (ng/mL)	29.6 ± 10.3	56.3 ± 61.9	121 ± 78.0	128 ± 80.2	189 ± 107	478 ± 408
T_{max} (h)	1.00	2.75	0.75	1.00	1.00	2.50
AUC_{0-24} (h*ng/mL)	51.2 ± 13.8	114 ± 52.0	254 ± 143	293 ± 155	621 ± 119	1,298 ± 1,209
$t_{1/2}$ (h)	3.25 ± 0.90	6.71 ± 5.41	6.67 ± 2.49	7.47 ± 2.45	5.48 ± 0.64	6.46 ± 1.38
Week 39						
C_{max} (ng/mL)	21.2 ± 2.72	31.0 ± 25.2	103 ± 59.8	123 ± 103	149 ± 189	299 ± 76.2
T_{max} (h)	2.25	2.75	3.00	2.00	2.50	1.00
AUC_{0-24} (h*ng/mL)	80.7 ± 34.2	106 ± 69.5	388 ± 100	318 ± 184	1,024 ± 169	1,144 ± 515
$t_{1/2}$ (h)	— ²	3.28 ± 2.05	7.19 ± 1.55	6.09 ± 1.38	6.51	5.17 ± 0.87

¹Mean ± standard deviation except for T_{max} for which the median is reported. If N = 1, then the individual value is reported.
²No values were estimated.

Best Possible Copy

**APPEARS THIS WAY
ON ORIGINAL**

α-HTBZ

Parameter ¹	1 mg/kg/day		3 mg/kg/day		10 mg/kg/day	
	Female	Male	Female	Male	Female	Male
Day 1						
C _{max} (ng/mL)	35.2 ± 12.7	10.3 ± 6.30	69.2 ± 58.0	51.6 ± 25.3	198 ± 132	215 ± 109
T _{max} (h)	0.75	1.75	1.00	1.50	1.26	1.50
AUC ₀₋₁ (h•ng/mL)	62.6 ± 33.8	37.6 ± 35.3	213 ± 230	154 ± 34.6	664 ± 474	803 ± 301
AUC ₀₋₂₄ (h•ng/mL)	69.1 ± 44.0	31.5	282 ± 270	172 ± 41.0	886 ± 377	849 ± 341
t _{1/2} (h)	2.12 ± 1.69	3.33	6.54 ± 2.40	4.54 ± 0.49	4.57 ± 1.85	5.02 ± 1.91
Week 13						
C _{max} (ng/mL)	18.4 ± 4.46	23.8 ± 17.17	68.6 ± 15.8	69.2 ± 43.9	131 ± 65.8	277 ± 159
T _{max} (h)	1.00	2.75	1.50	1.00	2.25	3.50
AUC ₀₋₂₄ (h•ng/mL)	40.3 ± 23.6	133 ± 91.2	298 ± 241	373 ± 277	748 ± 72.7	1,680 ± 1,801
t _{1/2} (h)	2.32 ± 1.04	13.1 ± 13.5	4.72 ± 1.17	3.59 ± 0.61	4.15 ± 0.35	3.53 ± 1.30
Week 39						
C _{max} (ng/mL)	21.7 ± 6.09	18.6 ± 17.4	109 ± 55.4	80.8 ± 41.6	171 ± 145	327 ± 67.0
T _{max} (h)	2.51	2.75	4.00	2.00	2.50	2.00
AUC ₀₋₂₄ (h•ng/mL)	80.1 ± 8.37	151 ± 179	561 ± 277	463 ± 261	1,429 ± 1,070	2,531 ± 1,976
t _{1/2} (h)	3.33	3.20 ± 1.71	5.05 ± 1.42	4.18 ± 0.72	4.06 ± 0.37	3.82 ± 0.69

¹Mean ± standard deviation except for T_{max} for which the median is reported. If N = 1, then the individual value is reported.

