CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 21-894

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 21-894
Submission date: 18-Jan-08 (response to Agency's approvable letter) and earlier submissions

Drug: tetrabenazine
Sponsor: Prestwick Pharmaceuticals, Inc.
Indication: chorea of Huntington's disease

Reviewing Division: Division of Neurology Products

Comments:
The first pharm/tox review of this NDA noted several significant deficiencies in the nonclinical data. As part of the first approvable letter, the applicant was asked to address some of these issues before approval. The sponsor subsequently addressed some of these issues. Other nonclinical issues are still outstanding; however, the division believes that the remaining issues could be addressed as post approval requirements. I agree.
The topics recommended to be addressed are
1) complete the 2 year carcinogenicity study in male rats
2) conduct a 2 year carcinogenicity study in female rats
3) conduct a study of fertility and early embryonic development
4) submit in vivo metabolism data from species used in nonclinical studies
5) conduct a neurotoxicity study in animals

Conclusions:
I concur with the Division pharm/tox conclusion that adequate labeling can be written with the information available at this time and that the above outstanding nonclinical issues can be addressed post approval. I concur with the pregnancy category of C and the labeling as proposed in the Jan. 7, 2008 supervisory memo.

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/s/

Paul Brown
7/23/2008 04:30:40 PM
PHARMACOLOGIST

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: March 18, 2008
From: Andrea M. Powell, Ph.D.
Pharmacologist

Subject: NDA 21-894 – January 18, 2008 Response to Approvable Letter (Amendment 0067)

Recommendations: From a Pharmacology/Toxicology standpoint, the sponsor has met the conditions for approval as delineated in the December 26, 2007 approvable letter.

Post Marketing Commitments: The December 26, 2007 approvable letter listed the four nonclinical post marketing commitments below as conditions for approval. The sponsor's commitments are noted in italics. The proposed timelines for these commitments are acceptable.

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 study 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.
    - *The final report for the ongoing carcinogenicity study in male rats will be submitted during the [redacted] of 2009.*
    - *The protocol for the carcinogenicity study in female rats will be submitted during the 1st [redacted], and assuming that the protocol is acceptable, the 2-year study would be initiated during the [redacted], with a final study report submitted during the [redacted]*

2. You have not conducted a study of fertility and early embryonic development (to implantation) for tetrabenazine. This study may be conducted post approval but you need to commit to a date for submission of the final study report for this study.
    - *The final report will be submitted during the [redacted] of 2009.*

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 [redacted] study no. CAM/35; Amendment 0056, 10/19/07) but 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.
    - *The final report for Study no. CAM/35 and additional relevant data will be submitted during the [redacted] of 2008.*

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 assessments does not appear to have been sufficiently sensitive to rule out potential neurotoxic effects.

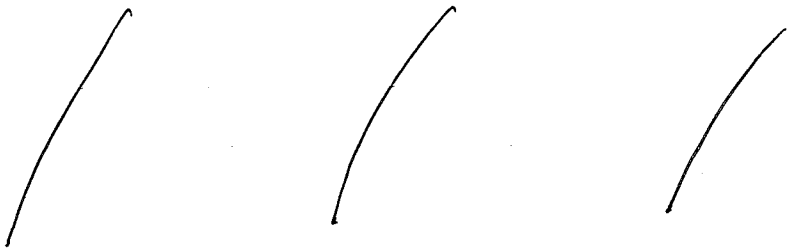
Based on our review and further internal discussions, we have concluded that a neurotoxicity study of tetrabenazine using methodology and a multiple dose regimen similar to that used by Satou et al. (2001) would provide the best evaluation. Consideration should be given to including a group in which 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.

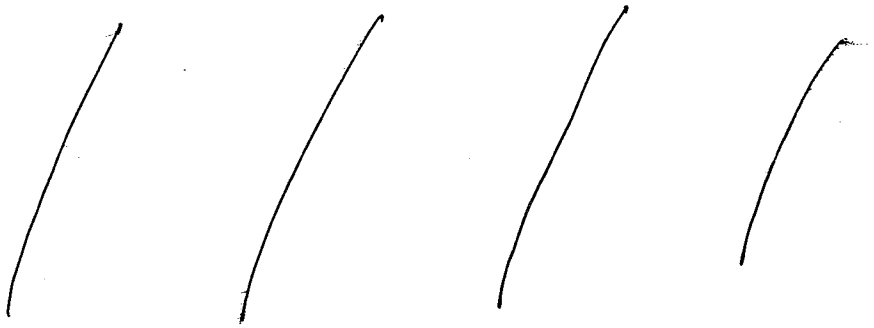
- *A draft study protocol will be submitted during the ——— 2008. Based on the assumption that the study can be initiated during the ——————— , the final study report will be submitted during the ———————*

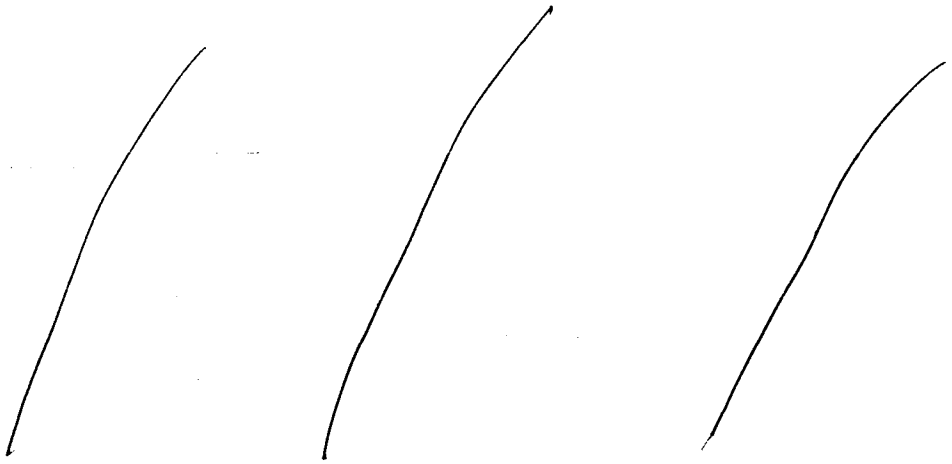
Labeling: The following changes to the labeling proposed with the December 26, 2007 approvable letter were negotiated with the sponsor and accepted by the sponsor and the Division on February 21, 2008 (strikeout = deletions, and underline = additions). The remaining nonclinical sections of the labeling are essentially unchanged from those proposed on December 26, 2007.

Pharmacodynamics:



During the February 21st labeling negotiations the sponsor noted that ———————





The following wording is recommended for the pharmacodynamics section of the labeling:

Pharmacodynamics

The precise mechanism by which tetrabenazine exerts its anti-chorea effects is unknown, but is believed to be related to its effect as a reversible depletor of monoamines (such as dopamine, serotonin, norepinephrine, and histamine) from nerve terminals. Tetrabenazine reversibly inhibits the human vesicular monoamine transporter type 2 (VMAT2) (Ki ≈ 100 nM), resulting in decreased uptake of monoamines into synaptic vesicles and depletion of monoamine stores. Human VMAT2 is also inhibited by dihydrotetrabenazine (HTBZ)

Z. α-HTBZ exhibits in vitro binding affinity to bovine VMAT2. Tetrabenazine exhibits weak in vitro binding affinity at the dopamine D2 receptor (Ki = 2100 nM).

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ON ORIGINAL

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/s/

Andrea Powell
3/18/2008 04:34:31 PM
PHARMACOLOGIST

Lois Freed
3/18/2008 04:35:45 PM
PHARMACOLOGIST
I concur.

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
CONTROLLED SUBSTANCE STAFF

Date: **March 6, 2008**

To: **Russell Katz, M.D., Director**
Division of Neurology Products (HFD-120)

Through: **Michael Klein, Ph.D., Acting Director**
Controlled Substance Staff (HFD-009)

From: **Katherine Bonson, Ph.D., Pharmacologist**
Controlled Substance Staff (HFD-009)

Subject: **Label review**
Xenazine (tetrabenazine)
NDA 21-894
Treatment for Huntington's Disease
Sponsor: Prestwick Pharmaceuticals, Inc.

Background:

The Division of Neurology Products (HFD-120) consulted CSS regarding the abuse potential of tetrabenazine (Xenazine). Tetrabenazine acts an inhibitor of the brain vesicular monoamine transporter type 2 (VMAT2), which induces the depletion of monoamines such as dopamine, norepinephrine, and serotonin. It is being reviewed for the indication of Huntington's Disease, under Orphan Drug status. The initial proposed therapeutic dose is 25 mg (p.o.), with increases in dose each week by increments of 12.5 mg (p.o.) up to 100 mg/day until satisfactory control of chorea is attained or until adverse events become intolerable.

The Sponsor proposes that the drug label state under the *Controlled Substance Class* subheading of *Drug Abuse and Dependence* that "tetrabenazine is not a controlled substance". In the *Physical and Psychological Dependence* subheading, the Sponsor proposes language stating that tetrabenazine

Conclusions and Recommendations:

Based on information provided by Prestwick Pharmaceuticals, Inc. in the NDA, CSS concludes that tetrabenazine is unlikely to have sufficient abuse potential that would warrant scheduling.

This conclusion is based on the following:

* Central nervous system (CNS) adverse events observed in clinical efficacy trials do not include euphoria or other positive subjective effects indicative of abuse potential.

* The clinical profile of patients who experienced overdose with tetrabenazine at doses up to 750 mg orally is typically limited to somnolence and cognitive impairment.

* Adverse events with tetrabenazine observed during post-marketing experience in countries other than the U.S. over the past 30 years are similar to those seen during the clinical trials for Huntington's Disease in the present NDA.

* Abrupt discontinuation of tetrabenazine did not produce adverse events other than a re-emergence of Huntington's Disease-associated choreas. This suggests that tetrabenazine does not produce a withdrawal syndrome.

* Tetrabenazine acts as a monoamine depleter because of its ability to block the vesicular monoamine transporter type 2 (VMAT2). Given that increases in dopamine are associated with abuse potential, the decrease in dopamine produced by tetrabenazine suggests a lack of abuse potential.

* Animal behavioral studies suggest that tetrabenazine does not produce effects similar to those produced by known drugs of abuse.

CSS recommends that the *Drug Abuse and Dependence* section (LINES 1054 – 1067) of the proposed label be revised according to the above conclusions, to read as follows:

LINE 1054 – 1067:

Clinical trials did not reveal any tendency for drug seeking behavior, though these observations were not systematic. Abuse has not been ———] from the postmarketing experience in countries where tetrabenazine has been marketed. Abrupt discontinuation of tetrabenazine from patients did not produce symptoms of withdrawal or a discontinuation syndrome; only symptoms of the original disease were observed to re emerge. As with any CNS-active drug, physicians should carefully evaluate patients for a history of drug abuse and follow such patients closely, observing them for signs of tetrabenazine misuse or abuse (such as development of tolerance, incrementation of dose, drug-seeking behavior).

I. Summary of Information Related to Abuse Potential from Clinical Studies

A. Clinical Studies Assessing Safety and Efficacy of Tetrabenazine

Clinical Adverse Events Indicative of Abuse Potential

The most frequently observed CNS-associated adverse events that differentiated from placebo included insomnia (22%), depression (15%), sedation (15%), restlessness aggravated (13%), irritability (9%), anxiety (7%), and somnolence (7%). There were no reports of euphoria or other adverse events that would suggest that the sedation observed during clinical trials represents an abuse potential signal.

During clinical trials, 15 of 54 patients (28%) cited sedation as the reason for limitation of upward titration or a decrease in dose. Sedation and somnolence are stated to be observed primarily during periods of dose increases.

Overdose Experience

The label notes that 8 cases of tetrabenazine overdose are reported in the scientific literature in the past 35 years since tetrabenazine was first marketed in countries outside the U.S. The doses in these cases ranged up to 1000 mg. The adverse events observed during overdose were similar to those observed in clinical trials in the present NDA, including somnolence and cognitive impairments.

Post-Marketing Experience in Foreign Countries

Tetrabenazine has been marketed in countries other than the U.S. for over 30 years as a treatment for chorea. The CNS adverse event profile observed in this post-marketing experience is parallel to that seen in the clinical trials for Huntington's Disease in the present NDA. These include: drowsiness, depression, movement disorder, anxiety, insomnia, irritability, confusion, and dizziness. This CNS profile is not associated with abuse potential in the absence of positive subjective effects such as euphoria.

Physical Dependence and Withdrawal Syndrome

A prospective study evaluated the adverse events associated with abrupt discontinuation of tetrabenazine in patients with chorea. Although there was a re-emergence of the chorea symptoms, there were no other signs or symptoms reported that the investigators associated with a withdrawal syndrome. Thus, it appears tetrabenazine does not produce physical dependence.

II. Summary of Information Related to Abuse Potential from Preclinical Studies

A. Receptor Binding

Tetrabenazine is described as an inhibitor of vesicular monoamine transporter type 2 (VMAT2). The functional effect of this pharmacological activity is to preferentially reduce the release of dopamine, as well as other monoamines such as norepinephrine and serotonin. A reduction in these monoamines is not associated with increased abuse potential.

Additionally, tetrabenazine is described as being a weak antagonist at dopamine D2 receptors. The D2-type receptors in the brain are associated with abuse potential, so antagonism at this site suggests that tetrabenazine does not have abuse potential through this dopaminergic mechanism.

Most drugs with abuse potential increase dopaminergic transmission in the brain, and this dopamine signal is associated with pleasurable subjective responses. In contrast, drugs that reduce dopaminergic transmission (such as antipsychotics) typically produce flattening of affect and other negative subjective responses. Thus, it is unlikely that tetrabenazine, a drug that reduces dopaminergic transmission, would produce positive subjective responses in humans that would lead to abuse of the drug.

B. Animal Behavioral Studies

The label [redacted]
In a rat intracranial self-stimulation test, administration of tetrabenazine was found to suppress self-stimulation. Typically, drugs with abuse potential increase self-stimulation in this test. Tetrabenazine also was able to block the effects of amphetamine on self-stimulation. In a separate study, tetrabenazine decreased the rate of responding in rats trained to press a lever to obtain water reinforcement. These results contrast with those produced by the stimulants amphetamine and methylphenidate, in which the two drugs increased the rate of response for water reinforcement.

Both of these rodent studies indicate that tetrabenazine does not produce behavioral responses similar to those produced by drugs with known abuse potential.

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/s/

Katherine Bonson
3/17/2008 09:52:23 AM
PHARMACOLOGIST

Michael Klein
3/17/2008 10:05:39 AM
PHARMACOLOGIST
Acting Director - Controlled Substance Staff

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**
SUBMISSION TYPE:	**Response to Approvable Letter**
DATE RECEIVED BY CENTER:	**05-April-07**
PRODUCT:	**Xenazine® (tetrabenazine) Tablets**
INTENDED CLINICAL POPULATION:	**Huntington's disease (for treatment of chorea of Huntington's disease)**
SPONSOR:	**Prestwick Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**Submissions (arranged by letter date):** • **16-March-06** • **09-February-07 (Amendment 30)** • **04-April-07 (Amendment 32)** • **28-Jun-07 (Amendment 37)**
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, M.D.**
PROJECT MANAGER:	**Susan Daugherty, R.N., B.S.N.**

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EXECUTIVE SUMMARY

Background:

NOTE: The official submission of the NDA is the paper submission; therefore, all amendment numbers and dates discussed in this review refer to the paper submission. There are notable discrepancies in the amendment numbers and submission dates between the paper (official) and the electronic (unofficial) submissions.

This is a review of Prestwick Pharmaceuticals' response to the Agency's Approvable letter of 24-March-06 for Xenazine® (tetrabenazine) Tablets for the treatment of chorea associated with Huntington's disease. The NDA was submitted on 26-September-05. I was the original reviewer for this NDA, and in my original NDA review dated 30-March-06, I concluded that from a Pharmacology/Toxicology standpoint, the package did not support approval. Dr. Lois Freed, the Pharmacology/Toxicology supervisor, provided secondary review in her memorandum dated 27-March-06, and Dr. Kenneth Hastings, Pharmacology/Toxicology Associate Director, provided tertiary review in his memorandum dated 23-March-06. The Agency issued an approvable letter on 24-March-06.

Pharmacology/Toxicology Comments from the 24-March-06 Approvable Letter:

Prior to approval the sponsor was asked to address the following six nonclinical issues:

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 in vivo 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 the animals.

2. The 26-week oral toxicity study is the only definitive toxicity study conducted in rats. Therefore, it is particularly important that you provide the data from this study in a complete and accurate manner. The following deficiencies were identified in the report of the study:

   a. The reporting of clinical signs is incomplete. For example, several instances of convulsions observed in two high-dose animals were not listed in the summary table. Similarly, instances of "lethargy" were noted in the summary table, but not in any animal individual line listing. You need to address the apparent discrepancies between the summary of clinical signs and the individual animal line listings.

   b. The study report did not include a signed Pathologist's Report. In order to document the gross pathology and histopathology findings in the chronic study, you need to provide a copy of this report.

3. You conducted a 14-day oral study of tetrabenazine to assess toxicokinetics and effects on serum prolactin in rats —— Study #7425-114). The toxicokinetics data have been provided, but the serum prolactin data have not. You need to submit a final report of the

serum prolactin data. These data are important for the interpretation of the results of the chronic toxicity study in rats.

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.

6. You need to commit to initiating carcinogenicity studies. Your protocol for a 26-week p53 transgenic mouse assay has been reviewed by the Division and the Executive CAC; minutes of the Executive CAC meeting were sent to you on October 27, 2005. You have recently submitted a protocol for a 2-year carcinogenicity study in rats that is currently under review. You need to commit to a timeline for the conduct of the studies and submission of final reports of these studies. Final reports would not be required prior to approval.

We asked the sponsor address the following issues as Phase 4 commitments:

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.

Although not needed prior to approval, we ask that you address these issues in a timely manner.

Submissions and Subsequent Communications: The following is a delineation of the Pharmacology/Toxicology related submissions from the review cut-off point during the original review cycle to the present.

- **Response to FDA: Information Request (dated 16-March-06): response to the Division's 24-January-06 request for Pharmacology/Toxicology information. With regard to the 26-week oral toxicity study in rat (——— study # 455738,) we asked for (1) clarification on the schedule for observation and reporting of clinical signs, (2) summary table and individual line listings of clinical observations by dose group, (3) day of sacrifice/death for each premature death. The response was labeled as an information request and not listed as an NDA amendment. This was not reviewed during the first review cycle.

- **Amendment 11 (dated 28-April-06):** End-of-Review Meeting package for the meeting that took place on 25-May-06 (meeting minutes issued on 01-September-06).

- **Amendment 12 (dated 28-July-06):** safety update after receipt of the Approvable letter. From a Pharmacology Toxicology standpoint the components of interest were listings of initiated or completed nonclinical studies.

- **Amendment 30 (dated 09-February-07**): response to the 24-March-06 approvable letter. The following study reports are relevant to Pharmacology/Toxicology.

  1. Study # CAM/05: Quantitative whole body autoradiography and excretion balance following a single oral administration of [^{14}C]-Tetrabenazine to partially pigmented rats.

  2. Study # CAM/08: Quantitative whole body autoradiography and excretion balance following a single oral administration of [^{14}C]-Tetrabenazine to male mice.

  3. Study # CAM/07: Study to investigate the pharmacokinetics of tetrabenazine following oral administration in beagle dogs.

  4. Study # CAM/11: [^{14}C]-Tetrabenazine: metabolite identification studies.

  5. Study # CAM/21: [^{14}C]-Tetrabenazine: Investigation of metabolites in plasma of rat, mouse, rabbit and dog after oral administration.

  6. Study # CAM/26: Comparative in vitro metabolism studies of [^{14}C]-Tetrabenazine (TBZ) with mouse, rat, dog, Cynomolgus monkey and human liver microsomes.

  7. Study # 7425-114: 14-day oral gavage study with tetrabenazine to assess toxicokinetics and prolactin levels in rats.

  8. Study # 20730: Cambridge rat 26-week 13- and 26-week oral repeat-dose study
     a. ——— report No. 20730, Tetrabenazine 26 week toxicity study in rats with twice daily dosing, administration by gavage and 13 week interim kill. [Study Pathologist's

statement (18-April-06) regarding the methodology employed in the original histologic examination of the brain in this study].

b. Tetrabenazine - 26 week study in rats with twice daily dosing, administration by Gavage and 13 week interim kill. Submission of a readable form of Appendix 38 – dose-related clinical signs: individual animals. This appendix was originally submitted on16-March-06, in response to a Pharmacology/Toxicology request for information. The original appendix was unreadable.

c. Final peer review statement: histological evaluation extension for the 26 week toxicity study in rats (—— project # 455738, —————— study # 457929).

d. Final supplemental pathology report: histological evaluation extension for the 26 week toxicity study in rats (—— project # 455738, study # 457929).

e. DRAFT study 27259: Tetrabenazine histological evaluation extension for the 26 week toxicity study in rats (—— project # 455738).

9. Study # 7425-109: 4-week dose range-finding oral gavage toxicity and toxicokinetic study with tetrabenazine in C57Bl/6 mice, final report, (not reviewed for this review cycle).

10. Evaluation of the rat bone marrow micronucleus test data for tetrabenazine.

- Division letter issued 16-March-07 stating that Amendment 30 was not considered to be a complete response to the 24-March-06 approvable action. The Pharmacology/Toxicology deficiencies from that letter are reproduced below:

1. A signed Pathologist's Report for the 26-week oral toxicity study in rat ———Study 20730) was not provided (# 2b in the approvable letter). The signed pathology report to which you refer in your response is limited to additional brain histopathology for the 26-week study in rat.

2. Issue # 4 from the approvable letter has not been fully addressed.

a. You have not addressed the potential for treatment-related neuropathology in the chronic toxicity study in dog. For the 9-month toxicity study in dog, you need to document that the microscopic examination of brain was conducted using techniques sensitive enough to have detected, if present, neuropathological findings similar to those reported by Satou et al. (2001) in rat.

b. You need to submit a final report for Study #27259 (Tetrabenazine Histological Evaluation Extension for the 26 Week Toxicity Study in Rats).

The letter also noted the following potential Pharmacology/Toxicology deficiency that was not part of the incomplete response decision:

1. It does not appear that you have adequately addressed the issue of interspecies comparisons of in vivo metabolism (#1 in the approvable letter). For each major

circulating metabolite in humans, you need to provide plasma exposure (AUC) data in humans and in the animal species/strains used in the pivotal toxicity studies.

- **Amendment 32 (dated 04-April-07):** to address the incomplete response issues delineated in the 16-March-07 incomplete response letter. The Pharmacology/Toxicology related reports are listed below. In addition, the sponsor stated "The Non-Clinical in vivo metabolism data will be addressed in an amendment to the NDA."

  1. Pathologist's report: 13-week interim kill animals (from Tetrabenazine – 26 week toxicity study in rats with twice daily dosing administration by gavage and 13 week interim kill ——— study # 455738)).

  2. Pathologist's report: 26-week terminal kill animals (from Tetrabenazine – 26 week toxicity study in rats with twice daily dosing administration by gavage and 13 week interim kill ——— study # 455738)).

  3. ——— Report # 27259: Tetrabenazine histological evaluation extension for the 26 week toxicity study in rats ——— study # 455738).

- Division letter (complete response) issued 18-May-07 stating that 05-April-07 is considered the date of submission.

- **Amendment 37 (dated June 28, 2007): additional data to respond to the Division's incomplete** response letter issued 16-March-07. The following study was the only component relevant to Pharmacology/Toxicology:

  1. ——— study # 7425-101: Neuropathology extension of 9-month toxicity study in dogs.

- The Division letter issued 15-August-07 acknowledging that the user fee goal date is extended to 05-January-07, due to the submission of a major amendment on 06-August-07.

- **Amendment 46 (dated 17-August-07):** final report for ——— udy # 7425-123: Oral gavage dose range-finding study with tetrabenazine in female rats. This study was not reviewed during this cycle.

- **Amendment 48 (dated 20-August-07):** response to our 13-August -07 request for information regarding the status of the carcinogenicity study in p53 transgenic mouse. The sponsor stated that the final report would be submitted by the end of September of 2007.

- **Amendment 51 (dated 26-September-07):** This amendment contains the final report for ——— study 7425-110: 26 Week oral gavage oncogenicity study with tetrabenazine in model P53N5-T (Heterozygous) mice. This study is a Phase 4 commitment and was not reviewed during this review cycle.

- **Amendment 54 (dated 05-October-07):** response to the Pharmacology/Toxicology potential deficiency noted Division letter that issued on 16-March-07.

  1. DRAFT report for Study # CAM/35: [^{14}C]-Tetrabenazine: studies to investigate the concentrations and pharmacokinetic properties of major circulating human plasma components in the plasma of rat, mouse, rabbit and dog after repeat oral administration. The draft report was not reviewed.

2. Analysis of major circulating human metabolites after tetrabenazine administration to healthy volunteers – Pharmacokinetic analysis and report.

NOTE:	In the review TBZ refers to tetrabenazine and HTBZ refers to the metabolite dihydroxytetrabenazine.

Overall Conclusions on Pharmacology/Toxicology Issues and Recommendation on Approvability:

From a pharmacology/toxicology standpoint, the NDA package does not meet the "usual" standard for approval for a chronic-use drug. The sponsor has not provided adequate data to characterize the toxicity of orally administered tetrabenazine in one of the most basic and pivotal assays, the chronic toxicity study in rodent. This will be further discussed below. However, the decision has been made that this NDA will be approved without the need for additional nonclinical data prior to approval.

The primary reason for my determination that this NDA does not meet the "usual" nonclinical standard for a chronic-use drug is the incomplete and unreliable reporting of the clinical signs in the chronic toxicity study in rat (Issue #2a from the approvable letter). Although the study was performed at a contract laboratory, it is the responsibility of the sponsor to ensure that the data are accurately and consistently reported and submitted in a readable form. Even with the guidance of our comments and requests for information, the sponsor did not provide an adequate report of the data. Furthermore, it took repeated attempts to obtain a readable presentation of the data.

The high dose in this study, which was associated with potentially treatment-related late-onset convulsions, morbidity, and death, is only approximately three-times the maximum recommended human dose on a mg/m^2 basis. The highest dose not associated with unexplained morbidity/mortality is the mid dose which is approximately 1.5 times the maximum recommended human dose on a mg/m^2 basis. It is not possible to establish a no-effect dose for the more serious treatment-related clinical observations because, to date, the sponsor has not submitted comprehensive, accurate line listings for individual animals, or an accurate summary of occurrence per group. (Further discussion of this issue is presented later in the review).

Adequate characterization of the clinical observations in the chronic toxicity studies is important for identifying premonitory signs for serious treatment-related toxicity, so that similar toxicities can be avoided in humans. An accurate and comprehensive report of treatment-related clinical signs may have provided some insight into the cause of death or moribund condition in the high dose rats with unscheduled deaths.

It should be emphasized that these data should already be available; it was not a request for the conduct of an additional study, but a request for accurate, comprehensive and reviewable data from a completed toxicity study. The need for these data, while considered one reason for lack of approvability during the first review cycle, appears to have been supplanted by the clinical experience, since the decision has been made that the NDA can be approved without this data. It is doubtful that these data, if obtained Phase 4, would be used to modify the clinical dosing regimen in Huntington's disease; thus, there would be no point in asking for this data as a Phase 4 commitment.

It should be noted that only three of the seven issues listed in the original approvable (AE) letter as needed prior to approval have been adequately addressed. [Note that nonclinical issue #2 had two components, each counted here as a separate issue]. These three issues are:

- AE Issue #2b - submission of the pathology report for chronic toxicity study in rats.
- AE Issue #3 - submission of the final report of the prolactin data from 14-day study in rat.
- AE Issue #6 – commitment to initiate carcinogenicity studies. [With regard to carcinogenicity studies, the sponsor has already submitted the results of the 26-week carcinogenicity study in p53 mice (Amendment # 51 - not reviewed during this cycle). The sponsor initiated a 2-year carcinogenicity study in male rats on 20-Jun-06, and has stated their intent to initiate a 2-year carcinogenicity study in female rats by ——— Formal commitments for the dates of submission of the final reports for the 2-year studies in male and female rats should be required prior to approval.]

Four of the seven nonclinical issues identified in the original approvable letter have not been adequately addressed. These issues are:

- AE Issue #1 - interspecies comparison of in vivo metabolism.
- AE Issue #2a - incomplete reporting of the clinical signs in the chronic toxicity study in rat.
- AE Issue #4 - drug-induced neuropathology issue related to the findings of Satou et al. (2001).
- AE Issue #5 - repeat of the in vivo micronucleus assay in rat.

Of these, AE Issues #1, #4, and #5 do not rise to a similar level of concern as AE Issue #2a (as discussed above). The extent to which these requested data (or lack of submitted data) would have significant potential impact on the clinical use of this drug will be discussed below.

AE Issue #1 - interspecies comparison of in vivo metabolism: During the first review cycle for the NDA we could not ensure that the pivotal nonclinical studies adequately characterized the toxicity of the major drug-related circulating products in humans after oral administration of TBZ. In humans, TBZ is extensively metabolized and the parent compound is either undetectable in the plasma or circulating at very low levels after oral administration. Based on the results of a mass balance study conducted in humans, the most abundant circulating component in humans was an unidentified peak, P16. The sponsor has now identified the major circulating drug-related compounds in humans after oral administration of TBZ. The major metabolic pathway in humans is the formation of the stereoisomeric metabolites, α- and β-HTBZ, via carbonyl reductase. These stereoisomers are further metabolized by CYP450 (predominantly CYP2D6) to mono O-dealkylated-HTBZ (also referred to as desmethyl-HTBZ). P16 is a mixture of up to four enantiomers (two derived from α-HTBZ, and two from β-HTBZ). These O-dealkylated (or desmethyl) derivatives are further metabolized by sulfate conjugation, which constitute additional major circulating components, and excreted.

The sponsor has not yet provided an adequate interspecies comparison of in vivo metabolism. The sponsor conducted a study (CAM/21) in which the in vivo metabolic profile of orally administered radiolabeled-tetrabenazine was evaluated in Sprague Dawley rats, C57BL/6 mice, Beagle dogs, and New Zealand White rabbits at 0.5, 2 and 6 hrs post dose. Based on the results of this study, all species tested had (among other metabolites) circulating levels of α-HTBZ, β-HTBZ, and O-dealkylated-HTBZ (the specific enantiomers were not clearly identified). In addition, rat, dog and human had circulating levels of sulfate of O-dealkylated dihydrotetrabenazine (specific enantiomers were not identified).

On 16-March-07, we informed that sponsor that the results of this study do not appear to adequately address the issue of interspecies comparisons of in vivo metabolism, and asked that they provide, for each major circulating metabolite in humans, plasma exposure (AUC) data in humans and in the animal species/strains used in the pivotal toxicity studies.

The sponsor has conducted an additional in vivo metabolism study to address this issue. This study (Study # CAM/35) was submitted as a DRAFT report to the NDA. The submission of a DRAFT report to an NDA is not acceptable, and the report was not reviewed.

The submission of the final report should be a Phase 4 commitment. These data are needed in order to determine the relevance (and adequacy) of the nonclinical studies to the assessment of human risk, especially with regard to the assessments of reproductive toxicity and the carcinogenicity, for which adequate relevant human data generally do not exist.

AE Issue #4 - drug induced neuropathology issue, related to the findings of Satou et al. (2001): There is some level of assurance in the fact that the additional evaluations of brain from the 26-week toxicity study in rat and the 9-month toxicity study in dog did not detect a signal for treatment-related neuropathology. However, these evaluations are inadequate to rule out the possibility that oral administration of tetrabenazine can induce histologic changes in the pars compacta/substantia nigra (SNpc), as reported in the literature after short term treatment of rat with TBZ by the intraperitoneal route (cf. Satou T et al. *Exp Toxicol Pathol* 53(4):303-308, 2001).

A key point of the initial request to the sponsor was the need to demonstrate that the techniques used for the histologic evaluation of the brain in the chronic toxicity studies in rat and dog were sensitive enough to have detected, if present, neuropathological findings similar to those reported by Satou et al. (2001). The differences in the processing of brain between Satou et al. and the sponsor are notable and expected, considering the different primary purposes of the studies (a focused neuropathology study versus a standard chronic toxicity study). The additional evaluations of the existing brain slides and additional sections conducted by the sponsor do not address this concern.

Furthermore, degenerated neurons are detectable for a finite period of time and that period of time can vary, based on the nature of the insult and the type of cell affected. If the vulnerable neurons were affected earlier in the course of the 26-week treatment, the ability to detect the effect could be diminished or absent at the time of evaluation (at least 26 weeks, or nine moths after the initiation of dosing for rat and dog, respectively).

Tetrabenazine has produced reversible parkinsonism in humans presumably by its pharmacodynamic effects. It would be important to determine if oral administration of tetrabenazine can induce neuropathology in the substantia nigra pars compacta. Loss of neurons in this area is known to be the cause of Parkinson's disease. In Huntington's disease patients, the intended clinical population, it would be very difficult to detect parkinsonian symptoms resulting from neurotoxicity.

Therefore, the sponsor should conduct a short-term neurotoxicity study in rat to determine whether the tetrabenazine induced-neuropathology demonstrated in rat by Satou et al. (2001) can be replicated, and whether oral administration of tetrabenazine to rats results in similar histopathology in the brain. This study should include a group treated according to the multi-dose paradigm used by Satou et al. (i.e., repeated administration of tetrabenazine at a dose of 1 mg/kg/day, i.p. for seven days), several groups treated with oral tetrabenazine using a range of doses up to a maximum tolerated dose, and appropriate control groups. Appropriate sections of the substantia nigra should be stained and evaluated for neurodegeneration. It is recommended that the sponsor submit the protocol for review by the Division prior to the conduct of the study. The conduct of this study should be a Phase 4 commitment.

AE Issue #5 - repeat of the in vivo micronucleus assay in rat: The sponsor did not conduct an additional study as requested. The sponsor submitted an expert opinion by [redacted], PhD, JD. In his opinion the study was negative in both males and females, and thus required no further evaluation. Dr. [redacted] arguments were not compelling (see the evaluation of his response for further details); therefore, without any additional new data, the results should still be interpreted as equivocal in females (and negative in males). Feedback from the Genetic Toxicology Subcommittee provided support for our original assertion of an equivocal response in female rats. The lack of the requested additional in vivo micronucleus assay

in rats to clarify the equivocal results in female rats will not have an impact on the clinical use of this drug, especially since it has already been established as notably genotoxic in the in vitro chromosomal aberration assays. Therefore, this issue does not need to be further addressed, except in labeling.

Original Phase 4 Commitments: It should be noted that only one of the three original Pharmacology/Toxicology Phase 4 commitments listed in the original approvable letter has been partially addressed.

- Issue #1 – submit the final study reports for carcinogenicity studies. The sponsor has already submitted the results of the 26-week carcinogenicity study in p53 mice (Amendment # 51 - not reviewed in this cycle). The sponsor initiated a 2-year carcinogenicity study in male rats on 20-Jun-06. and has stated their intent to initiate a 2-year carcinogenicity study in female rats by the ——— Formal commitments for the dates of submission of the final reports for the 2-year studies in male and female rats should be required prior to approval.

The two additional original Phase 4 issues have not yet been addressed.

- Issue #2 – conduct a fertility and early embryonic development (to implantation) study. This should continue to be a Phase 4 commitment.
- Issue #3 – address the apparent discrepancies in the study report for the completed pre- and post-natal development study in rat. 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. This is further discussed later in the review.

Additional Nonclinical Information: Although not requested by the Agency, the sponsor submitted reports of acute-dose tissue distribution studies conduced with [^{14}C] -TBZ in pigmented rat and albino mouse which indicated that TBZ and/or its metabolites bind to melanin-containing tissues. This suggests that there could be accumulation in these tissues over time, possibility resulting in toxicity 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 should be included in the product labeling.

Recommendations for Nonclinical Studies:

The decision was made that this NDA could be approved with no further nonclinical data required prior to approval. The conduct of the following studies and/or submission of the following reports should be Phase 4 commitments.

1. Submission of final study report for the ongoing 2-year carcinogenicity study in male rats. The sponsor should commit to a timeline for the submission of the final report.

2. Conduct of a 2-year carcinogenicity study in female rats. The sponsor should commit to a timeline for conduct of the study and submission of the final study report.

3. Conduct of a fertility and early embryonic development (to implantation) study. The sponsor should commit to a timeline for conduct of the study and submission of the final study report.

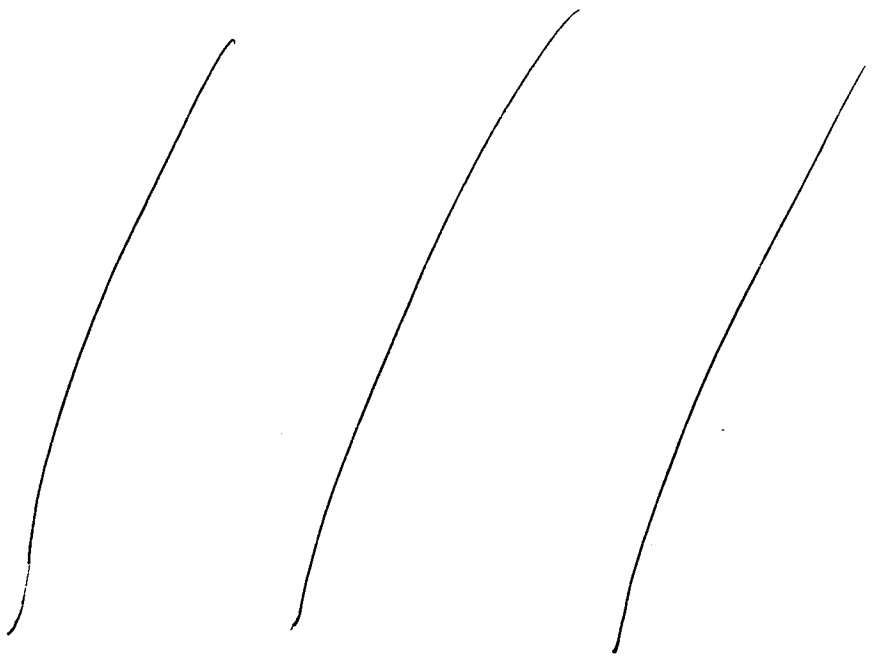
4. The neuropathology assessment conducted on brain from the 26-week toxicity study in rat and the 9-month toxicity study in dog is inadequate to rule out the possibility that oral administration of tetrabenazine can induce histologic changes in the pars compacta/substantia nigra, as reported in

the literature after short term treatment of rat by the intraperitoneal route (cf. Satou T et al. *Exp Toxicol Pathol* 53(4):303-308, 2001). Therefore, to address our concern that tetrabenazine may produce neuropathologic changes in the pars compacta/substantia nigra, the sponsor should conduct a short-term neurotoxicity study in rat to determine whether the tetrabenazine induced-neuropathology demonstrated in rat by Satou et al. (2001) can be replicated, and whether oral administration of tetrabenazine to rats results in similar histopathology in the brain. This study should include a group treated according to the multi-dose paradigm used by Satou et al. (i.e., repeated administration of tetrabenazine at a dose of 1 mg/kg/day, i.p. for seven days), several groups treated with oral tetrabenazine using a range of doses up to a maximum tolerated dose, and appropriate control groups. Appropriate sections of the substantia nigra should be stained and evaluated for neurodegeneration. It is recommended that the sponsor submit the protocol for review by the Division, prior to the conduct of the study.