β-HTBZ

Parameter ¹	1 mg/kg/day		3 mg/kg/day		10 mg/kg/day	
	Female	Male	Female	Male	Female	Male
Day 1						
C _{max} (ng/mL)	97.1 ± 28.7	40.4 ± 28.1	218 ± 220	185 ± 105	578 ± 367	693 ± 382
T _{max} (h)	0.75	1.50	1.00	1.50	1.25	1.25
AUC ₀₋₁ (h•ng/mL)	135 ± 65.6	120 ± 104	447 ± 489	318 ± 58.8	1,446 ± 968	2,091 ± 758
AUC ₀₋₂₄ (h•ng/mL)	139 ± 68.8	177 ± 167	468 ± 493	329 ± 71.9	1,512 ± 1,177	2,127 ± 780
t _{1/2} (h)	2.18 ± 1.49	2.10 ± 1.52	4.49 ± 1.33	3.02 ± 0.25	5.91 ± 1.86	3.86 ± 1.37
Week 13						
C _{max} (ng/mL)	68.2 ± 20.6	52.6 ± 29.6	229 ± 47.5	227 ± 128	420 ± 86.8	972 ± 826
T _{max} (h)	1.00	2.25	1.51	0.76	2.25	3.25
AUC ₀₋₂₄ (h•ng/mL)	98.7 ± 35.3	166 ± 157	852 ± 669	735 ± 411	2,635 ± 1,465	6,688 ± 9,470
t _{1/2} (h)	2.07 ± 1.03	1.49 ± 0.75	3.99 ± 1.54	5.23 ± 1.07	4.02 ± 1.10	4.54 ± 0.36
Week 39						
C _{max} (ng/mL)	35.7 ± 10.5	72.3 ± 70.8	232 ± 159	257 ± 136	480 ± 409	1,001 ± 261
T _{max} (h)	2.00	2.75	3.00	2.00	2.75	2.25
AUC ₀₋₂₄ (h•ng/mL)	139	303 ± 306	789 ± 584	845 ± 249	4,053 ± 4,428	7,022 ± 5,522
t _{1/2} (h)	— ²	1.49 ± 0.40	2.33 ± 1.18	4.79 ± 0.87	3.07 ± 1.45	3.33 ± 0.03

¹Mean ± standard deviation except for T_{max} for which the median is reported. If N = 1, then the individual value is reported.
²No values were estimated.

Best Possible Copy

APPEARS THIS WAY
ON ORIGINAL

Histopathology Inventory

Study	13/26 wk	39-wk
Species	rat	dog
Adrenals	x*	x*
Aortic arch or aorta	x	x
Bone Marrow smear (femur)	onr	
Bone (femur)		
Brain	x*^	x*\$
Cecum	x	x
Cervix		
Colon	x	x
Duodenum	x	x
Epididymis	x*	x*
Esophagus	x	x
Eye	x (1)	
Fallopian tube		
Gall bladder		x
Gross lesions	x	x
Harderian gland		
Heart	x*	x*
Ileum	x	x
Injection site		
Jejunum	x	x
Kidneys	x*	x*
Lachrymal gland		
Larynx		
Liver	x*	x*
Lungs	x*	x
Lymph nodes, cervical		
Lymph nodes submandibular	x	
Lymph nodes, mesenteric	x	x
Mammary Gland	x	x (f)
Nasal cavity		
Optic nerves	x (1)	
Ovaries	x*	x*
Pancreas	x	x
Parathyroid	x*	x
Peripheral nerve		
Pharynx		
Pituitary	x*	x
Prostate	x*	x
Rectum	x	x
Rib		
Salivary gland (mandibular)	x*	x
Sciatic nerve	x	x
Seminal vesicles	x	
Skeletal muscle	x	x
Skin	x	
Spinal cord (3 levels)	x	x
Spleen	x*	x*

Study	13/26 wk	39-wk
Species	rat	dog
Sternum (including marrow)	x	x
Stomach	x	x
Testes	x*	x*
Thymus	x*	x*
Thyroid	x*	x*
Tongue	x	
Trachea	x	x
Urinary bladder	x	x
Uterus	x*	x
Vagina	x	
Zymbal gland		

X, histopathology performed

onr - obtained, not read

^ - forebrain, midbrain, cerebellum and pons

\$ - no details except that it includes substantia nigra

*, organ weight obtained

**APPEARS THIS WAY
ON ORIGINAL**

2.6.6.4 Genetic toxicology

Study title: Tetrabenazine – testing for mutagenic activity with *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2uvrA

Key findings: negative

Study no.: _____ Report # 19082

Volume #19, module 4

Conducting laboratory and location: _____

Date of study initiation: 31-Jul-00

GLP compliance: yes, OECD-GLP

QA reports: yes (x) no () The QA statement indicates that they relied on process inspections for this type of short term study. The report was QAed and "...is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data."