The sponsor should commit to a timeline for conduct of the study and submission of the final study report.

5. Submission of the final report for study # CAM/35: [^{14}C] –Tetrabenazine: Studies to Investigate the Concentrations and Pharmacokinetic Properties of Major Circulating Human Plasma Components in the Plasma of Rat, Mouse, Rabbit and Dog after Repeat Oral Administration.

Recommendations on Labeling: [The following recommendations were made on 16-December-07. The labeling included in the 26-December-07 Approvable letter was revised based on subsequent internal discussions and discussions with the sponsor.]



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✓ Trade Secret / Confidential

_____ Draft Labeling

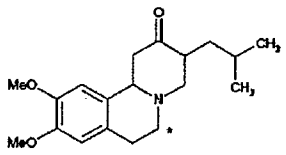
_____ Deliberative Process

ORIGINAL STUDY REPORTS

Comparative In Vitro Metabolism Studies of [^{14}C]-Tetrabenazine (TBZ) with Mouse, Rat, Dog, Cynomolgus Monkey and Human Liver Microsomes (Study # CAM/26) (Amendment 30, module 4/volume 2)

This study was conducted at [redacted] according to GLP regulations with appropriate QA statements. The study initiation date was 01-August-06.

Methodology: [^{14}C]-TBZ at a final concentration of 5 µM was incubated with liver microsomes from the species indicated below for 0, 5, 10, 20, 30 and 60 minutes. HPLC with on-line radiodetection was used for sample analysis, and the metabolite profile for a subset of samples was determined by LC-MS/MS. The position of the label is depicted below.



* position of ^{14}C-label

Commercially prepared liver microsomes were used in this study as follows: pooled male and female human, male CD1 mouse, male Sprague Dawley rat, male Beagle dog, and male Cynomolgus monkey. The original assay was conducted at a protein concentration of 0.2 mg microsomal protein/mL of incubation mixture. According to the sponsor, the degree of metabolism was too extensive (data not reported), and a second assay was conducted using at a lower protein concentration (0.05 mg/mL, 5 µM) for which the data were reported. After a five minute preincubation period, metabolism was initiated by the addition of the NADPH co-factor solution.

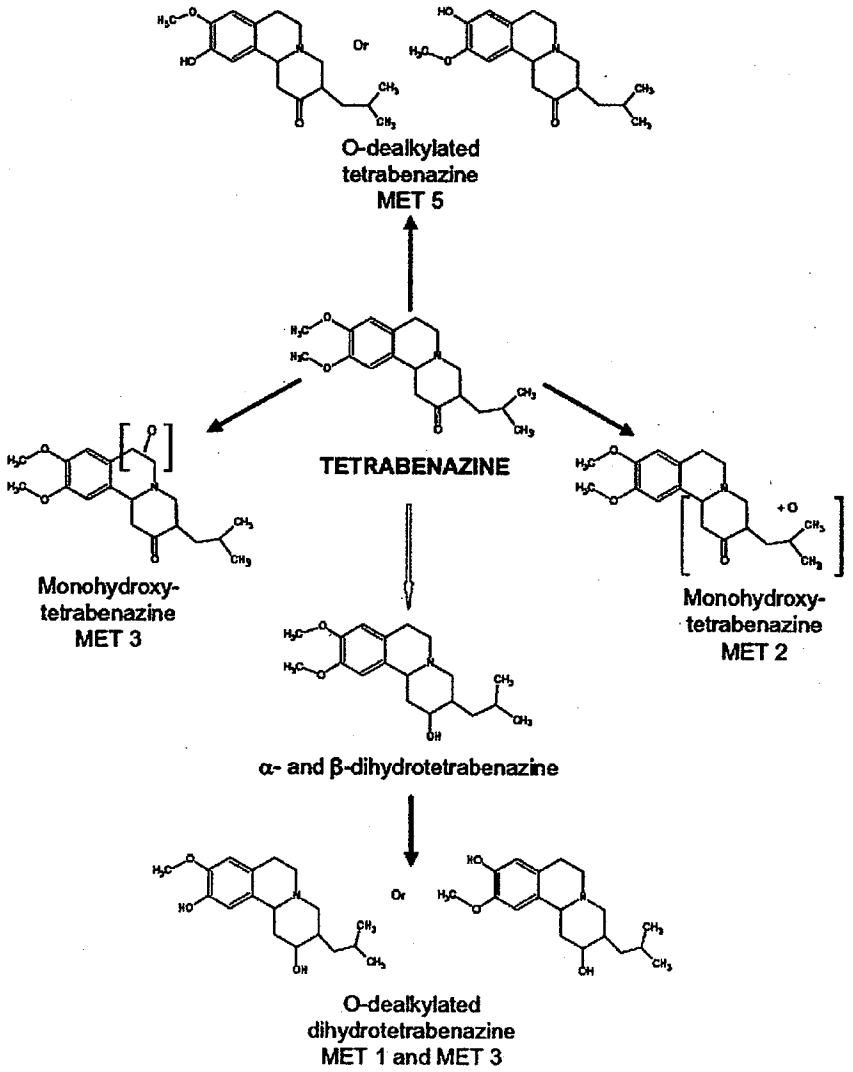
Results: TBZ is metabolized in vitro by liver microsomes to up to seven radioactive components. The results are summarized in the following reviewer-generated table:

In vitro metabolism of [14-C]-TBZ (expressed as percentage of total eluted material at 60 minutes)						
	identity (some are multi-component)	CD1 (♂) mouse	SD (♂) rat	(♂) Beagle dog	(♂) Cyno. monkey	pooled human
MET 1	• Mono-hydroxy O-dealkylated TBZ • O-dealkylated HTBZ • Bis-O-dealykylated HTBZ	1.65%	1.77%	0.00%	0.00%	0.00%
MET 2	• Mono-hydroxy TBZ	6.92%	6.90%	2.80%	11.88%	1.31%
MET 3	• β-HTBZ (listed as major component) • 2nd isomer of Mono-hydroxy TBZ • O-dealkylated HTBZ (1st regioisomer)	17.62%	16.05%	5.67%	14.79%	11.32%
α-HTBZ	• confirmed α-HTBZ	4.85%	15.71%	2.60%	3.30%	9.81%
MET 4	• [redacted]	0.00%	1.93%	0.00%	0.00%	2.63%
MET 5	• [redacted] • O-dealkylated TBZ	16.31%	6.08%	5.51%	11.27%	10.21%
MET 6	• 2nd isomer of TBZ	2.52%	2.91%	5.40%	2.69%	3.90%
	% TBZ metabolized	60.24%	56.86%	19.89%	61.54%	43.85%

- According to the sponsor, "No metabolite formation was evident in the no-cofactor control samples."
- "In all incubations, a relatively minor additional radiolabelled component (MET 6) was detected eluting just after the parent, TBZ. It has been suggested in previous non-related studies that this component comprises another stereo-isomer of parent TBZ. Results from the no-protein control incubation indicated that this stereo-isomer of TBZ was formed non-enzymatically."

Conclusion: Based on this in vitro data, the sponsor has stated that metabolism is qualitatively similar among species (and is depicted in the following schematic provided by the sponsor); however, this appears true only for major radioactive components. The rate of metabolism in this in vitro system was similar for monkey, mouse, and rat, less for human, and notably less for dog. Rate was defined as the "amount of parent compound metabolized and/or metabolite formed per minute per mg microsomal protein." In this system, no species specific radioactive component was identified.

The proposed biotransformation pathway for ^{14}C-tetrabenazine in mouse, rat, dog, Cynomolgus monkey and human liver microsomes is shown below:



[^{14}C]-Tetrabenazine: Metabolite Identification Studies (Study # CAM/11) (Amendment 30, module 4/volume 1)

This study was conducted at [redacted] according to GLP regulations with appropriate QA statements (GLP only covers this study, not the acquisition of samples from previous studies). The study initiation date was 03-Oct-05.

Metabolite identification by HPLC was conducted on urine samples collected from male Lister Hooded rats, male albino mice, and male Beagle dog administered single oral doses of [^{14}C]-Tetrabenazine. The metabolites were compared to those identified in human urine (Study CAM/06).

One male Listar Hooded rat [redacted]Lis) was administered a single oral (gavage) dose of [^{14}C]-TBZ/TBZ in corn oil at a dose of 5 mg/kg. One non-naïve male Beagle dog (27 months old, weighing 13.34 kg on day of dosing) was administered a single oral (gavage) dose of [^{14}C]-TBZ/TBZ dissolved in (2-hydroxypropyl)–β-cyclodextrin at a dose of 25 mg free base. Urine and feces were collected as follows:

- urine: pre-dose, 0-6, 6-14, 24-48, and 48-72 hrs
- feces: predose, 0-24, 24-48, and 48-72 hrs

In addition, 0-6 hr urine from male albino mice (study CAM/08), and concentrated 0-4 hr urine from humans (study CAM/06) were also available.

Urine evaluation: urine from rat (0-6 and 6-14 hr), dog (0-6 hr), mouse (0-24 hr), and human (0-4 hr) were evaluated HPLC with on-line radiodetection and by co-chromatography with human urine.

HPLC analysis of urine from the different species identified numerous metabolites in each species:

- Mouse: The 0-24 hr sample contained 28.8% of the radioactivity administered. There were at least 21 possible metabolites, with the most abundant component accounting for 4.96% of the administered dose.
- Rat: The 0-6 hr sample contained 8.22% of the radioactivity administered. There were at least 21 possible metabolites, with the most abundant component accounting for 0.89% of the administered dose. The 6-24 hr sample contained 12.3% of the radioactivity administered. There were at least 20 possible metabolites, with the most abundant component accounting for 2.35% of the administered dose.
- Dog: The 0-6 hr sample contained 13% of the radioactivity administered. There were at least 19 possible metabolites, with the most abundant component accounting for 1.72% of the administered dose.
- Human: The 0-4 hr sample contained < 16.55 % of the radioactivity administered (16.55% was noted for the 0-6 hr sample; however this study used the 0-4 hr portion). There were at least 20 possible metabolites, with the most abundant component accounting for 3.65% of the administered dose.

The sponsor's summary Table #1 follows. In this table the sponsor has presented a comparison of the radioactive urinary components detected in rat, dog and mouse to 14 of the 22 detected in human. According to the sponsor, the radioactive components were qualitatively similar across species and the "major" components detected in human urine were also detected in rat, mouse and dog. ("Major" was not defined).

Conclusion: From this study it can be concluded that the urinary metabolic profiles are complex in humans and the animal species tested. It should be noted that the strain of rat evaluated (Lister Hooded) is not the strain used in the pivotal toxicity studies in rat (SD), and without further information, the strain of mouse (identified only as albino) may also not be one of those used in the pivotal toxicity studies.

Summary Table 1 Cross species comparison of radioactive urinary components

		% Dose		
Component assigned in CAM/06	Assignment (from CAM/06)	Mouse	Rat	Dog
U1	Monohydroxy-dihydro tetrabenazines and Glucuronides of O-dealkylated dihydro tetrabenazine(s)	0.17	0.19	0.32
U3		-	0.42	0.81
U4		3.06	-	0.39
U5		1.22	-	0.40
U6		4.21	0.85	0.97
U7		-	0.77	-
U8		2.93	0.89	0.76
U9		-	0.35	1.54
U10	Monohydroxy dihydrotetrabenazine	-	0.31	-
U11	Sulphate conjugates of O-dealkylated dihydro tetrabenazine(s)	4.96	0.77	0.94
U13		2.73	0.71	1.72
U15	O-dealkylated dihydro tetrabenazine and/or β-dihydro tetrabenazine	0.98	0.10	-
U17		1.03	0.07	0.31
U18	α-dihydrotetrabenazine	0.96	0.06	0.73

- component not present

APPEARS THIS WAY
ON ORIGINAL

[^{14}C]-Tetrabenazine: Investigation of Metabolites in Plasma of Rat, Mouse, Rabbit and Dog After Oral Administration (Study # CAM/21) (Amendment 30, module 4/volume 2)

This study was conducted at [redacted] according to GLP regulations with appropriate QA statements. The study initiation date was 18-May-06.

Methodology: Single oral doses of [^{14}C]-tetrabenazine were administered to C57BL/6 mice, SD rats, New Zealand White rabbits, and non-naïve Beagle dogs. Blood samples were obtained at 0.5, 2 and 6 hrs post dose. Plasma was separated and analyzed twice as described by sponsor:

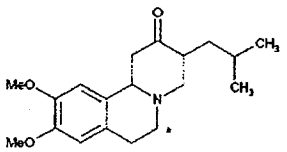
- "Plasma samples (mouse, rat, rabbit and dog) were analysed by HPLC with on-line radiodetection. Available reference compounds were also analysed by HPLC for comparison. The proportions of radioactive components have been calculated. Values assigned to reference compounds have been based on comparison of retention times and may therefore be over/underestimated.

  Plasma samples (mouse, rat, rabbit and dog) were also analysed by using LC-MS/MS techniques for the presence of components corresponding to the available reference compounds and for the presence of metabolites identified in a previous study [redacted] study number CAM/06[1]). Additional metabolites identified in a previous study [redacted] study number CAM/16[2]) were also included in the metabolite screen."

Animals: Animals, as specified below, were acclimatized for only 2 days prior to dosing (except dogs which were acclimated for 6 days). In addition to the number of animals listed below, it appears that additional mice and/or rats were needed to provide sufficient data at 0.5 and 2 hrs post dose. Blood samples from mice, rats and rabbits were terminal samples, whereas dogs were sampled serially.

- Mouse: C57BL/6 (6/sex) from [redacted]. Animals were 6-10 weeks old and weighted 17-25 grams at dosing.
- Rat: Sprague Dawley (9/sex) from [redacted] Animals were approximately 7 weeks old and weighted 194-267 grams at dosing.
- Rabbit: non-pregnant New Zealand White (3 females) from [redacted] Animals were 2-3 kg at dosing.
- Dog: non-naïve Beagle [redacted]OBE) (1/sex) from [redacted] Animals were 30-36 months old at dosing.

[^{14}C]-Tetrabenazine: TBZ (position of label depicted below) was dissolved in corn oil and administered by oral gavage. For rabbits and dogs, the administration of TBZ was followed by a water flush (5 and 20 ml, respectively). For mouse, rat and rabbit, TBZ was administered at a dose of 5 mg/kg and a dose volume of 5 ml/kg. For dogs, TBZ was administered at a dose of 25 mg/animal at a dose of 5 ml/kg. A separate solution was used for dog. The position of the label is depicted below.



* position of ^{14}C-label

Results: Multiple radioactive components were detected in the plasma from each species as discussed below. According to the sponsor, for all of the species tested, "Generally, all components accounting for >10% plasma radioactivity were identified with the exception of component 2 which has been assigned a polar material."

Rat: According to the sponsor, at least 14 radioactive components were detected by HPLC and these data are summarized in the reviewer-generated summary below. The qualitative results of the multiple reaction monitoring (MRM) screening are quoted directly from the sponsor:

- "MRM analyses confirmed that tetrabenazine was present in all samples analysed. MRM analyses also confirmed the presence of at least 9 additional components, including O-dealkylated dihydrotetrabenazine (NSJ 39), O-dealkylated dihydrotetrabenazine (NSJ 3B) and O-dealkylated tetrabenazine (NSJ 25A/NSJ 25B), beta-dihydrotetrabenazine and alpha-dihydrotetrabenazine.

  The following components were also present in one or more of the timepoints examined [sic] glucuronide of O-dealkylated dihydrotetrabenazine, mono-hydroxy O-dealkylated tetrabenazine, mono-hydroxy dihydrotetrabenazine, sulfate of O-dealkylated dihydrotetrabenazine, mono-hydroxy O-dealkylated tetrabenazine and mono-hydroxy Tetrabenazine."

Radioactive Components in Rat Plasma [expressed as ng equiv/g or (% sample radioactivity)]						
Component	0.5 hrs		2 hrs		6 hrs	
	male	female	male	female	male	female
1		-		4.7 (0.6)		-
2	24.4 (10.0)	30.5 (3.4)	38.0 (8.2)	60.4 (7.7)	32.2 (9.0)	62.6 (11.0)
3		4.5 (0.5)		-		-
4	-	92.2 (10.3)	13.4 (2.9)	37.7 (4.8)	15.0 (4.2)	121.8 (21.4)
5	37.4 (15.3)	-	78.3 (16.9)	-	22.9 (6.4)	-
6	19.8 (8.1)	142.5 (15.9)	69.0 (14.9)	164.8 (21.0)	32.2 (9.0)	192.9 (33.9)
7	52.3 (21.4)	75.3 (8.4)	69.0 (14.9)	120.8 (15.4)	53.0 (14.8)	-
8		69.9 (7.8)		174.2 (22.2)		-
9	28.8 (11.8)	201.6 (22.5)	48.2 (10.4)	174.2 (22.2)	16.5 (4.6)	158.2 (27.8)
10	-	201.6 (22.5)	37.5 (8.1)	-	14.7 (4.1)	12.5 (2.2)
11	50.1 (20.5)	27.8 (3.1)	101.0 (21.8)	14.1 (1.8)	41.2 (11.5)	9.1 (1.6)
12	-	13.4 (1.5)	4.6 (1.0)	8.6 (1.1)	-	-
13/14	-	36.7 (4.1)	4.6 (1.0)	24.4 (3.1)	127.9 (35.7)	11.4 (2.0)
15	31.5 (12.9)		-		137.9 (38.5)	
16	-		-		-	

- component 1: polar material
- component 2: polar material
- component 3: polar material
- component 4: co-chromatographs with glucuronide of O-dealkylated HTBZ using LC-MS/MS
- component 5: co-chromatographs with bis-O-dealkylated HTBZ (NSJ 11) using HPLC
- component 6: co-chromatographs with O-dealkylated HTBZ (NSJ 3B) using HPLC and LC-MS/MS
- component 7: co-chromatographs with mono-hydroxy O-dealkylated TBZ using LC-MS/MS
- component 8: co-chromatographs with β-HTBZ using HPLC and LC-MS/MS
- component 9: co-chromatographs with O-dealkylated HTBZ (NSJ 39) using HLPC and LC-MS/MS
- component 10: co-chromatographs with α-HTBZ using HLPC and LC-MS/MS
- component 11: co-chromatographs with bis-O-dealkylated TBZ (NSJ 9) using HPLC
- component 12: co-chromatographs with O-dealkylated TBZ (NSJ 25A / NSJ 25B) using HPLC and LC-MS/MS
- component 13/14: co-chromatographs with TBZ using HPLC and LC-MS/MS
- component 15: identity not confirmed
- component 16: not observed

Mouse: According to the sponsor, at least 11 radioactive components were detected in the 0.5 hr plasma sample by HPLC. No data were presented for 2 hrs and 6 hrs, and no reason for the lack of comparable data at these time points was provided. The data are summarized in the reviewer-generated summary below. The qualitative results of the multiple reaction monitoring (MRM) screening are quoted directly from the sponsor:

- "MRM analyses confirmed that tetrabenazine was present in all samples analysed. MRM analyses also confirmed the presence of at least 8 additional components, including O-dealkylated dihydrotetrabenazine (NSJ 39) and O-dealkylated tetrabenazine (NSJ 25A/NSJ 25B), beta-dihydrotetrabenazine and alpha-dihydrotetrabenazine.