Drug, lot #, and % purity: Tetrabenazine, batch # 99501, chromatographic purity by HPLC is 99.9%. TBZ was dissolved in and diluted with DMSO.

Methods

Strains/species/cell line: *Salmonella typhimurium* strains: TA1535, TA1537, TA98, and TA100.
Escherichia coli strain: WP2uvrA.

Doses used in definitive study: 10, 33.3, 100, 333.3, 1000 and 3333.3 µg per plate.

Basis of dose selection: toxicity test in strain TA 100 (single cultures) at 17, 50, 167, 500, 1667 and 5000 µg/plate in the presence and absence of metabolic activation. At 5000 µg/plate (+S9, -S9) lawn thinning was noted, as was precipitation ("Unable to count colonies...due to heavy precipitation." The next highest concentration tested, 1667 µg/plate, was not associated with precipitate or toxicity).

Vehicle control: DMSO

Positive controls:

agent	solvent	concentration	S9	strains
sodium azide	water	1 µg/plate	- S9	TA1535, TA100
9-aminoacridine	DMSO	80 µg/plate	- S9	TA1537
2-nitrofluorene	DMSO	1 µg/plate	- S9	TA98
N-ethyl-N-nitro-N-nitrosoguanidine	DMSO	2 µg/plate	- S9	WP2 uvrA
2-aminoanthracene	DMSO	0.5, 2 or 20 µg/plate	+ S9	TA98, TA100, TA1535, TA1537 & WP2 uvrA

Metabolic activation system: S9 from the livers of male Fischer 344 rats treated with Aroclor 1254 (prepared and stored frozen until use). Enzyme activity of batch FL1091 was tested in TA 1538 with a variety of pre-mutagens in 20-Apr-00, (≈ 3 months prior to assay). No concurrent testing with any mutagen but 2-AA (although at a variety of doses).

Incubation and sampling times: For the direct plating method the incubation time was 2 or 3 days at 37°C. For the preincubation method the incubation period was 20 minutes prior to plating then 2 or 3 days at 37°C.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

- For each of the two main assays, plates were run in triplicate.
- "... the colonies were counted using a _____ Colony Counter _____) set a maximum sensitivity, i.e., colonies of 0.1 mm or more were counted. The plates were also examined for precipitates and, microscopically, for microcolony growth."
- The sponsor's evaluation criteria for a mutagenic response follows:

- i) for *S. typhimurium* strains TA 1535, TA 1537, and TA 98 and for *E. coli*, at least a doubling of the mean concurrent vehicle control values at some concentration of the test material. For *S. typhimurium* strain TA 100, a 1.5-fold increase over the control value was considered significant. If the mean colony count on the vehicle control plates was less than 10, then a value of 10 was assumed for assessment purposes. In such cases, a minimum count of 20 was required before a significant mutagenic response was registered.
- ii) a dose related response, although at high dose levels this relationship could be inverted because of, for example, (1) toxicity to the bacteria generally, (2) specific toxicity to the mutants and (3) inhibition of foreign compound metabolising enzymes where mutagens require metabolic activation by the liver.
- iii) a reproducible effect in independent tests.

Study outcome: The assays were adequate and negative, although, there were some technical problems with the testing of WP2uvrA (see the reviewer-generated summary tables for details). There were no results for WP2uvrA in the first assay due to an error in agar preparation that seems to have affected only this one bacterial strain. In the second assay (employing the pre-incubation method), there were problems with the positive control for WP2uvrA; (1) in the absence of metabolic activation two of the three triplicate plates had revertant counts below the lab's historical control (hc) range (102, 112 and 152 compared to hc range of 125-804; 387 ± 147), and (2) in the presence of metabolic activation one the three triplicate plates also had a revertant count below the lab's historical control range (36, 284, and 261 compared with the hc range of 149-859; 551 ± 156). In this assay, in the presence of metabolic activation there appears to be a concentration related increase in mean revertants compared to the vehicle control with a the mean response that was 2.2x the vehicle control at the TBZ concentration of 333.3 $\mu\text{g}/\text{plate}$. The two higher concentrations, 1000 and 3333.3 $\mu\text{g}/\text{plate}$, were noted with a slight thinning of the lawn and/or precipitate (there was no statement that the presence of the precipitate interfered with the scoring), and the mean revertant counts were 1.6x and 1.5x the vehicle control, respectively. Although in isolation, the revertant count at 333.3 $\mu\text{g}/\text{plate}$ would be considered a positive response, there was no evidence of a positive response at the higher doses. In addition, the retest of the direct plate method in this strain did not produce any evidence of a positive response.