  The following components were also present in one or more of the timepoints examined [sic] Glucuronide of O-dealkylated dihydrotetrabenazine, mono-hydroxy dihydrotetrabenazine, mono-hydroxy O-dealkylated tetrabenazine and mono-hydroxy tetrabenazine."

Radioactive Components in Mouse Plasma [expressed as ng equiv/g or (% sample radioactivity)]						
Component	0.5 hrs		2 hrs		6 hrs	
	male	female	male	female	male	female
1	-	-				
2	74.0 (9.3)	153.2 (15.4)				
3	19.1 (2.4)	21.9 (2.2)				
4	50.9 (6.4)	102.5 (10.3)				
5	-	-				
6	168.6 (21.2)	307.4 (30.9)				
7	-	-				
8	84.3 (10.6)	106.4 (10.7)				
9	28.6 (3.6)	51.7 (5.2)				
10	112.9 (14.2)	165.1 (16.6)				
11	66.8 (8.4)	32.8 (3.3)				
12	31.8 (4.0)	-				
13/14	158.3 (19.9)	54.7 (5.5)				
15	-	-				
16	-	-				

- component 1: not observed
- component 2: polar material
- component 3: polar material
- component 4: co-chromatographs with glucuronide of O-dealkylated HTBZ using LC-MS/MS
- component 5: not observed
- component 6: co-chromatographs with O-dealkylated HTBZ (NSJ 3B) using HPLC
- component 7: not observed
- component 8: co-chromatographs with β-HTBZ using HPLC and LC-MS/MS
- component 9: co-chromatographs with O-dealkylated HTBZ (NSJ 39) using HPLC and LC-MS/MS
- component 10: co-chromatographs with α-HTBZ using HPLC and LC-MS/MS
- component 11: co-chromatographs with bis-O-dealkylated TBZ (NSJ 9) using HPLC
- component 12: co-chromatographs with O-dealkylated TBZ (NSJ 25A / NSJ 25B) using HPLC and LC-MS/MS
- component 13/14: co-chromatographs with TBZ using HPLC and LC-MS/MS
- component 15: not observed
- component 16: not observed

Rabbit: According to the sponsor, at least 10 radioactive components were detected by HPLC and these data are summarized in the reviewer-generated summary below. The qualitative results of the multiple reaction monitoring (MRM) screening are quoted directly from the sponsor:

- "MRM analyses confirmed the presence of at least 8 components, including O-dealkylated dihydrotetrabenazine (NSJ 39, beta-dihydrotetrabenazine and alpha-dihydrotetrabenazine.

  The following components were also present in one or more of the timepoints examined glucuronide of dihydrotetrabenazine, glucuronide of O-dealkylated dihydrotetrabenazine, mono-hydroxy dihydrotetrabenazine, mono-hydroxy O-dealkylated tetrabenazine and mono-hydroxy tetrabenazine."

Radioactive Components in Rabbit Plasma [expressed as ng equiv/g or (% sample radioactivity)]						
Component	0.5 hrs		2 hrs		6 hrs	
	male	female	male	female	male	female
1	[redacted]	-	[redacted]	-	[redacted]	-
2	[redacted]	139.6 (12.9)	[redacted]	326.1 (33.5)	[redacted]	245.6 (38.0)
3	[redacted]	53.0 (4.9)	[redacted]	40.9 (4.2)	[redacted]	-
4	[redacted]	130.9 (12.1)	[redacted]	207.3 (21.3)	[redacted]	179.7 (27.8)
5	[redacted]	268.4 (24.8)	[redacted]	128.5 (13.2)	[redacted]	89.2 (13.8)
6	[redacted]	23.8 (2.2)	[redacted]	182.0 (18.7)	[redacted]	49.8 (7.8)
7	[redacted]	118.0 (10.9)	[redacted]	76.9 (7.9)	[redacted]	82.1 (12.7)
8	[redacted]	61.7 (5.7)	[redacted]	11.7 (1.2)	[redacted]	-
9	[redacted]	18.4 (1.7)	[redacted]	-	[redacted]	-
10	[redacted]	15.2 (1.4)	[redacted]	-	[redacted]	-
11	[redacted]	-	[redacted]	-	[redacted]	-
12	[redacted]	-	[redacted]	-	[redacted]	-
13/14	[redacted]	253.2 (23.4)	[redacted]	-	[redacted]	-
15	[redacted]	-	[redacted]	-	[redacted]	-
16	[redacted]	-	[redacted]	-	[redacted]	-

- component 1: not observed
- component 2: polar material
- component 3: polar material
- component 4: co-chromatographs with glucuronide of HTBZ and glucuronide of O-dealkylated HTBZ using LC-MS/MS
- component 5: co-chromatographs with bis-O-dealkylated HTBZ (NSJ 11) using HPLC analysis
- component 6: co-chromatographs with O-dealkylated HTBZ (NSJ 3B) using HPLC and LC-MS/MS
- component 7: co-chromatographs with mono-hydroxy O-dealkylated TBZ using LC-MS/MS
- component 8: co-chromatographs with β-HTBZ using HPLC and LC-MS/MS
- component 9: co-chromatographs with O-dealkylated HTBZ (NSJ 39) using HPLC and LC-MS/MS
- component 10: co-chromatographs with α-HTBZ using HPLC and LC-MS/MS
- component 11: not observed
- component 12: not observed
- component 13/14: co-chromatographs with TBZ using HPLC and LC-MS/MS
- component 15: not observed
- component 16: not observed

Dog: According to the sponsor, at least 13 radioactive components were detected by HPLC and these data are summarized in the reviewer-generated summary below. The qualitative results of the multiple reaction monitoring (MRM) screening are quoted directly from the sponsor:

- "MRM analyses confirmed that tetrabenazine was present in all samples analyzed. MRM analyses also confirmed the presence of at least 10 components, including O-dealkylated dihydrotetrabenazine (NSJ 39), O-dealkylated dihydrotetrabenazine (NSJ 3B) and O-dealkylated tetrabenazine (NSJ 25A/NSJ 25B), beta-dihydrotetrabenazine and alpha-dihydrotetrabenazine.

  The following components were also present in one or more of the timepoints examined [sic] glucuronide of O-dealkylated dihydrotetrabenazine, mono-hydroxy O-dealkylated tetrabenazine, sulphate of O-dealkylated dihydrotetrabenazine, mono-hydroxy O-dealkylated tetrabenazine and mono-hydroxy tetrabenazine."

Radioactive Components in Dog Plasma [expressed as ng equiv/g or (% sample radioactivity)]						
Component	0.5 hrs		2 hrs		6 hrs	
	male	female	male	female	male	female
1	-	-	-	-	-	-
2	15.6 (8.5)	27.6 (20.7)	14.5 (4.2)	6.8 (2.6)	13.0 (4.7)	41.5 (8.2)
3	-	-	-	-	-	-
4	-	-	9.0 (2.6)	20.3 (7.7)	12.2 (4.4)	58.2 (11.5)
5	-	-	27.7 (8.0)	-	15.0 (5.4)	-
6	33.1 (18.0)	63.5 (47.7)	29.4 (8.5)	37.4 (14.2)	36.0 (13.0)	130.6 (25.8)
7	-	-	-	-	-	-
8	-	-	68.2 (19.7)	109.8 (41.7)	60.1 (21.2)	139.7 (27.6)
9	70.2 (38.2)	42.1 (31.6)	-	-	-	-
10	-	-	43.6 (12.6)	25.0 (9.5)	17.2 (6.2)	54.7 (10.8)
11	-	-	69.2 (20.0)	52.9 (20.1)	49.8 (18.0)	7.6 (1.5)
12	-	-	30.1 (8.7)	-	26.6 (9.6)	2.5 (0.5)
13/14	53.1 (28.9)	-	54.7 (15.8)	7.9 (3.0)	42.9 (15.5)	71.4 (14.1)
15	-	-	-	-	3.9 (1.4)	-
16	12.0 (6.5)	-	-	3.2 (1.2)	-	-

- component 1: not observed
- component 2: polar material
- component 3: not observed
- component 4: co-chromatographs with glucuronide of O-dealkylated HTBZ using LC-MS/MS
- component 5: co-chromatographs with bis-O-dealkylated HTBZ (NSJ 11) using HPLC
- component 6: co-chromatographs with O-dealkylated HTBZ (NSJ 3B) using HPLC and LC-MS/MS
- component 7: not observed
- component 8: co-chromatographs with β-HTBZ using HPLC and LC-MS/MS
- component 9: co-chromatographs with O-dealkylated HTBZ (NSJ 39) using HPLC and LC-MS/MS
- component 10: co-chromatographs with α-HTBZ using HPLC and LC-MS/MS
- component 11: co-chromatographs with bis-O-dealkylated TBZ (NSJ 9) using HPLC
- component 12: co-chromatographs with O-dealkylated TBZ (NSJ 25A / NSJ 25B) using HPLC and LC-MS/MS
- component 13/14: co-chromatographs with TBZ using HPLC and LC-MS/MS
- component 15: identity not confirmed
- component 16: identity not confirmed

Conclusion: Multiple radioactive components were identified in each species (at least 14 in rat, at least 11 in mouse, at least 10 in rabbit and at least 13 in dog.

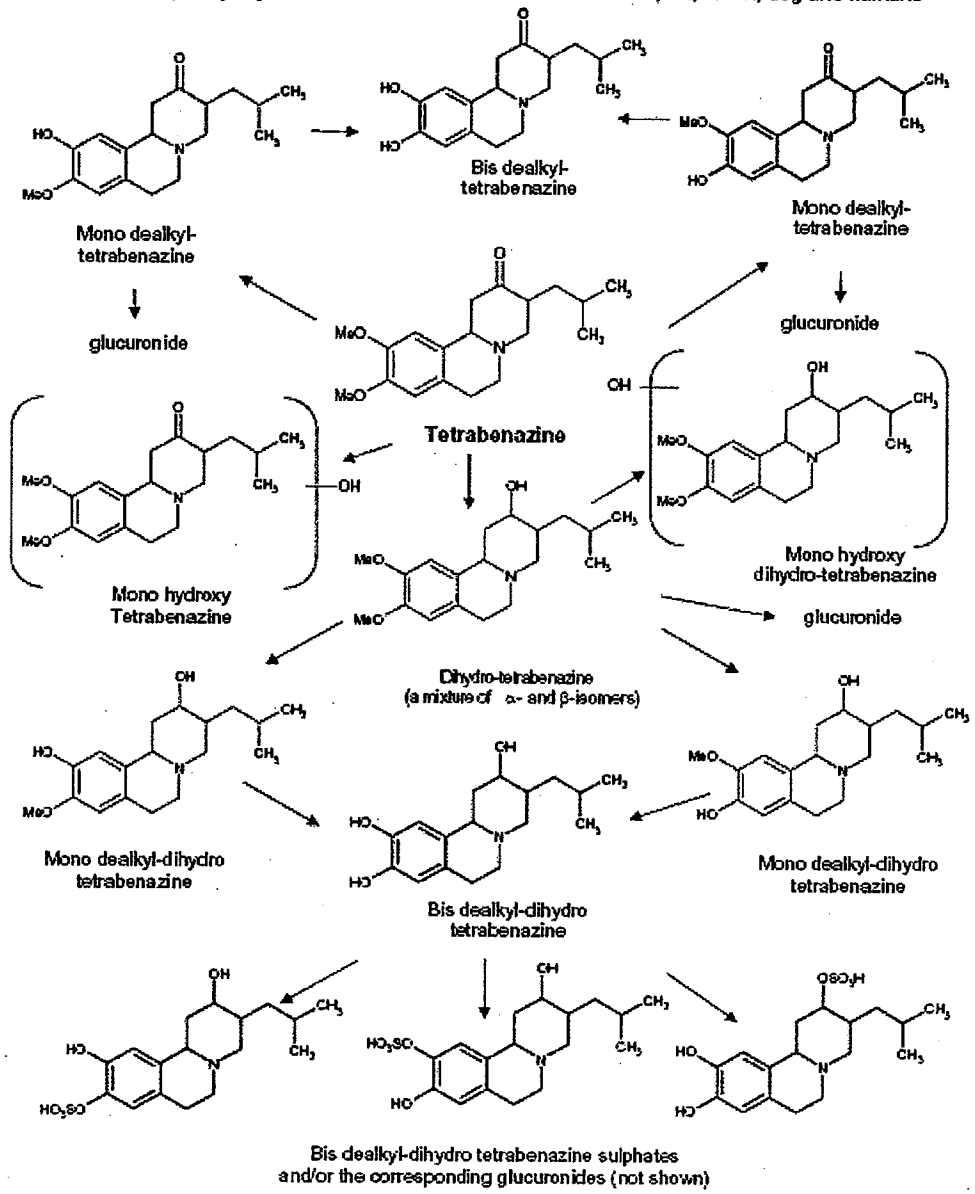
The sponsor provided the following summary table that compared the results for the rat, dog and mouse (sexes combined) and female rabbits to data derived in humans (from study CAM/06). Based on this summary, all of the six plasma radioactive components that were detected in humans (some of which are unresolved mixtures of enantiomers), were also detected in rat, dog, mouse and rabbit, except the sulfated conjugates of O-delakylated-HTBZ, which are not present in the plasma from mouse and rabbit. Based on these data, the sponsor proposed the following common metabolic pathway.

Component observed	Nominal retention time (minutes)	Mouse	Rat	Rabbit	Dog	Human (CAM/06[1])
Glucuronide of dihydrotetrabenazine (or Glucuronide of Mono-hydroxy O-dealkylated Tetrabenazine)	9.2-10.7	-	-	++	-	-
Glucuronide of O-dealkylated dihydrotetrabenazine	10.7-11.5	++	++	+	++	+
Mono-hydroxy dihydrotetrabenazine	12.2-15.7	+	+	+	+	+
Sulphate of O-dealkylated dihydrotetrabenazine	13.8-16.1	-	+	-	+	++
Bis-O-dealkylated dihydrotetrabenazine (NSJ 41A)	16.1	-	-	-	-	-
Mono-hydroxy O-dealkylated Tetrabenazine	15.2-16.2	+	++	++	+	-
Mono-hydroxy Tetrabenazine	16.3	+	+	+	+	-
Beta-dihydrotetrabenazine	16.5	++	++	+	++	+
O-dealkylated dihydrotetrabenazine (NSJ 3B or NSJ 39)	15.6-19.2	++	++	++	++	++
Alpha-dihydrotetrabenazine	22.4	++	++	+	++	++
O-dealkylated Tetrabenazine(NSJ 25A / NSJ 25B)	28.0	+	+	-	+	-
Tetrabenazine	31.4-34.1	++	++	-	++	-

Nominal retention times taken from MRM analysis of mixed reference samples and from several samples

\+ = Component present
++ = Component present at >10% plasma radioactivity (see main report tables 3 – 8)
\- = Not present or below limit of detection based on HPLC with radiodetection

Proposed pathways of [^{14}C]-tetrabenazine biotransformation in mouse, rat, rabbit, dog and humans



Tetrabenazine Histological Evaluation Extension for the 26 Week Toxicity Study in Rats

[redacted] Report # 27259 based on [redacted] Project # 455738) (Amendment 32, module 4 volume 1 of 1)

Objective: "The object of this study was to re-evaluate the brain tissue from a subset of the Control and High Dose groups using special stains to detect evidence of nigrostriatal neurodegeneration as suggested by Satou et al. (Satou T *et al.* Exp Toxicol Pathol 53(4): 303-308 2001)."

This reevaluation of brain tissue from the 26-week toxicity study in rats was conducted according to GLP (with QA). Study initiation for this additional brain evaluation was 29-June-06.

Animals: Sprague-Dawley [redacted] :CD®(SD) IGS BR) from [redacted]

Dosing regimen from the 26-week oral (gavage) toxicity study in rat				
group	treatment	dose	# sacrificed after 13 wks	# sacrificed after 26 wks
control	vehicle	0 mg/kg, bid = 0 mg/kg/day	10/sex	20/sex
low dose	tetrabenazine	2.5 mg/kg, bid = 5 mg/kg/day	10/sex	20/sex
mid dose	tetrabenazine	7.5 mg/kg, bid = 15 mg/kg/day	10/sex	20/sex
high dose	tetrabenazine	15 mg/kg, bid = 30 mg/kg/day	10/sex	20/sex
• Test articles administered as bid oral gavage, with doses separated by approximately 12 hrs, except on days designated for TK sampling, on which a single dose was administered.				

This study report is comprised of three separate evaluations as described below. All three evaluations were based on brain tissue from the same subset of the control and high dose animals that survived until the scheduled sacrifice after 26 weeks of treatment.

Neuropathology Examination			
Group	Dose	Males	Females
control	0 mg/kg, bid x 26 weeks	# sacrificed after 26 wks = 19 # examined for neuropathology = 15 animals: 11-22, 24-26	# sacrificed after 26 wks = 17 # examined for neuropathology = 15 animals: 134-140, 142-148, 246
high dose	15 mg/kg, bid x 26 weeks	total sacrificed after 26 wks = 16 # examined for neuropathology = 15 animals: 102, 103, 105-109, 111-115, 117-119	total sacrificed after 26 wks = 19 # examined for neuropathology = 15 animals: 221-227, 229-236

Initial neuropathology evaluation: This part of the evaluation was conducted at [redacted] by reviewing pathologist [redacted] BSc, BVSc, MAnimSc, FRIPH, DipRCPath., MRCVS, MRCPath. The description of the methodology was inadequate. Brain tissue from a subset of animals (15/sex/group) sacrificed after 26 weeks of treatment (as designated in the table above) were processed. No further details of the processing, or sectioning were provided. Sections stained with H&E, anti-GFAP, FluoroJade B, anti-tyrosine hydroxylase and Bielschowsky's silver were evaluated by light microscopy.

According to Dr. [redacted] "The animals in which substantia nigra sections were available did not show any evidence of damage." Dr. [redacted] concluded that there was no effect of treatment. The pathologist's statement is unclear, since there was no delineation of the presence or absence of substantia nigra in the examined sections for each animal. A copy of the study pathologist's summary table follows.

Tetrabenazine
Histological Evaluation Extension for the 26 Week Toxicity Study
In Rats - Project No. 455738

Table 1 Summary of Histological Findings: Brain

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS Males Grp 1 0 mg/kg /bid	Males Grp 4 15 mg/kg /bid	Females Grp 1 0 mg/kg /bid	Females Grp 4 15 mg/kg /bid
NERVOUS SYSTEM					
BRAIN		(15)	(15)	(15)	(15)
No abnormality detected		14	15	15	15
Gliosis, cerebellar peduncle, level III, focal, unilateral		1	0	0	0
FluoroJade B stain examined		15	15	15	15
Bielschowsky stain examined		15	15	15	15
GFAP stain examined		15	15	15	15
Tyrosine hydroxylase stain examined		15	15	15	15

Figures in brackets represent the number of animals from which this tissue was examined microscopically

Peer review neuropathology evaluations: Peer review was conducted at [redacted] by [redacted] DVM&S, MSc, DLAS, MRCVS, MRCPath.