It should be noted that according to the current OECD guidelines for this assay, "2-Aminoanthracene should not be used as the sole indicator of the efficacy of the S-19 mix. If 2-aminoanthracene is used, each batch of S9 should also be characterized with a mutagen that requires metabolic activation by microsomal enzymes, e.g., benzo(a)pyrene, dimethylbenzanthracene." This was done with the lot of S9 used, but it was not done concurrent to the assay. It was conducted approximately 3 months prior to the conduct of the assay.

1 st Assay – direct plate method (mean revertant colony count per plate ± SD)						
test substance	concentration (µg/plate)	TA 98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
absence of metabolic activation						
Vehicle	0	10 ± 6	72 ± 12	8 ± 2	2 ± 2	n.c.a.
TBZ	10	8 ± 3	91 ± 2	8 ± 4	4 ± 2	n.c.a.
TBZ	33.3	9 ± 1	84 ± 11	8 ± 1	4 ± 3	n.c.a.
TBZ	100	10 ± 0	92 ± 14	9 ± 5	4 ± 1	n.c.a.
TBZ	333.3	9 ± 1	58 ± 9	8 ± 4	3 ± 2	n.c.a.
TBZ	1000	10 ± 3	59 ± 9	6 ± 2	4 ± 3	n.c.a.
TBZ	3333.3 (P)	10 ± 3 (STL)	64 ± 5	5 ± 1	1 ± 1 (STL)	n.c.a.
positive control	variable	472 ± 25	745 ± 14	223 ± 23	2708 ± 132	n.c.a.
presence of metabolic activation						
Vehicle	0	15 ± 4	87 ± 18	8 ± 2	5 ± 1	n.c.a.
TBZ	10	14 ± 1	95 ± 7	12 ± 3	4 ± 1	n.c.a.
TBZ	33.3	15 ± 1	84 ± 4	9 ± 4	4 ± 3	n.c.a.
TBZ	100	14 ± 1	91 ± 13	9 ± 3	4 ± 1	n.c.a.
TBZ	333.3	11 ± 2	104 ± 6	8 ± 2	7 ± 1	n.c.a.
TBZ	1000	18 ± 5	99 ± 14	7 ± 4	5 ± 1	n.c.a.
TBZ	3333.3 (P)	5 ± 1 (STL)	98 ± 9	4 ± 2	1 ± 1 (STL)	n.c.a.
positive control	variable	328 ± 35	863 ± 60	277 ± 15	326 ± 49	n.c.a.

n.c.a. – “no counts available for WP2*uvrA* due to an error in the agar preparation.”
(TL) – thin lawn; (STL) – slightly thin lawn; (VTL) – very thin lawn; (P) – precipitation

2 nd Assay – pre-incubation method (mean revertant colony count per plate ± SD)						
test substance	concentration (µg/plate)	TA 98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
absence of metabolic activation						
vehicle	0	13 ± 4	114 ± 17	26 ± 9	7 ± 4	6 ± 3
TBZ	10	11 ± 2	112 ± 4	16 ± 5	6 ± 3	7 ± 1
TBZ	33.3	15 ± 4	110 ± 2	15 ± 1	4 ± 2	7 ± 1
TBZ	100	11 ± 2	104 ± 4	27 ± 8	6 ± 3	11 ± 6
TBZ	333.3	9 ± 1	124 ± 12	20 ± 9	6 ± 0	8 ± 3
TBZ	1000 (P)	14 ± 5 (TL)	111 ± 6 (TL)	21 ± 4 (STL)	6 ± 3 (TL)	5 ± 2 (TL)
TBZ	3333.3 (P)	10 ± 3 (VTL)	104 ± 8 (VTL)	21 ± 12 (TL)	4 ± 3 (VTL)	5 ± 1 (VTL)
positive control	variable	452 ± 21	639 ± 18	247 ± 20	2023 ± 357	122 ± 26
presence of metabolic activation						
vehicle	0	18 ± 4	124 ± 9	12 ± 1	7 ± 3	5 ± 2
TBZ	10	24 ± 5	124 ± 3	12 ± 4	9 ± 4	6 ± 4
TBZ	33.3	28 ± 10	137 ± 8	9 ± 4	11 ± 4	7 ± 2
TBZ	100	29 ± 1	134 ± 22	13 ± 4	12 ± 5	8 ± 3
TBZ	333.3	22 ± 8	116 ± 15	14 ± 3	9 ± 2	11 ± 3
TBZ	1000 (P)	17 ± 2 (STL)	108 ± 10 (STL)	13 ± 6	8 ± 3 (STL)	8 ± 2
TBZ	3333.3 (P)	20 ± 6 (TL)	119 ± 12 (TL)	7 ± 1 (STL)	7 ± 4 (TL)	9 ± 3 (STL)
positive control	variable	489 ± 18	753 ± 35	219 ± 40	207 ± 19	194 ± 137