Initial peer review: According to the report, all of the necropsy and histology procedures were conducted in the Ediburgh facilities, with stained slides submitted for peer review. The methods section is reproduced below with reviewer annotations in square brackets [ ].

Brain sections from all study animals that survived until 26-Week sacrifice were examined. From each animal (except animal 138), multiple stains of slides 20 and 35 were examined. Only slide 20 was submitted from animal 138. Slide 20 contained three sections of brain. There was slight variation in sectioning but in general, slide 20 contained the usual three brain sections taken and examined in general toxicology studies. Specifically, the three sections on slide 20 were as follows:

> [Full coronal] Section 1: Level of the basal nuclei, including the caudate nucleus (or a portion of the caudate nucleus and the putamen), the corpus callosum and the cerebral cortex (cingulate, frontal, parietal, insular and piriform cortical areas)
>
> [Full coronal] Section 2: Level of the posterior thalamus, including the thalamus, hypothalamus, hippocampus, internal capsule, and cerebral cortex (cingulate, occipital, parietal, temporal, rhinal and piriform cortical areas)
>
> [Full coronal] Section 3: Level of the medulla oblongata and cerebellum

Slide 35 contained a single [full coronal] brain section. For most females and a lesser number of males, this section was taken from the midbrain at the level of the substantia nigra and included rostral colliculus, aqueduct, cerebral peduncles, and cerebral cortex (including the cingulate, occipital, temporal, rhinal and piriform cortical areas). For many males, this section was taken further caudally, at the level of the pons. These sections generally did not contain sufficient substantia nigra for evaluation. For animals 011, 016, 017, 018, 019, 021, 022, 024, 026, 102, 105, 106, 107, 108, 111, 113, 114, 115, 138, 231 and 246, block 20 was reembedded and sections cut and stained with H&E, anti-tyrosine hydroxylase, Bielschowsky's silver, anti-GFAP and Fluoro-Jade B.

- [note: according to the report, "The substantia nigra was considered present for evaluation if at least six TH positive neurons were present on at least one slide."]

In the data tables in Appendix 1, the presence or absence of the substantia nigra in the brain sections is specifically recorded.

Slides 20 and 35 were stained as follows:

- Hematoxylin and eosin
- Immunohistochemical stain for Tyrosine hydroxylase (TH) (a stain that specifically stains/identifies dopaminergic neurons)
- Negative control immunohistochemical stain for tyrosine hydroxylase
- Immunohistochemical stain for glial fibrillary acidic protein (GFAP) (a stain that specifically stains astrocytes and serves as a general marker for central nervous system damage)
- Cytochemical silver stain (Bielschowsky's) (a stain that specifically stains axons and neurofilaments)
- Cytochemical Fluoro-Jade B stain (a stain that specifically identifies degenerating and necrotic neurons). These slides were examined with a fluorescent microscope.

Positive control slides were provided for all stains.

Results: According to the report, all of the positive control slides were stained appropriately. The peer review pathologist (Dr [redacted] noted that the ventral tegmental area (VTA) "is closely aligned morphologically and functionally with substantia nigra" and that the presence/absence of the VTA was noted for each animal. The results of this initial peer review are summarized below:

- General: there were no abnormalities in the brain sections (with any stain).
- H&E: "no evidence of neuronal degeneration or necrosis" in any animals.
- Fluoro-Jade B: "no evidence of neuronal degeneration or necrosis" in any animals.
- GFAP: "did not reveal any reactive astrocytosis, indicating no ongoing damage in the brain."
- Silver: "normal, indicating no alterations in axons or neurofilaments."
- TH: "no difference in the control and high dose brain sections…"
- There were no morphologic differences between control and high dose animals with regard to substantia nigra, ventral tegmental area and striatal (caudate nucleus/putamen) areas (noted to be areas which contain TH positive neurons or projections these neurons). The peer review pathologist noted that this was based on a subset of 13/14-control females, 15/15-high dose females, 6/15-control males and 7/15-high dose males; all animals that had sufficient substantia nigra on the slides for evaluation.
- "For those animals (control and high dose) where the substantia nigra was present in the sections, there was considerable variability in the number of neurons present. Again, this was due to sectioning."
- "There was complete agreement between the Study Pathologist [Dr [redacted]] and the Reviewing Pathologist [Dr [redacted] on the lack of morphologic differences in the brain between the control and high dose animals."

With regard to the difference in histologic evaluation techniques used in this study versus those used in the referenced article published by Satou et al. (2001), the peer review pathologist states:

> "Morphometrics were not conducted on the current study. But TH staining was conducted, and this technique allowed for the specific identification of dopaminergic neurons in the substantia nigra. In the opinion of the RP [peer review pathologist], using hematoxylin and eosin stained sections to define neurons belonging to the substantia nigra would be a very subjective process. In

the current study, based on light microscopy observations of the TH and GFAP stained sections, there were no differences between the control and test article treated animals."

Supplemental peer review pathology report: This supplemental study describes the additional histopathologic evaluation conducted by the peer review pathologist (Dr ___ to increase the number of control males and high dose males from which substantia nigra could be evaluated. Additional wet tissue and paraffin blocks were obtained from the (15) control males and (15) high dose males that were previously evaluated for neuropathology.

Wet tissue from eight control males (011, 015, 016, 017, 019, 022, 024, and 026) and six high dose males (102, 107, 111, 112, 113, and 114) were determined to contain possible substantia nigra tissue. These sections were trimmed, embedded in paraffin, sectioned (5 microns) and stained with H&E. Substantia nigra was present in all but high dose male 107. Serial sections were obtained for the eight control males and the remaining five high dose males and these sections were stained for Fluoro-Jade B, GFAP, Bielschowsky's silver and TH; however, it is not clear if each stain was associated with a unique section, or if all four stains were tested on a single section.

The original paraffin blocks of brain (block 35) were evaluated to determine if re-embedding would yield addition sections of substantia nigra. It was determined that six control males and three high dose males should be re-embedded and sectioned; however, the resulting H&E stained sections did not provide any additional sections containing substantia nigra, and these sections were not further processed.

Copies of the peer review pathologist's revised data tables follow. The results of the supplemental evaluation were incorporated into the original peer review summary table. The supplemental evaluations appear in the table with a black background.

The study pathologist (Dr. ___ did not review the supplemental slides generated during the second (supplemental) peer review. The conclusion of the peer review pathologist (Dr ___ lid not change from his original conclusion after reviewing the supplemental slides. This final evaluation was based on light microscopic evaluation of substantia nigra (unilateral or bilateral) from 13 of 15 control males, 11 of 15 high dose males, 13 of 15 control females and 15 of 15 high dose females. The following caveat should be noted in the interpretation of this data, according Dr ___ substantia nigra was considered present and sufficient for evaluation if at least six TH positive neurons were present on at least one slide.

General consensus (study pathologist and peer review pathologist) conclusion: "Based on the light microscopic, morphologic evaluation of haematoxylin and eosin, anti-glial fibrillary acidic protein, anti-tyrosine hydroxylase, Fluoro-Jade B and silver (Bielschowsky's) stains, there were no differences in the brain, including the substantia nigra and striatal areas, between the control animals and high dose animals, who received oral tetrabenazine at 15 mg/kg twice daily for 26 weeks."

Tissue/Diagnosis	Group 1, 0 mg/kg Saline																Group 4, 15 mg/kg															
Sex	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 4	0 2 5	0 2 6	J N C	1 0 2	1 0 3	1 0 5	1 0 6	1 0 7	1 0 8	1 0 9	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 1 7	1 1 8	1 1 9	I N C
BRAIN	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
H&E stained sections	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
anti-Tyrosine hydroxylase stained sections	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
anti-GFAP stained sections	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Fluoro-Jade B stained sections	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Bielschowsky's silver staining	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Substantia nigra, Present Bilateral	X	-	X	X	X	X	X	-	-	X	-	X	.	X	X	10	X	X	-	-	-	X	X	X	X	X	X	.	-	-	X	9
Substantia nigra, Present Unilateral	-	X	-	-	-	-	-	-	X	-	-	-	X	-	-	3	-	-	-	-	-	-	-	-	-	-	-	X	X	-	2
Substantia nigra, Tyrosine hydroxylase staining	N	N	N	N	N	N	N	-	N	N	-	N	N	N	N	13	N	N	-	-	-	N	N	N	N	N	-	N	N	N	11
Ventral Tegmental Area, Present	-	X	X	X	X	X	X	-	X	X	-	X	X	X	X	12	X	X	-	X	-	-	-	N	X	X	X	-	X	N	X	10
Ventral Tegmental Area, Tyrosine hydroxylase staining	-	N	N	N	N	N	N	-	N	N	-	N	N	N	N	12	N	N	-	N	-	-	-	N	N	N	N	-	N	N	N	10
Thalamus, Tyrosine hydroxylase staining	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Striatal area, Tyrosine hydroxylase staining	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15	N	N	N	N	N	N	N	N	N	N	N	N	-	N	N	14

Tissue/Diagnosis	Group 1, 0 mg/kg Saline																Group 4, 15 mg/kg															
Sex	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F		F	F	F	F	F	F	F	F	F	F	F	F	F	F		
	1 3 4	1 3 5	1 3 6	1 3 7	1 3 8	1 3 9	1 4 0	1 4 2	1 4 3	1 4 4	1 4 5	1 4 6	1 4 7	1 4 8	2 4 6	1 N C	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7	2 2 9	2 3 0	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 3 6	1 N C
BRAIN	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
H&E stained sections	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
anti-Tyrosine hydroxylase stained sections	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
anti-GFAP stained sections	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Fluoro-Jade B stained sections	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Bielschowsky's silver staining	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Substantia nigra, Present Bilateral	X	-	X	-	-	X	X	X	X	-	X	X	-	-	-	8	X	X	X	-	X	-	-	X	X	X	X	X	X	X	X	12
Substantia nigra, Present Unilateral	-	X	-	X	-	-	-	-	-	X	-	-	X	X	-	5	-	-	-	X	-	X	X	-	-	-	-	-	-	-	-	3
Substantia nigra, Tyrosine hydroxylase staining	N	N	N	N	-	N	N	N	N	N	N	N	N	N	-	13	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Ventral Tegmental Area, Present	X	X	X	X	-	X	X	X	X	-	X	X	X	X	-	12	X	X	X	X	X	X	X	X		X	X	X	X	X	X	14
Ventral Tegmental Area, Tyrosine hydroxylase staining	N	N	N	N	-	N	N	N	N	-	N	N	N	N	-	12	N	N	N	N	N	N	N	N	-	N	N	N	N	N	N	14
Thalamus, Tyrosine hydroxylase staining	N	N	N	N	-	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15
Striatal area, Tyrosine hydroxylase staining	N	N	N	N	-	N	N	N	N	N	N	N	N	N	N	14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	15

Neuropathology Extension of 9-Month Toxicity Study in Dogs (——Study # 7425-101)
(submitted in amendment #037, module 4/volume 1)

This additional examination of the brain tissue from the original 9-month general toxicity study was conducted at ——— according to GLP regulations (with QA). There was an exception to GLP; the results of this report were not reported to the original study director of the 9-month general toxicology study conducted at ——— and this report will not be filed as an amendment to the original toxicology study report generated by ——— Thus, this neuropathology evaluation exists as a separate report. Study initiation of the additional brain evaluation was approximately 03-May-07.

Animals: purebred Beagle dogs from ———

Dosing regimen used in the 9-month oral (capsule) toxicity study			
group	treatment	dose	# sacrificed after 9-months
control	vehicle	0 mg/kg/day, qd	4/sex
low dose	tetrabenazine	1 mg/kg/day, qd	4/sex
mid dose	tetrabenazine	3 mg/kg/day, qd	4/sex
high dose	tetrabenazine	10 mg/kg/day, qd	4/sex

Objective: "The objective of this portion of the study was to provide a more comprehensive microscopic evaluation of the brain of the study dogs to ascertain if the findings of Satou et. al. ..., including neuronal loss in the substatia nigra, could be confirmed in study 7425-101 [9-month toxicity study in dog]."

Methods: The methods section is reproduced below with reviewer annotations in square brackets [ ].

PAI received residual wet tissue of the brain from all study animals. In addition, the original paraffin blocks (blocks 1 to 4) and hematoxylin and eosin stained brain sections (slides 1 to 4) were sent to PAI.

- [note: appendix 1 lists only three bocks: Oversized block #1 ("Full coronal section taken from the level of the caudate putamen or rostral thalamus (including the cerebral cortex)"), Oversize block #2 ("Full coronal section taken from the level of the midbrain (including the cerebral cortex)"), and Standard size block #3 ("Section of the cerebellum and caudal pons").]

At PAI, the original paraffin blocks prepared by ——— were re-sectioned to yield at least four serial sections.

Residual wet tissue of the brain was trimmed as per the embedding scheme in Appendix 1. This produced an additional eight paraffin blocks per brain. From each of these blocks, at least five serial sections were cut.

- [note: appendix 1 lists the blocks as follows:
  1. "Section through the frontal cortex"
  2. "Section through the caudate putamen area"
  3. "Section of the cerebral cortex at the level of the caudate putamen area"
  4. "Section of the thalamus/hypothalamus area (landmark: third ventricle)"
  5. "Midbrain* (landmark aqueduct)" (* should contain a section of substantia nigra)
  6. "Midbrain* (landmark aqueduct)" (* should contain a section of substantia nigra)
  7. "Cerebral cortex, level of the midbrain"
  8. "Cerebellum and medulla oblongata (emphasis on the medulla oblongata) and an additional section of the caudal medulla oblongata if available."]

Serial sections from the blocks produced at PAI were stained with H&E, anti-glial fibrillary acidic protein (GFAP), anti-tyrosine hydroxylase (TH), Fluoro-Jade B or Bielschowsky's silver (each section received one of the stains).

Serial sections from the blocks produced at ——— were stained with the above stains with the exception of H&E since H&E stained sections were previously produced.

H&E is the standard stain utilized to evaluate microscopic tissue sections in the majority of general toxicity studies.

Fluoro-Jade B is a stain that is very specific and sensitive for necrotic neurons. Use of Fluoro-Jade B stain significantly increases the ability to detect necrotic neurons at microscopic examination.

Anti-GFAP selectively stains intermediate filament in astrocytes. Because astrocytes respond (by increasing in size and/or number) to a variety of central nervous system injuries, and increase in GFAP staining is a sensitive indicator of central nervous system lesions.

Anti-TH selectively stains dopaminergic neurons and the cell processes and connections of those neurons. Staining is most intense in the substantia nigra nuclear area (which is populated by numerous dopaminergic neurons) and the caudate/putamen area (where those neurons project). Scattered staining exists in other portions of the brain. Because anti-TH selectively stains dopaminergic neurons and their projections, the use of this stain allows for specific examination of this particular group of neurons.

Bielschowsky's silver stains filamentous structures in nervous system tissue, including axons. The use of this stain allows for a specific and sensitive microscopic examination of axons throughout the brain.

Results: There was no effect of treatment on brain histopathology under the conditions of the assay. Copies of the sponsor's summary tables follow.

According to the review pathologist:

- "The extensive sectioning and staining scheme allowed for a very comprehensive morphologic evaluation of the brain including the frontal cortex, cerebral cortex, caudate/putamen, thalamus, midbrain (including the substantia nigra and pons), cerebellum, medulla oblongata and other structures."
- "There were no microscopic lesions in the brain related to the test article."
- "[Caudate/Putamen and Substantia Nigra] ... were normal for all animals in which it was examined. The substantia nigra was not examined in Animals H40633 (control male), H40641 (3 mg/kg males) and H40643 (10 mg/kg male). Findings by Satou et. al. ... in rats were not confirmed in this study (7425-101) with dogs."

Sponsor's/Pathologist's conclusion:

- The use of an extensive sectioning scheme of the brain combined with special staining procedures for astrocytes (glial acidic fibrillary protein), axons (Bielschowsky's silver), necrotic neurons (Fluoro-Jade B) and dopaminergic neurons (tyrosine hydroxylase) allowed for a thorough evaluation of the brain from all the study animals.

  Oral (capsule) administration of tetrabenazine at doses up to 10 mg/kg/day to dogs for 9 months was not associated with any test article related microscopic lesions in the brain, including the substantia nigra and caudate putamen (striatal) areas.

	Group 1 - 0 mg/kg/day									Group 2 -1 mg/kg/day								
	M	M	M	M	F	F	F	F		M	M	M	M	F	F	F	F	
Tissue/Diagnosis	H 4 0 6 3 1	H 4 0 6 3 2	H 4 0 6 3 3	H 4 0 6 3 4	H 4 0 6 4 7	H 4 0 6 4 8	H 4 0 6 4 9	H 4 0 6 5 0	INC	H 4 0 6 3 5	H 4 0 6 3 6	H 4 0 6 3 7	H 4 0 6 3 8	H 4 0 6 5 1	H 4 0 6 5 2	H 4 0 6 5 3	H 4 0 6 5 4	INC
Brain (FJB staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Brain (anti-GFAP staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Brain (silver staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Brain (anti-TH staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Caudate/Putamen	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Substantia Nigra	N	N	U	N	N	N	N	N	7	N	N	N	N	N	N	N	N	8
Brain (H&E Staining)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cerebral Cortex (including Frontal Cortex)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	-	N	N	7
Gliosis, Focal	-	-	-	-	-	-	-	-	0	-	-	-	-	-	1	-	-	1
Caudate/Putamen	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Thalamus/Midbrain (including the substantia nigra)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Cerebellum	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Medulla Oblongata/Pons	N	N	N	N	N	N	-	-	6	-	-	N	N	N	-	N	N	5
Nucleus of Cranial Nerve 8, Axonal Spheroids	-	-	-	-	-	-	1	1	2	-	1	-	-	-	1	-	-	2
Pons, Axonal Spheroids	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	0
Perivascular, Infiltrates, Lymphocytes	-	-	-	-	-	-	-	-	0	1	-	-	-	-	-	-	-	1

	Group 3 - 3 mg/kg/day									Group 4 - 10 mg/kg/day								
	M	M	M	M	F	F	F	F		M	M	M	M	F	F	F	F	
Tissue/Diagnosis	H 4 0 6 3 9	H 4 0 6 4 0	H 4 0 6 4 1	H 4 0 6 4 2	H 4 0 6 5 5	H 4 0 6 5 6	H 4 0 6 5 7	H 4 0 6 5 8	INC	H 4 0 6 4 3	H 4 0 6 4 4	H 4 0 6 4 5	H 4 0 6 4 6	H 4 0 6 5 9	H 4 0 6 6 0	H 4 0 6 6 1	H 4 0 6 6 2 *	INC
Brain (FJB staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Brain (anti-GFAP staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Brain (silver staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Brain (anti-TH staining)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Caudate/Putamen	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Substantia Nigra	N	N	U	N	N	N	N	N	7	U	N	N	N	N	N	N	N	7
Brain (H&E Staining)					-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cerebral Cortex (including Frontal Cortex)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Gliosis, Focal	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	0
Caudate/Putamen	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Thalamus/Midbrain (including the substantia nigra)	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Cerebellum	N	N	N	N	N	N	N	N	8	N	N	N	N	N	N	N	N	8
Medulla Oblongata/Pons	N	N	-	N	N	N	N	N	7	N	N	-	N	N	N	N	-	6
Nucleus of Cranial Nerve 8, Axonal Spheroids	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	1	1
Pons, Axonal Spheroids	-	-	-	-	-	-	-	-	0	-	-	1	-	-	-	-	-	1
Perivascular, Infiltrates, Lymphocytes	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	0

14-Day Oral Gavage with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats (Final Report) [redacted] study # 7425-114) (Amendment 30, module 4/volume 3)

This study was conducted at [redacted] according to GLP regulations. The study report was QAed, except for the TK anaylsis performed by [redacted] This study was initiated on 14-February-05.

It should be noted that a draft report of the in-life portion of this study was submitted to the NDA during the original review cycle (original submission, module 4, volume 11), and revised a TK report was submitted to the NDA in Amendment #0005. Serum prolactin levels were not submitted with the previous reports.

This study was conducted to provide a TK assessment in rats for α-HTBZ and β-HTBZ, stereoisomeric metabolites of TBZ. The TK assessment in the chronic toxicity studying rats evaluated TBZ and HTBZ using a non-chiral assay.

Test article: tetrabenazine (lot # 105481, stated purity 100.3%) was formulated in 0.1% (v/v) Tween 80® and 0.5% (w/v) carboxymethylcellulose (CMC) in reverse osmosis water. Dosing formulations (30 mg/ml) were prepared weekly and stored under refrigeration. According to the sponsor, the formulations were stable for 10 days under refrigeration. Homogeneity was assessed during Week 1 (91.4 - 94.9% of nominal) and concentration verification of the dosing solution was conducted in Week 2 (100.5% nominal).

Animals: CD®(SD)IGS BR rats from [redacted] At initiation of treatment the animals were 48-54 days old and weighed 258-306 grams for males and 149-191 grams for females. Animals were housed singly.

Dosing: Animals (12/sex) were administered TBZ (by oral gavage) for 2-weeks 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). Each dose was administered in a volume of 0.5 ml/kg. No concurrent control groups were included.

Results

Mortality: there were no unscheduled deaths. All animals were sacrificed after the last blood sample was obtained and discarded without necropsy.

Clinical observations: Animals were observed for mortality/morbidity twice daily and cage-side observations were conducted daily approximately 60-90 min post morning dose only. Detailed observations were conducted once prior to the initiation of treatment, then Day 1, 8 and 14, and prior to sacrifice. The following signs noted in the report: hypoactivity in all animals (noted on 6-13 days per animal), squinting of eyes in all animals except one female (noted on 1-9 days per affected animal), vocalization in two males and two females (noted on 1-5 days per affected animal) and sensitivity to touch in one male and one female (noted on 1 day for each affected animal). Although not noted in the main portion of the study report, the delineation of protocol deviations stated that on Day 14 all males "appeared to have fine muscle twitching similar to shivering" during blood collection.

Body weights: assessed prior to initiation of treatment, and on Days 1, 8 and 14. All animals gained weight throughout the study. For males, the mean (± SD) change in body weight was 20 ± 6.8 g, 25 ± 7.7 g, and 45 ± 13.1 g for days 1-8, 8-14 and 1-14, respectively. For females the mean (± SD) change in body weight was 26 ± 6.4 g, 18 ± 7.5 g, and 43 ± 10.0 g for days 1-8, 8-14 and 1-14, respectively.

Food consumption: assessed on Days 1, 8 and 14. For males, the mean (± SD) food consumption is 169 ± 7.7g, and 146 ± 9.7g for days 1-8 and 8-14, respectively. For females the mean (± SD) food consumption was 121 ± 14.0g, and 117 ± 14.2g for days 1-8 and 8-14, respectively.

Serum prolactin levels: blood samples were obtained prior to the morning dose on Day 14. The protocol notes that the animals were not disturbed for at least one hr prior to sampling. Serum was separated and shipped to ——— for analysis.

Day 14 Serum Prolactin Levels (ng/mL)		
Sex	Mean ± SD	Range
female	16.03 ± 19.72	———
males	14. 82 ± 10.86	——— 5
• —	reference range for rats: 5 – 100 ng/ml	

The prolactin concentrations in all samples were either less than or within the normal reference range for rats provided by ———

Note: The TK evaluation was reviewed in the original NDA review; the data are reproduced here.

TK: blood was collected via the jugular vein on Days 1 and 14 (from 3/sex at the following time points: predose and approximately 0.5, 1, 2, 4, 8 12, and 24* hrs post dose). (*-24 hr sample was not collected on Day 14). Only the morning dose was administered on Days 1 and 14. Plasma was separated and shipped to —— for analysis by LC-MS/MS.

According to the TK report, "Interfering peaks were found to be present in the tetrabenazine chromatograms... and tetrabenazine plasma concentrations were reported for information only. Consequently, the tetrabenazine 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.

α-HTBZ

Figure 2: Plasma concentrations of α-HTBZ in composite rats on Days 1 and 14 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).

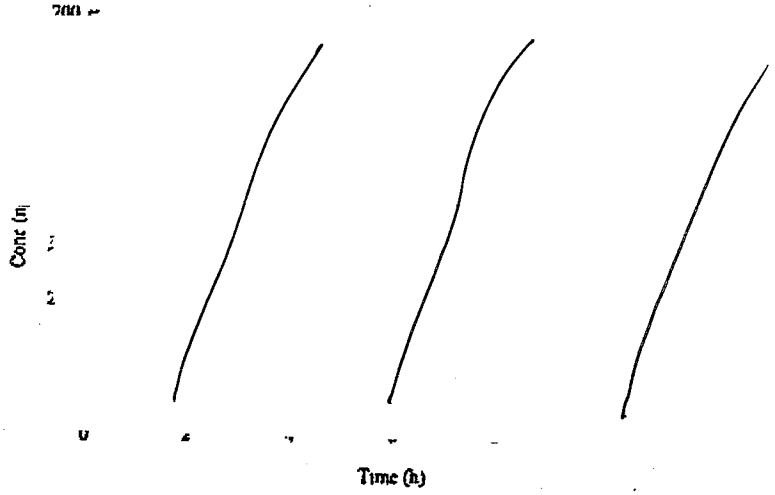


Table 2: Summary of toxicokinetic parameters for α-HTBZ during oral gavage administration of tetrabenazine to male and female rats for 14 days (15 mg/kg on Days 1 and 14 and 15 mg/kg/b.i.d on Days 2-13)

Parameter	Day 1		Day 14	
	Female	Male	Female	Male
Cmax (ng/mL)	137	392	40	614
Tmax (h)	0.50	1.00	0.50	1.00
AUC$_{0-t}$ (h·ng/mL)	381	962	192	1,788

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β-HTBZ

Figure 3: Plasma concentrations of β-HTBZ in composite rats on Days 1 and 14 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).

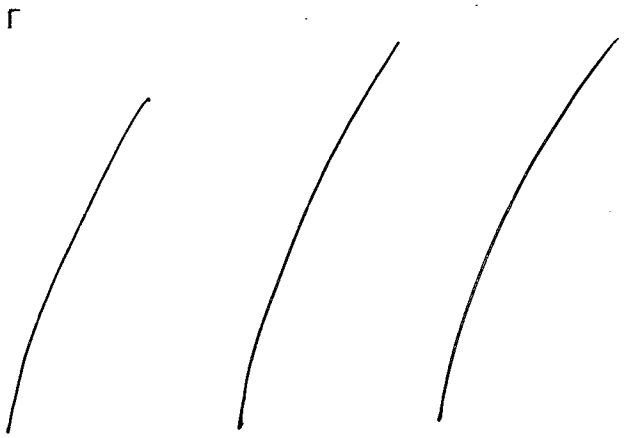


Table 3: Summary of toxicokinetic parameters for β-HTBZ during oral gavage administration of tetrabenazine to male and female rats for 14 days (15 mg/kg on Days 1 and 14 and 15 mg/kg/b.i.d on Days 2-13).

Parameter	Day 1		Day 14	
	Female	Male	Female	Male
Cmax (ng/mL)	10.1	4.5	5.2	11.7
Tmax (h)	0.50	1.00	0.50	1.00
AUC$_{0-t}$ (h•ng/mL)	9.36	4.91	7.51	20.5

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$_{0-t}$ Ratio	Day 1		Day 14	
	Female	Male	Female	Male
α/β	40.7	195.8	25.5	87.3

Quantitative Whole Body Autoradiography and Excretion Balance Following a Single Oral Administration of [^{14}C]-Tetrabenazine to Partially Pigmented Rats. (Study # CAM/05) (Amendment 30, module 4/volume1)

This study was conducted at [redacted] according to GLP regulations with appropriate QA statements. The study initiation date was 22-March-05.

[^{14}C]-TBZ and TBZ were dissolved in corn oil to a concentration of 1 mg/ml.

Animals: male Lister hooded (partially pigmented) rats from [redacted] Animals were acclimated for at least one day prior to use and were approximately 7 weeks old and weighed 151-226 grams at dosing.

Excretion: This portion of the study was conducted in 3 males. Animals were administered a single oral dose of 5 mg/kg (oral gavage). Sampling was conducted at the following intervals:

- urine: prior to dosing, 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hrs.
- feces: prior to dosing, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hrs.
- expired air: prior to dosing, 0-24, 24-48, and 48-72 hrs.
- carcass and cage wash: 168 hrs

The results are presented in the following sponsor-provided summary table. The results are expressed as percentage of dose administered.

Sample/ Timepoint (hours)	Mean (n=3)
Urine	
0-6	7.58
6-24	14.2
24-168	3.92
Total Urine	25.7
Faeces	
0-24	48.9
24-48	12.2
48-168	3.56
Total Faeces	64.7
Expired Air	ND
Cagewash	1.33
Carcass	1.34
Total Recovery	93.1

Tissue distribution: Seven animals were administered a single oral dose of 5 mg/kg (oral gavage) and at each of the following time points, one animal was sacrificed and subjected to whole body autoradiography: 2 hrs, 8 hrs, 1, 3, 7, and 21 days post dose. The remaining (spare) animal was also sacrificed at day 21. Prior to sectioning, the right eyeball was removed from each animal and radioactivity was assessed after combustion.

The results are demonstrated in the sponsor's summary table that follows. Radioactivity was extensively distributed and present in all tissues at 2 hrs (except pigmented fur). At 8 hrs, radioactivity was present in all tissues at concentrations greater than that found in blood with the following exceptions: bone, brain, muscle, and white fat. On day 1, radioactivity was still present and quantifiable in most tissues, but was below the limits of quantification (LOQ) in blood. By day 3, radioactivity continued to decline in all

tissues except the eye. By day 7, radioactivity was below the LOQ for most tissues, but was notably present (but decreasing) in eye, pigmented skin, and uveal tract, and present for the first time in pigmented fur. Radioactivity was also detected in caecum contents, kidney cortex, and liver at levels slightly greater than the LOQ. By Day 21 radioactivity was present only in the eye, uveal tract and pigmented fur.

Table 2 Mean concentrations of radioactivity in the tissues of male Lister Hooded (partially pigmented) rats following single oral administrations of [^{14}C]-Tetrabenazine (nominal 5 mg free base/kg, 0.37 MBq/rat)

Results are expressed as µg equivalents /g

Tissue	1M 2 Hours	2M 8 Hours	3M 1 Day	4M 3 Days	5M 7 Days	6M 21 Days
Adrenal gland	1.65	1.55	0.590	0.205	BLQ	BLQ
Aorta	0.786	0.280	BLQ	BLQ	BLQ	BLQ
Blood	0.479	0.146	BLQ	BLQ	BLQ	BLQ
Bone	0.047	BLQ	BLQ	BLQ	BLQ	BLQ
Bone marrow	1.10	0.354	0.047	BLQ	BLQ	BLQ
Brain	0.379	0.135	BLQ	BLQ	BLQ	BLQ
Bulbo-urethral gland	1.18	0.396	0.081	BLQ	BLQ	BLQ
Caecum contents	30.6	58.3	3.76	0.261	0.042	BLQ
Caecum mucosa	5.69	5.94	0.754	0.073	BLQ	BLQ
Epididymis	0.926	0.786	0.219	0.042	BLQ	BLQ
Ex-orbital lachrymal gland	2.01	0.681	0.145	BLQ	BLQ	BLQ
Eye[a]	11.2	12.1	12.2	4.79	2.37	1.66
Fat (brown)	0.682	0.251	0.042	BLQ	BLQ	BLQ
Fat (white)	0.240	0.123	BLQ	BLQ	BLQ	BLQ
Harderian gland	1.80	0.824	0.307	BLQ	BLQ	BLQ
Intra-orbital lachrymal gland	2.40	0.716	0.180	BLQ	BLQ	BLQ
Kidney (cortex)	3.08	1.32	0.641	0.212	0.051	BLQ
Kidney (medulla)	1.53	0.526	0.480	0.040	BLQ	BLQ
Large intestine contents	NS	19.9	4.83	0.408	BLQ	BLQ
Large intestine mucosa	0.988	3.71	0.641	BLQ	BLQ	BLQ
Liver	5.68	2.69	0.965	0.331	0.060	BLQ
Lung	0.632	0.235	0.056	BLQ	BLQ	BLQ
Lymph nodes	0.965	0.389	0.048	BLQ	BLQ	BLQ
Muscle	0.477	0.138	BLQ	BLQ	BLQ	BLQ
Myocardium	0.941	0.246	0.060	BLQ	BLQ	BLQ
Nasal mucosa	0.432	0.547	0.292	0.350	BLQ	BLQ
Pancreas	4.13	1.63	0.281	0.058	BLQ	BLQ
Peridontal membrane	0.996	0.313	0.087	BLQ	BLQ	BLQ
Pigmented fur	BLQ	BLQ	BLQ	BLQ	8.09	19.0
Pineal body	0.719	0.419	BLQ	BLQ	BLQ	BLQ
Pituitary gland	1.12	0.481	0.075	BLQ	BLQ	BLQ
Preputial gland	0.669	0.362	0.143	0.047	BLQ	BLQ
Prostate	0.879	0.334	0.076	BLQ	BLQ	BLQ
Salivary gland	1.59	0.738	0.118	BLQ	BLQ	BLQ
Seminal vesicles	0.957	0.376	0.054	BLQ	BLQ	BLQ
Skin (non-pigmented)	0.548	0.215	0.050	0.044	BLQ	BLQ
Skin (pigmented)	1.65	1.28	1.00	0.910	0.306	BLQ
Small intestine contents	96.5[b]	19.1	1.50	0.259	BLQ	BLQ
Small intestine mucosa	9.65	1.83	0.666	BLQ	BLQ	BLQ
Spinal cord	0.485	0.166	0.044	BLQ	BLQ	BLQ
Spleen	1.35	0.397	0.083	BLQ	BLQ	BLQ
Stomach contents	8.40	4.12	0.485	BLQ	BLQ	BLQ
Stomach mucosa	1.63	1.67	0.214	BLQ	BLQ	BLQ
Testis	0.856	0.548	0.168	0.071	BLQ	BLQ
Thymus	0.941	0.271	0.045	BLQ	BLQ	BLQ
Thyroid gland	2.08	0.443	0.261	0.112	BLQ	BLQ
Tongue	0.737	0.227	0.056	BLQ	BLQ	BLQ
Tooth pulp	0.725	0.278	0.049	BLQ	BLQ	BLQ
Urinary bladder	13.4	5.46	1.47	0.113	BLQ	BLQ
Uveal tract	45.1	66.1	48.9	25.0	12.7	6.93

Distribution of radioactivity was determined using a bio-image analyser and quantified using associated Tina and SeeScan software see section 2.5.2.3.

BLQ Below limit of quantification (0.040 µg equiv./g)
NS No Sample
a Radioactivity in the eye quantified by combustion and liquid scintillation analysis
b Measurement above upper limit of quantification (68.0 µg equiv./g). Extrapolated value reported.

Quantitative Whole Body Autoradiography and Excretion Balance Following a Single Oral Administration of [^{14}C]-Tetrabenazine to Male Mice (Study # CAM/08) (Amendment 30, module 4/volume 1)

This study was conducted at [redacted] according to GLP regulations with appropriate QA statements. The study initiation date was 30-August-05.

[^{14}C]-TBZ and TBZ were dissolved in corn oil to a concentration of 1 mg/ml.

Animals: male albino mice (not otherwise specified) from [redacted] Animals were acclimated for at least 35 days prior to use and were approximately 9 weeks old and weighed 36 – 46 grams at dosing.

Excretion: This portion of the study was conducted in 3 males. Animals were administered a single oral dose of 5 mg/kg (oral gavage). Sampling was conducted at the following intervals:

- urine: prior to dosing, 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hrs.
- feces: prior to dosing, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hrs.
- expired air: prior to dosing, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hrs.
- carcass and cage wash: 168 hrs

The results are presented in the following sponsor-provided summary table. The results are expressed as percentage of dose administered. (NS= no sample, and BLD = below limit of detection).

Sample	Time (hours)	Male mice n=3
Urine	0 – 6	NS
	6 – 24	28.77
	24 – 48	4.69
	48 – 72	1.26
	72 – 96	0.63
	96 – 120	0.28
	120 – 144	0.52
	144 – 168	0.14
	Sub-total	36.29
Faeces	0 – 24	35.43
	24 – 48	8.59
	48 – 72	4.11
	72 – 96	1.62
	96 – 120	1.42
	120 – 144	0.93
	144 – 168	0.49
	Sub-total	52.59
CO2 Trap 1	0 - 24	0.13
	24 – 48	0.05
	48 – 72	0.02
	72 – 96	BLD
	96 – 120	BLD
	120 – 144	BLD
	144 – 168	BLD
	Sub-total	0.20
CO2 Trap 2	0 - 24	0.03
	24 – 48	BLD
	48 – 72	BLD
	72 – 96	BLD
	96 – 120	BLD
	120 – 144	BLD
	144 – 168	BLD
	Sub-total	0.03
Cagewash	168	2.09
Carcass	168	0.35
Total		91.55

Tissue distribution: Seven animals were administered a single oral dose of 5 mg/kg (oral gavage) and at each of the following time points, one animal was sacrificed and subjected to whole body autoradiography: 2 hrs, 8 hrs, 1, 3, 7, and 21 days post dose. The remaining (spare) animal was also sacrificed at day 21.

The results are demonstrated in the sponsor's summary table that follows. Radioactivity was extensively distributed and present in all tissues at 2 hrs. At 8 hrs, radioactivity was present in all tissues at concentrations greater than that found in blood with the following exceptions: bone, brain, eye, muscle, fat (brown and white) and spinal cord. On day 1, radioactivity was still present and quantifiable in all tissues except bone and white fat (periodontal membrane, tongue, and tooth pulp were not evaluated at this time point). By day 3, radioactivity was below the limits of quantification (LOQ) in blood and levels in tissues continued to decline except for a slight increase in stomach content (thyroid was not assessed at this time point). By day 7, radioactivity was below the LOQ for all tissues assessed except liver (epididymis, and large intestine mucosa/content were not assessed at this time point). Quantifiable radioactivity was not present in any tissue assessed at 21 days post dose (cartilage was not assessed at this time point).