(TL) – thin lawn; (STL) – slightly thin lawn; (VTL) – very thin lawn; (P) – precipitation

Retest of E-Coli in the direct plate method - (mean revertant colony count per plate \pm SD)						
test substance	concentration (μ g/plate)	TA 98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
absence of metabolic activation						
vehicle	0	-	-	-	-	6 \pm 1
TBZ	10	-	-	-	-	5 \pm 1
TBZ	33.3	-	-	-	-	9 \pm 2
TBZ	100	-	-	-	-	6 \pm 1
TBZ	333.3	-	-	-	-	9 \pm 4
TBZ	1000	-	-	-	-	11 \pm 3
TBZ	3333.3 (P)	-	-	-	-	7 \pm 3
positive control	variable	-	-	-	-	462 \pm 19
presence of metabolic activation						
vehicle	0	-	-	-	-	6 \pm 2
TBZ	10	-	-	-	-	8 \pm 4
TBZ	33.3	-	-	-	-	8 \pm 5
TBZ	100	-	-	-	-	7 \pm 2
TBZ	333.3	-	-	-	-	7 \pm 3
TBZ	1000	-	-	-	-	4 \pm 2
TBZ	3333.3 (P)	-	-	-	-	5 \pm 1
positive control	variable	-	-	-	-	636 \pm 48

(P) designates concentration in which there was precipitation

APPEARS THIS WAY
ON ORIGINAL

Study title: Tetrabenazine – Chromosomal aberration assay with Chinese Hamster ovary cells *in vitro*

Key finding: positive in the presence of metabolic activation

Study no.: — report # 19406

Volume # 19, module 4

Conducting laboratory and location: _____

Date of study initiation: 31-Jul-00

GLP compliance: OECD-GLP compliant

QA reports: yes (x) no ()

Drug, lot #, and % purity: Tetrabenazine (TBZ) batch # 99501, chromatographic purity by HPLC 99.9%. No information was supplied about the stability of TBZ in the vehicle, or when the dosing solution was prepared relative to its use.

Methods

Strains/species/cell line: Chinese hamster ovary cells (CHO) (generation time of 12 hrs and modal chromosome number of 21)

Metabolic activation system: S9 from Aroclor 1254 treated male Fischer rats (“Enzymatic activity of each batch of S9 was characterized by testing selected pre-mutagens in an Ames test with *S. typhimurium* TA 1538.” [batches FLI 091 and 092 were characterized; the batch(s) used in this study were not identified].

Doses used in definitive study: see the following reviewer-generated tables for details.

Vehicle for test article: dimethylformamide “This solvent gave the best dispersion characteristics when compared with tissue culture medium, dimethylsulphoxide and ethanol.” According to the sponsor, tetrabenazine in vehicle did not alter the osmolality of the culture medium, (tested only in the presence of S9 in test 1) and did not change the color of the medium (therefore no further testing of pH), but did produce precipitation in the presence and absence of metabolic activation at concentrations of 312 – 5000 µg/ml.

Basis of dose selection: In test 1, a full range of test article concentrations were tested from 20 µg/ml – 5000 µg/ml. The concentrations evaluated for chromosomal aberrations were selected based on treatment-induced cytotoxicity (see reviewer generated summary tables that follow for details). For test 2, the concentrations of TBZ were chosen based on the results of test 1, and the concentrations evaluated for chromosomal aberrations were based on treatment-induced cytotoxicity. The concentrations selected for testing and evaluation in test 3 were selected based on the results of test 2 and the treatment-induced cytotoxicity.

Negative controls: untreated control and dimethylformamide (vehicle)

Positive controls: cyclophosphamide (CPH) (+S9), and methyl methanesulphonate (MMS) (-S9).