Table 1 **Concentrations of radioactivity in the tissues of male mice following single oral administrations of [^{14}C]-Tetrabenazine at a nominal dose level of 5 mg/kg**

Results expressed as µg equivalents /g

Tissue	4M 2 hours	5M 8 hours	6M 1 day	7M 3 days	8M 7 days	9M 21 days
Adrenal gland	1.59	0.656	0.111	BLQ	BLQ	BLQ
Aorta	0.199	0.148	0.021	BLQ	BLQ	BLQ
Blood	0.182	0.115	0.019	BLQ	BLQ	BLQ
Bone	0.020	BLQ	BLQ	BLQ	BLQ	BLQ
Bone marrow	0.410	0.311	0.032	BLQ	BLQ	BLQ
Brain	0.062	0.060	0.014	BLQ	BLQ	BLQ
Caecum contents	19.6	28.8[a]	1.14	0.042	BLQ	BLQ
Caecum mucosa	2.66	3.16	0.138	0.023	BLQ	BLQ
Cartilage	0.224	0.204	0.031	BLQ	BLQ	NS
Epididymis	0.103	0.192	0.026	BLQ	NS	BLQ
Epimysium	0.186	0.143	0.041	BLQ	BLQ	BLQ
Ex-orbital lachrymal gland	0.507	0.694	0.069	0.017	BLQ	BLQ
Eye	0.113	0.102	0.020	BLQ	BLQ	BLQ
Fat (brown)	0.082	0.103	0.017	BLQ	BLQ	BLQ
Fat (white)	0.259	0.064	BLQ	BLQ	BLQ	BLQ
Harderian gland	0.609	0.507	0.088	0.020	BLQ	BLQ
Intra-orbital lachrymal gland	0.783	0.604	0.065	0.016	BLQ	BLQ
Kidney	1.19	1.00	0.054	0.014	BLQ	BLQ
Kidney cortex	1.22	1.05	0.056	BLQ	BLQ	BLQ
Kidney medulla	0.942	0.900	0.042	BLQ	BLQ	BLQ
Large intestine contents	9.65	35.1[a]	2.45	0.041	NS	BLQ
Large intestine mucosa	1.02	1.47	0.146	0.013	NS	BLQ
Liver	3.90	3.08	0.465	0.115	0.031	BLQ
Lung	0.341	0.264	0.024	BLQ	BLQ	BLQ
Lymph nodes	0.210	0.435	0.033	BLQ	BLQ	BLQ
Muscle	0.127	0.093	0.015	BLQ	BLQ	BLQ
Myocardium	0.146	0.199	0.024	BLQ	BLQ	BLQ
Nasal mucosa	0.266	0.199	0.014	BLQ	BLQ	BLQ
Pancreas	0.912	0.937	0.057	0.017	BLQ	BLQ
Peridontal membrane	0.243	0.400	NS	0.027	BLQ	BLQ
Pituitary gland	0.331	0.278	0.031	BLQ	BLQ	BLQ
Salivary gland	0.472	0.373	0.056	0.018	BLQ	BLQ
Seminal vesicles	0.168	0.150	0.036	BLQ	BLQ	BLQ
Skin (non-pigmented)	0.222	0.158	0.021	0.013	BLQ	BLQ
Small intestine contents	25.9[a]	30.6[a]	1.54	0.070	BLQ	BLQ
Small intestine mucosa	4.13	1.28	0.430	0.039	BLQ	BLQ
Spinal cord	0.059	0.068	0.016	BLQ	BLQ	BLQ
Spleen	0.709	0.477	0.038	0.014	BLQ	BLQ
Stomach contents	32.1[a]	28.8[a]	0.107	0.133	BLQ	BLQ
Stomach mucosa	1.66	1.02	0.054	BLQ	BLQ	BLQ
Testis	0.106	0.240	0.034	0.019	BLQ	BLQ
Thymus	0.256	0.237	0.033	BLQ	BLQ	BLQ
Thyroid gland	0.211	0.199	0.055	NS	BLQ	BLQ
Tongue	0.180	0.132	NS	BLQ	BLQ	BLQ
Tooth pulp	0.156	0.142	NS	BLQ	BLQ	BLQ
Urinary bladder	22.3[a]	23.2[a]	0.522	0.058	BLQ	BLQ

Distribution of radioactivity was determined using a) Bio-image analyser and quantified using associated Tina and SeeScan software (see section 2.5.2.3).

BLQ Below limit of quantification (<0.013 µg equiv./g)
NS No Sample
a Measurement above upper limit of quantification (>21.7 µg equiv./g). Extrapolated value reported.

- General comment for all studies: "Where concentrations are reported as µg equivalents/g, radioactivity is assumed to be associated with [^{14}C]-Tetrabenazine or with components of the same molecular weight.

Study to Investigate the Pharmacokinetics of Tetrabenazine Following Oral Administration in Beagle Dogs (Study # CAM/07) (Amendment 30, module 4/volume 1)

This non-GLP/nonQAed study was conducted at [redacted]
The study initiation date was 12-May-05.

Animals: four male Beagle dogs [redacted] HCT:DOBE) from [redacted] (animals were approximately 22 months old, and weighed 14-17 kg at dosing)

For each test article (tablet or solution) the target dose was 25 mg free base/dog. The tablet was supplied by the sponsor and administered with a water wash. The test solution was prepared as [^{14}C]-TBZ/TBZ dissolved in (2-hydroxypropyl)-β-cyclodextrin to a concentration of 0.25 mg/ml, and administered as an oral gavage. Each animal had two dosing sessions (duration of washout not stated); first with tablet and the second with solution.

Blood was sampled prior to dosing, 15, 30, 60 and 90 min, 2, 4, 6, 8, 12 and 24 hrs post dose. Plasma was separated and frozen until analysis by LC-MS/MS. Plasma concentrations of TBZ, α-HTBZ and β-HTBZ were measured and standard PK parameters assessed.

The results (mean) are presented in the following sponsor supplied summary table.

Parameter	Tetrabenazine		α-Dihydro-Tetrabenazine		β-Dihydro-Tetrabenazine	
	Tablet	Solution	Tablet	Solution	Tablet	Solution
T_{max}	0.5	0.5	1.5	0.5	0.5	0.5
C_{max}	50.2	63.0	27.5	39.6	106.3	148.4
AUC_{last}	74.4	83.7	96.4	103.3	245.5	249.1
AUC_{inf}	78.2	89.5	98.3	106.5	259.4	253.7
t½	8.79	4.42	2.17	2.74	11.48	3.20

"From these results it can be seen that the solution dose results in slight increase in the absorption of tetrabenazine and exposure to the major metabolites when compared to the tablet dose. These results indicate that the use of a solution dose for the human metabolism study will not result in a notably increased exposure to drug related material."

APPEARS THIS WAY
ON ORIGINAL

SUMMARY AND EVALUATION

Original Approvable Issue #1 - Interspecies Comparison of In Vivo Metabolism:

Background: During the first review cycle of the NDA we could not ensure that the pivotal nonclinical studies adequately characterize the toxicity of the major drug-related circulating products in humans after oral administration of TBZ. During the nonclinical development of TBZ, it was assumed that the two major circulating metabolites in humans after oral administration of TBZ were α-HTBZ and β-HTBZ. Therefore, the toxicokinetic assessments in the pivotal nonclinical toxicology studies were based on monitoring plasma levels of TBZ and the stereoisomeric metabolites α-HTBZ and β-HTBZ, or HTBZ (as measured using a non-chiral assay in the earlier studies).

In the original submission, the sponsor had conducted a minimal, inadequate assessment of the *in vivo* metabolic profiles in some of the animal species and strains used for the pivotal toxicity studies (beagle dog, NZW rabbit and CD1 mouse) with rat notably missing from the analysis. Furthermore, the sponsor had not linked the designations assigned to the metabolites in this initial study with the designations assigned to the metabolites used in the in vivo mass balance study in humans.

According to the original OCBP review for this NDA conducted by Dr. Sally Yasuda, in humans TBZ is extensively metabolized and the parent compound is either undetectable in the plasma or circulating at very low levels after oral administration. Based on the results of a mass balance study conducted in humans, the most abundant circulating component in humans was an unidentified peak, P16. Dr. Yasuda concluded that P16 should be resolved and the extent to which the other individual metabolites (including the mono-and bis-dealkyltetrabenazine metabolites) circulate should be clarified.

In response to the 24-March-06 Approvable Letter, the sponsor submitted the following study reports in Amendment 30 (dated 09-February-07):

- Study # CAM/11: [^{14}C]-Tetrabenazine: metabolite identification studies.
- Study # CAM/21: [^{14}C]-Tetrabenazine: Investigation of metabolites in plasma of rat, mouse, rabbit and dog after oral administration.
- Study # CAM/26: Comparative in vitro metabolism studies of [^{14}C]-Tetrabenazine (TBZ) with mouse, rat, dog, Cynomolgus monkey and human liver microsomes.

Prior to review of this submission, the sponsor was informed that we had identified their response to comparative metabolism issue as potentially deficient. The sponsor had provided an interspecies comparison of metabolites based only on an average exposure over the three time points assessed (0.5, 2 and 6 hrs post dose) for all animals. This was identified as review issue, and was conveyed to the sponsor in the 16-March-07 Incomplete Response letter to the sponsor, as follows:

> "It does not appear that you have adequately addressed the issue of interspecies comparisons of the in vivo metabolism (#1 in the approvable letter). For each major circulating metabolite in humans, you need to provide plasma exposure (AUC) data in humans and in the animal species/strains used in the pivotal toxicity studies."

In the 04-April-07 response to the incomplete letter (Amendment 32), the sponsor stated that they would address the comparative metabolism issue in an additional amendment to the NDA.

On 27-April-07 the sponsor sent an e-mail requesting concurrence on their plans to calculate in rat and dog the AUCs for the major drug-related circulating components in humans, as a way of addressing this issue. The Division's response (sent by e-mail on at least 22-May-07) is provided below:

- For each major circulating metabolite in humans, you need to provide plasma exposure (Cmax, AUC) data in humans and in the animal species/strains (i.e., dog, rat, mouse, and rabbit) used in the pivotal in vivo toxicology studies (including chronic, reproduction, carcinogenicity, and genotoxicity). It is important that you document that the toxicity of each major circulating metabolite in humans has been adequately tested in animals.

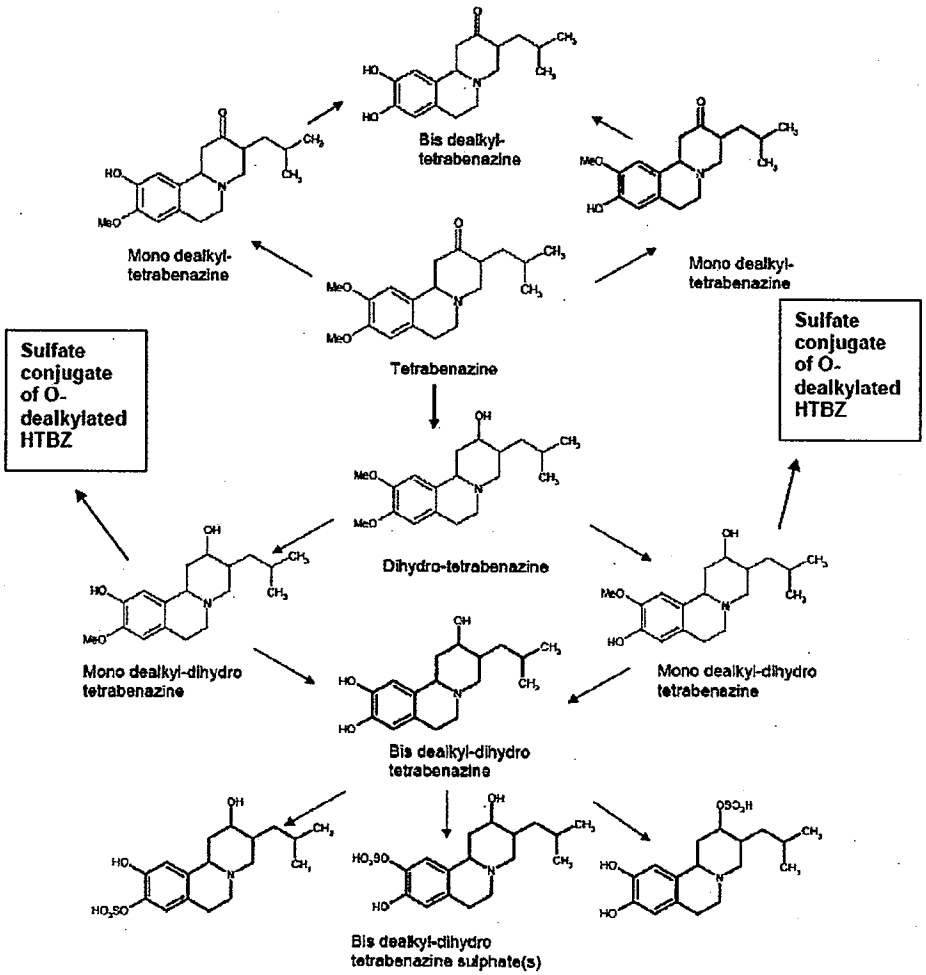
In the 05-October-07 amendment to the NDA (Amendment 54) the sponsor submitted the following reports, both considered by the Agency as preliminary:

- DRAFT report for Study # CAM/35: [^{14}C]-tetrabenazine: studies to investigate the concentrations and pharmacokinetic properties of major circulating human plasma components in the plasma of rat, mouse, rabbit and dog after repeat oral administration.

- Analysis of major circulating human metabolites after tetrabenazine administration to healthy volunteers – Pharmacokinetic analysis and report.

Evaluation: (see the individual study reviews for details).

Metabolism in humans: The following discussion of the metabolism of TBZ by humans is based on Dr. Sally Yasuda's Clinical Pharmacology Review (12-December-07). The sponsor has identified P16, the major circulating radioactive component after oral administration of TBZ to humans, as mono O-dealkylated HTBZ (a mixture of up to four unresolved enantiomers). It should be noted that O-dealkylated HTBZ can also be referred to as O-desmethylated HTBZ. O-dealkylated HTBZ is formed by the O-desmethylation of α-HTBZ and β-HTBZ, each of which can be O-desmethylated in two adjacent positions, yielding a total of four enantiomers. The O-dealkylated enantiomers are referred to as 1-desmethyl HTBZ, 2-desmethyl HTBZ, 3-desmethyl HTBZ, and 4-desmethyl HTBZ. The numbering system (1, 2, 3, and 4) is based on retention time and not the position of the reaction. 1-desmethyl HTBZ and 2-desmethyl HTBZ are derived from β-HTBZ. 3-desmethyl HTBZ, and 4-desmethyl HTBZ are derived from α-HTBZ. Dr. Yasuda has amended the sponsor's proposed metabolic scheme with the addition of the sulfated metabolites. This amended version of the sponsor's metabolic scheme follows.



Dihydrotetrabenazine is the same as HTBZ. Mono dealkyl-dihydro tetrabenazine is the same as O-dealkylated HTBZ.

Thus, the major metabolic pathway in humans appears to be metabolism of TBZ by carbonyl reductase to form α-HTBZ and β-HTBZ, which are subsequently metabolized by CYP450 (predominantly CYP2D6) to form up to four enantiomers of mono O-dealkylated HTBZ (or O-desmethyl HTBZ). These metabolites are subsequently sulfated and excreted.

In response to the Division's request to address the issue of interspecies comparisons of in vivo metabolism of TBZ, the sponsor re-analyzed the data from the mass balance study conducted in humans (Study # CAM/06). Dr. Yasuda referred to the following sponsor's summary table of the circulating drug-related compounds that account for greater than 10% of the circulating radioactivity after a single oral dose of 25 mg of radiolabeled TBZ (from Efficacy Information Amendment #31, 16-February-07).

Table 3 **Metabolites Greater Than 10% in Plasma Following Single Oral Dosing of [^{14}C]-Tetrabenazine to Six Male Human Subjects**

Component	Identity	Metabolite Type	0.25 – 1.5 hours	2 – 3 hours	4 – 8 hours	Mean
P11	Sulfate conjugate of *O*-dealkylated-HTBZ	tertiary	23.44 (16.67)	29.52 (20.41)	15.23 (16.90)	22.73 (17.99)
P13	Sulfate conjugate of *O*-dealkylated-HTBZ	tertiary	26.73 (19.01)	41.77 (28.88)	24.22 (26.88)	30.91 (24.92)
P16	*O*-dealkylated-HTBZ	secondary	49.54 (35.24)	50.11 (34.65)	19.77 (21.95)	39.81 (30.61)
P17	β-HTBZ	primary	13.65 (9.71)	6.54 (4.52)	4.48 (4.97)	8.22 (6.40)
P18	α-HTBZ	primary	25.73 (18.30)	16.70 (11.55)	14.05 (15.60)	18.83 (15.15)

Source: Modified from Table 15 in CAM/06 (5.3.1.1)
Results expressed as ng equivalents/mL. Values in ( ) are % sample radioactivity
HTBZ = dihydrotetrabenazine

Thus, P16, an unresolved mixture of O-dealkylated HTBZ represents 22-35% of radioactivity, two sulfated conjugates of O-dealkylated HTBZ, P13 and P11, represent 19-29% and 17-20% of radioactivity, respectively, and α-HTBZ and β-HTBZ represent 12-18% and 5-10% of radioactivity, respectively.

Dr. Yasuda also refers to additional preliminary exposure data regarding the circulating levels of O-dealkylated HTBZ (desmethyl HTBZ) after a single oral administration of 50 mg TBZ in healthy volunteers submitted in Amendment 54 (03-October-07). The results of this additional analysis are considered by Dr. Yasuda "to be preliminary because the supporting analytical method has not been provided". These data are based on a reanalysis of existing plasma samples from six healthy volunteers administered obtained up to 48 hrs after a single 50 mg oral dose of TBZ (note that this is not a reanalysis of data from the mass balance study, in which subjects were administered radiolabeled TBZ at a dose of 25 mg). It appears that the only drug-related compounds evaluated were TBZ, α- and β-HTBZ, and the four resolved enantiomers of O-dealkylated HTBZ (listed as desmethyl-HTBZ). A copy of the sponsor's summary table follows.

Table 1: Summary of pharmacokinetic parameters for α-HTBZ, β-HTBZ, 1-desmethyl HTBZ, and 3-desmethyl HTBZ after oral administration of a single 50 mg dose of tetrabenazine to healthy volunteers.

Parameter[1]	α-HTBZ	β-HTBZ	1-Desmethyl[2] HTBZ	3-Desmethyl[2] HTBZ
Cmax (ng/mL)	87.3 ± 22.7 (6)	46.8 ± 30.6 (6)	26.9 ± 8.41 (6)	9.53 ± 3.28 (5)
Tmax (h)	1.25 (6)	1.50 (6)	2.00 (6)	2.00 (5)
AUC(0-t) (h*ng/mL)	491 ± 269 (6)	219 ± 251 (6)	313 ± 113 (6)	59.3 ± 19.7 (4)
AUC(inf) (h*ng/mL)	506 ± 276 (6)	224 ± 252 (6)	466 ± 161 (6)	73.8 (1)
λz (h^{-1})	0.1017 ± 0.0340 (6)	0.1710 ± 0.0592 (6)	0.0631 ± 0.0209 (6)	0.1260 (1)
t½ (h)	7.33 ± 1.94 (6)	4.58 ± 1.86 (6)	12.2 ± 4.65 (6)	5.50 (1)

[1]Mean ± standard deviation (N) except for Tmax for which the median (N) is reported.
[2]The numbers correspond to the order of elution.

Comparison of metabolism across species: The sponsor evaluated in vitro metabolism by liver microsomes from pooled male and female human, male CD1 mouse, male Sprague Dawley rat, male Beagle dog, and male Cynomolgus monkey (study #CAM/26). The results of this study indicated that TBZ is metabolized to up to seven radioactive components. There was a qualitative similarity among

species for the major components and the rate of metabolism was similar for monkey, mouse, and rat, less for human, and notably less for dog.