Incubation information and sampling times: (Based on a sponsor-supplied table)

S9 mix	Test	Treatment period	Recovery period	Colcemid	Harvest
+ S9	test 1, 2 & 3	0-6 hrs	6-22 hrs	22-24 hrs	24 hrs
- S9	test 1 only	0-6 hrs	6-22 hrs	22-24 hrs	24 hrs
- S9	test 2 only	0-22 hrs	none	22-24 hrs	24 hrs
- S9	test 2 only	0-22 hrs	22-46 hrs	46-48 hrs	48 hrs

Cultures established approximately 20 hrs prior to exposure period

ResultsStudy validity (comment on replicates, counting method, criteria for positive results, etc.):

Vehicle controls, negative controls and test article treated cultures were run in duplicates. Each of the two concentrations of the positive controls was run as single culture. According to the sponsor, chromosomal aberrations were evaluated in up to 50 metaphases per slide (2-3 slides per culture) and where possible, in 100 metaphase cells per culture. Slides were evaluated blinded to treatment.

The sponsor defines cytotoxicity as "... cell count reduced to less than 50% of the mean vehicle control values or if consistent evidence of changes to cell morphology was observed."

Sponsor's criteria for clastogenicity:

The results for test item and positive control treated cultures are evaluated by comparison with the concurrent vehicle control cultures and with historical negative control data.

A negative response was recorded if responses from the test item treated cultures are within the 95% confidence limits for the historical negative control data.

The response at a single dose was classified as significant if the percent of aberrant cells is consistently greater than the 99% confidence limits for the historical negative control data or greater than double the frequency of an elevated vehicle or untreated control culture if appropriate.

A test was positive if the response in at least one acceptable dose level is significant by the criterion described above.

A test item was positive if one test was positive, as described above or if one of the tests was positive after another test gave indications of activity. These indications may be suspicious levels of aberrant cells (between 95% and 99% confidence limits).

Experiments that met in part the criteria for a positive response, or marginally met all the criteria, were classed as inconclusive.

According to the sponsor, the untreated controls and vehicle controls "had levels of structural and numerical aberrations within the 95% confidence limit of the historical negative control data." Examination of the data revealed an exception, i.e., Test 2 in the presence of metabolic activation, the percentage of cells noted with endoreduplication and polyploidy were greater than the historical control. In fact they would fall in the range of a positive response.

Study outcome: See the reviewer generated summary tables that follow. Test 1 was conducted in the presence (Table #2) and absence (Table #3) of metabolic activation. Under both conditions, drug-induced cytotoxicity was evident and followed a bell shaped curve, such that the degree of toxicity seen at the highest concentrations tested was less than that demonstrated at lower concentrations. However, the initial doses demonstrating significant toxicity were used as the highest doses for evaluation of chromosomal aberrations. According to the sponsor, the results of Test 1 in the presence and absence of metabolic activation were negative (aberrations frequency was within 95% confidence limits for a negative response). Examination of the data reveals that Test 1 in the presence of metabolic activation was negative; however at the highest concentration evaluated in the absence of metabolic activation there appear to be some signal of potential clastogenic activity compared to concurrent controls (but within historical control range). The concentrations of test article employed in Test 2 were selected to more closely examine the effects at the concentrations closest to the initial levels of cytotoxicity. There was no evidence of test article induced clastogenicity in the absence of metabolic activation (Table #5). Concentrations in the range of the high concentration from Test #1 could not be evaluated based on cytotoxicity (no metaphase cells present on slides). In Test 2, in the presence of metabolic activation (Table # 4), there was an increase in the aberration frequency and aberrant cell frequency at the highest concentration tested, as well as an increase in endoreduplication at the low and mid concentrations. Test 3 was carried out (in the presence of metabolic activation only) (Table #6), to confirm the positive response seen in Test 2 (+S9). There was a concentration related increase in the aberration frequency and aberrant cell frequency at the mid and high concentrations tested. The sponsor stated that "Due to the positive response in the level of structural aberration, the extra assessment of polyploidy in 300 metaphase cells was not carried out."

The sponsor concludes, "... Tetrabenazine was clastogenic when tested with Chinese hamster ovary cells *in vitro*. This response was observed in the presence of S9 mix and at concentration levels that were deemed toxic to the cells."

Conclusion: In two independent tests, TBZ is clastogenic in the presence of metabolic activation. In these tests, TBZ also caused an increase in aneuploidy and/or endoreduplication.

APPEARS THIS WAY
ON ORIGINAL