The sponsor submitted a urine metabolite identification study (# CAM/11) in Amendment 30. In this study, the metabolic profile from humans (from study #CAM/06, the in vivo mass balance study) based on 0-4 hr urine samples, was compared to urinary metabolites found in Listar Hooded rat (0-6 and 6-14 hr samples), beagle dog (0-6 hr sample), and albino mouse (0-24 hr sample) after single oral doses of [^{14}C]-TBZ. These urine samples accounted for a small percentage of the dose of radioactivity administered, i.e., 28.8% in mouse, 13% in dog, 20.52% in rat, and less than 16.55% in human, and this limits the conclusions that can be drawn. A further limitation is that only one dog and one rat were evaluated.

From this study it can be concluded that the urinary metabolic profiles are complex for TBZ in the three animal species tested and humans. It should be noted that the strain of rat evaluated (Lister Hooded) is not the strain used in the pivotal toxicity studies (SD), and without further information, the strain of mouse (identified only as albino) may not be the same as those used in the pivotal toxicity studies.

The sponsor also submitted an interspecies comparison of plasma metabolites (study # CAM/21) to Amendment #30. In this study, single oral doses of [^{14}C]-TBZ were administered to C57BL/6 mice, SD rats, New Zealand White rabbits, and non-naïve Beagle dogs. Blood samples were obtained at 0.5, 2 and 6 hrs post dose. Plasma was separated and analyzed using HPLC and multiple reaction monitor (MRM) screening. Multiple radioactive components were identified in each species (at least 14 in rat, at least 11 in mouse, at least 10 in rabbit and at least 13 in dog). The sponsor then provided a semi-quantitative comparison of the data for the rat, dog and mouse (sexes combined) and female rabbits to data derived in humans (from study CAM/06, the vivo mass balance study in humans). Based on this summary, all of the six plasma radioactive components that were detected in humans (some of which are unresolved mixtures of enantiomers), were also detected in rat, dog, mouse and rabbit, except the sulfated conjugates of O-dealkylated-HTBZ, which are not present in the plasma from mouse and rabbit.

In addition to the two nonclinical in vivo metabolism studies discussed above, the sponsor submitted an integrated summary and the following summary table (Table 1) comparing the mean plasma concentrations of the radioactive plasma components in human, SD rat, C57BL/6 mouse, Beagle dog, and non-pregnant New Zealand White rabbit after a single oral administration of [^{14}C]-TBZ. It should be noted that in this table the component number (e.g., P10) refers to the component numbering system used in the nonclinical study CAM/21 and not the system used for evaluations in humans. The numerical value listed for each component in animals was based on an average exposure over the three time points assessed (0.5, 2 and 6 hrs post dose) for all animals (sexes combined).

Based on the data in Table 1, the animals have circulating levels of α-HTBZ, β-HTBZ, and O-dealkylated HTBZ (specific enantiomer or mix of enantiomers not provided), which constitute three of the five major drug-related products in humans. The remaining two major circulating drug-related components in humans are sulfated conjugates of O-dealkylated HTBZ (specific enantiomer or mix of enantiomers not yet established), which, according to this table were not detected in any animal species. It should be noted that in the summary table from Study # CAM/21, sulfated conjugates of O-dealkylated HTBZ (specific enantiomer or mix of enantiomers not yet established) were noted in rat and dog.

While these data are useful from a qualitative standpoint, it does not allow for the necessary quantitiative comparison across species and does not allow for an evaluation of potential differences in metabolism between sexes for rat, dog and mouse.

As part of the integrated summary, the sponsor also submitted the following summary table (Table 2) comparing urinary radioactive components in human, Listar Hooded rat, Beagle dog, and albino mouse

after a single oral administration of [^{14}C]-TBZ. The data in this table have limited relevance for the following four reasons, (1) the O-delakylated-HTBZ and related conjugates have not been resolved into the appropriate enantiomers, (2) the strain of rat evaluated (Lister Hooded) is not the strain used in the pivotal toxicity studies in rat (SD), (3) without further information, the strain of mouse, identified only as albino, may also may not be one of those used in the pivotal toxicity studies, and (4) the data were derived in a small number of animals (e.g., one rat and one dog).

Table 1 **Plasma Components of [^{14}C]-Tetrabenazine in Mouse, Rat, Rabbit, Dog and Human as Determined by Radiometric HPLC and LC/MS/MS (Multiple Reaction Monitoring)**

Tetrabenazine metabolites	Component number in lab animals	Mouse (CAM/21)	Rat (CAM/21)	Rabbit (CAM/21)	Dog (CAM/21)	Human (CAM/06)
Unidentified polar metabolites	P1-P3	134	42.1	268	19.8	ND
Glucuronide conjugate of O-dealkylated HTBZ	P41	76.7	46.7	173	16.6	4.11 (P6, P8) 2
Sulfate conjugate of O-dealkylated HTBZ	-	ND	ND	ND	ND	53.6 (P11, P13)
Bis-O-dealkylated HTBZ	P5	ND	23.1	162	7.1	ND
O-Dealkylated HTBZ	P6, P9	278	208	91.3	73.7	39.8 (P16)
Mono-hydroxy O-dealkylated tetrabenazine	P7	ND	61.7	92.3	ND	ND
Beta-HTBZ	P8	95.4	40.7	24.5	63.0	8.2 (P17)
Alpha-HTBZ	P10	139	44.4	5.1	23.4	18.8 (P18)
Bis-O-dealkylated tetrabenazine	P11	49.8	40.6	ND	29.9	ND
O-Dealkylated tetrabenazine	P12	15.9	4.43	ND	9.9	ND
Tetrabenazine	P13, P14	106.5	34.2	84.4	38.3	ND

Source: CAM/21 (Section 4.2.2.4) Table 3 (mouse), Tables 4 and 5 (rat), Table 6 (rabbit), Tables 7 and 8 (dog)
CAM/06 (Section 5.3.1.1) Table 15 (human)

Values are mean plasma concentrations in units of ng equivalents/mL.

Components in ( ) are from CAM/06

* In rabbit plasma, this component (P4) also contained a glucuronide of dihydrotetrabenazine.

† Human plasma contained two additional components identified as glucuronide conjugates (namely P3 and P5) but these minor components were not quantified (see Figure 21 in CAM/06).

HTBZ = dihydrotetrabenazine; ND = not detected.

Table 2 **Urinary Metabolites of [^{14}C]-Tetrabenazine in Mouse, Rat, Dog and Human – Values Expressed as Percent of Administered Dose (and Percent of Sample Radioactivity)**

Identity	Urinary Component	Mouse (6-24 h)	Rat (0-6 h)	Dog (0-6 h)	Human (0-4 h)	Human (0-48 h)
Glucuronide conjugates of O-dealkylated-HTBZ	U1-U9	11.59% (40.27%)	3.16% (38.45%)	4.67% (35.99%)	3.95% (23.80%)	17.74% (26.95%)
Sulfate conjugates of O-dealkylated-HTBZ	U11 and U13	7.69% (26.73%	1.48% (18.00%)	2.66% (20.47%)	7.28% (43.96%)	29.77% (45.22%)
β-HTBZ and/or O-dealkylated-HTBZ	U15 and U17	1.03% (3.57%)	0.07% (0.91%)	0.31% (2.40%)	1.53% (9.22%)	5.02% (7.63%)
α-HTBZ	U18	0.96% (3.33%)	0.06% (0.69%)	0.73% (5.62%)	0.17% (1.01)	0.43% (0.65%)
Total percentage of administered dose		28.8%	8.22%	13.0%	16.55%	65.82%

Source: CAM/11 (Section 4.2.2.4) and CAM/06 (Section 5.3.1.1).
For mouse, the values are for a 6-24 h urine collection period (Tables 3 and 8, CAM/11).
For rat, the values are for a 0-6 h urine collection period (Tables 4 and 8, CAM/11).
For dog, the values are for a 0-6 h urine collection period (Tables 5 and 8, CAM/11).
For human, the values are for a 0-4 h urine collection period (Tables 6 and 8, CAM/11) or for a 0-48-h urine collection period (Table 12 in CAM/06)

Conclusion: The sponsor has not yet provided an adequate interspecies comparison of in vivo metabolism.

On 16-March-07, we informed that sponsor that the results of this study do not appear to adequately address the issue of interspecies comparisons of in vivo metabolism, and asked that they provide, for each major circulating metabolite in humans, plasma exposure (AUC) data in humans and in the animal species/strains used in the pivotal toxicity studies.

The sponsor has conducted an additional in vivo metabolism study to address this issue. This study (CAM/35) was submitted as a DRAFT report to the NDA. The submission of a DRAFT report to an NDA is not acceptable, and the report was not reviewed. Furthermore, the sponsor provided an additional PK analysis of major circulating human metabolites in healthy volunteers, the results of which are considered preliminary by the OCPB reviewer.

Although it has been determined that this NDA will be approved without these data, the submission of the final report should be made a Phase 4 commitment. These data are needed in order to determine the relevance (and adequacy) of the nonclinical studies to the assessment of human risk, especially with regard to the assessments of reproductive toxicity and the carcinogenicity, for which adequate relevant human data generally do not exist.

Original Approvable Issue #2a – Clinical Observations in the Chronic Toxicity Study in Rat:

- **Refers to 26 Week Toxicity Study in Rats with Twice Daily Dosing Administration by Gavage and 13 Week Interim Kill —— Project # 244738)**

Background: During the initial review of this study, there were concerns regarding the adequacy of the report. For example, one of the high animals was sacrificed in moribund condition during week 23. The cause of morbidity was listed by the pathologist as chronic dermatitis (area of chronic dermatitis on the muzzle). The sponsor did not discuss this animal further. Examination of the (incomplete) clinical observation line listings for this animal revealed that this animal was noted with convulsions on Days 133 (prior to the am dose), 142 (immediately after the pm dose), 154 (before the pm dose) and 159 (before

and immediately after the am dose). This animal was sacrificed on Day 159. An additional high dose animal that survived until terminal sacrifice was noted to have had convulsions on days 172 and 176. Convulsions were not mentioned in the general discussion of clinical observations and did not appear in the summary table. Furthermore, the sponsor's summary table presents only "selected" post dose clinical signs and is labeled as such and was focused on lethargy, hyperactive behavior, and aggressive behavior. Examination of the individual animal data suggested that the clinical observation data sets for each animal may not have been complete.

On January 24, 2006, the sponsor was contacted (via email) and asked to (1) clarify the schedule for observation and reporting of clinical signs, (2) address the apparent discrepancies between the clinical observation data presented in the summary table and the individual line listings, (3) confirm that the individual line listings are complete for each animal, (4) provide a new summary table of clinical observations, and (5) provide the day of sacrifice for each animal.

In a submission dated 16-March-06, the sponsor submitted a response to the request for information; however, the original action date for the NDA was 24-March-06, and this data was not reviewed during the first cycle.

As noted in the 24-March-06 Approvable Letter, resolution of the apparent discrepancies in the original report was needed prior to approval.

> "The reporting of clinical signs is incomplete. For example, several instances of convulsions observed in two high-dose animals were not listed in the summary table. Similarly, instances of "lethargy" were noted in the summary table, but not in any animal individual line listing. You need to address the apparent discrepancies between the summary of clinical signs and the individual animal line listings."

This issue was further discussed at the 25-May-06 End of Review Meeting, and documented in the minutes that issued on 05-September-06.

> "The Sponsor was advised that the March 16, 2006 response to a pharmacology/toxicology information request is unacceptable for review based on the quality of the submission (e.g., appendix 38 [a 33 page summary table of clinical signs] is unreadable). It would appear, however, that the original deficiencies may not have been entirely corrected. For example, Table 37 still does not provide a comprehensive summary of clinical signs; the convulsions that occurred in animals #110 and #111 are still not listed."

Evaluation of the sponsor's response: The description of the methodology employed to assess clinical observations changed notably between the original submission and each subsequent related response to requests for additional information (i.e., 16-March-06 response to the original request for information and the 09-February-07 response to the approvable letter) as delineated below:

Original Submission (includes report amendment #1)
The following description of the methodology is quoted directly from the sponsor: All animals were checked early in the morning and as late as possible each day for viability and at frequent intervals throughout each day for any signs of ill health or reaction to treatment. The onset, intensity and duration of any clinical sign was recorded. Once each week all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. Once each week, from Week 13 onwards, all animals received a detailed palpation. The size, appearance, position and duration of any mass detected was recorded. Towards the end of the final week of dosing, a detailed review of clinical signs was carried out. Clinical signs recorded for welfare reasons have not been reported.
• No deviations from protocol were delineated or discussed

16-March-06 Submission (Response to Reviewer's Request for Information)
The following description of the methodology is quoted directly from the sponsor: There were three purposes to undertaking the clinical signs assessments on this study **Viability and welfare checks – twice daily cage side check** of animal condition, with any developing clinical signs are also recorded as required **Clinical Signs – weekly removal from cage followed by a detailed examination and recording of condition and** behaviour when handled. **Dose Related Signs – these were assessed on a varying frequency both during the working day and during the** course of the study. Frequency was altered to ensure that maximum useful data was collected to give a picture of how effects developed without putting unnecessary burdens on the data collecting personnel and reporting procedures. All three objectives are covered in the wording in section 3.5.2 of the report Raw data for dose related clinical signs were not collected in Weeks 2, 6, 7 and 14, whereas data were collected twice during Weeks 8 and 9. This was due to either local holidays, intensive work schedules during those weeks or some other animal management reason. These omissions and additions do not affect the integrity of the study data and evaluation
Deviations From Protocol • The entire discussion above is listed as a "Deviation from Protocol" • Notably missing from the discussion of deviations from protocol (above) was that fact that during Weeks 1, 3, 4 and 5 observations were made only after the second dose of the day (pm dose). (This was noted on the cover sheet for the newly submitted Appendix 38.)

09-February-07 submission – Response to Approvable Letter (Amendment 30, Module 1/Volume1)
The following is based on the sponsors submission: • For the first six days of dosing, clinical observations were conducted at the following eight time points "to document and determine the duration of these clinical signs": (1) prior to the morning dose, (2) immediately after the morning dose, (3) 30 min post morning dose, (4) 1 hr post morning dose, (5) 6 hrs post morning dose, (6) prior to the evening dose, (7) immediately after the evening dose, and (8) 15 min post evening dose. [The results of these observations are presented in appendix 6 of the original report] • During the weekly detailed observations animals are removed from the cage and detailed observations including palpitations for tumors are conducted. • "After collection of the predetermined post dose observations on Days 1-6, it was apparent that some clinical observations (i.e. "lethargy") were persistent and did not appear to habituate or diminish over time. Thus in order to determine the persistence/duration of clinical signs following day 6 of the study, additional post dose clinical observations were collected at specified time points which varied from week to week. ... Therefore, from study day 7 to the termination of the study post dose observations for each week were collected predose or immediately post dose and then at only 1 predetermined post dose interval." • This one post dose observation period was scheduled as follows: Wks 1-5 conducted immediately after the evening dose, Wks 8, 15 & 22 conducted at 0.5 hrs post dose, Wks 9, 13, 19 & 26 conducted at 4 hrs post dose, Wks 10 & 20 conducted at 5 hrs post dose, Wks 11 & 21 conducted at 6 hrs post dose, Wk 12 conducted at 7 hrs post dose, Wks 16 & 23 conducted at 1 hr post dose, Wks 17 & 24 conducted at 2 hrs post dose, and wks 18 & 25 conducted at 3 hrs post dose.
• No deviations from the protocol are specifically noted; however, the discussion of what was done reflects the only two deviations which have been noted (officially and unofficially) to date.

The most recent (09-February-07) of the three versions listed above provides the most understandable description of the methodology employed; however, it is not clear that this version accurately reflects both the study protocol and the evaluation that was actually conducted, since a comprehensive delineation of protocol deviations has not been submitted for this study. (The original study report had no list of protocol deviations. The 16-March-06 submission clearly listed some deviations and noted others in a less obvious place in the report). A clear delineation of all deviations should have been submitted for review.

There have been three submissions of clinical observations data as described in the following reviewer-generated table.

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ON ORIGINAL

Clinical Observation Data Submitted to Date		
Submission	Source of Data	Comments
original submission (*) (GLP/QA)	Table 1: "Incidence of Selected Post Dose Clinical Signs: Males and Females"	Only listings are: lethargy, hyperactivity, aggressiveness to handler or cage mates
	Appendix 6: "Individual Clinical Signs: Males and Females"	Line listings are inconsistent with summary table. Includes notable findings that are not discussed in the summary text or table.
16-March-06	(new) Table 37: "Dose Related Clinical Signs: Male and Female"	Only listings are: lethargy, hyperactivity, aggressiveness to handler or cage mates, excessive rearing, excessive grooming, burrowing, agitation
	(new) Appendix 38:"Dose Related Clinical Signs: Individual Animals"	Original submission unreadable; resubmitted in readable form in 09-Feb-07 submission. Only listings are: lethargy, hyperactivity, aggressiveness to handler or cage mates, excessive rearing, excessive grooming, burrowing, agitation
09-February-07	(new) Table 38: "Summary of Dose Related Clinical Signs (Days 1-6): Male and Female"	Only listings are: lethargy, subdued, agitated
	(new) Table 39: "Summary of Weekly Detailed Clinical Signs (Weeks 1 – 26): Males and Females"	Appears to be a more complete summary table; however, it has notable problems and shortcomings (discussed below)
(*) refers to the amended study report that was used as the basis of the original NDA review (submitted in NDA Amendment 04)		

The sponsor's original summary table (Table 1) presented only "selected" post dose clinical signs (i.e., lethargy, hyperactivity, aggressiveness to handler and/or cage mates) and was labeled as such. Examination of the individual line listings (Appendix 6) indicated that there were many other potentially treatment-related clinical signs (e.g., convulsions, tremors, head shaking, and abnormal vocalization) that were not discussed in the report or presented in the summary table. Furthermore, the individual line listings did not appear to be complete. Clarifications were requested during the first NDA review cycle; however, the 16-March-06 response was not received in time for the first cycle decision (Approvable letter issued 24-March-06). The sponsor's response to this request for information was submitted as report Amendment 3, and does not contain a GLP or QA statement. Obvious deficiencies in this response were discussed at the "End of Review" meeting for the original review cycle, held on 25-May-06 (see meeting minutes dated 05-Sept-06). The sponsor was informed that the newly submitted individual line listings (Appendix 38) were unacceptable for review (data were unreadable), and that it appeared that deficiencies may not have been entirely corrected (e.g., convulsions that occurred in animals #110 and #111 are not listed in the summary table). The sponsor submitted a readable copy of Appendix 38 as part of the 07-February-07 response to the approvable letter. The sponsor did not provide any explanation of the discrepancies between Table 1 and Appendix 6, nor an explanation of the source of the two new data summaries submitted (Table 37 and Appendix 38). Included in these new data, there appears to be additional clinical signs that were not listed in either Appendix 6 or Table 1 from the original submission (e.g., excessive grooming, excessive rearing, burrowing). Thus, the summary table and individual animal line listings submitted in the 16-March-06 response to request for information are also incomplete and inadequate.

In the 09-February-07 response to the approvable letter the sponsor submitted two additional summaries of clinical observations, Tables 38 and 39. These new summaries did not have a GLP or QA statement. Table 38 is listed as a "Summary of Dose Related Clinical Signs (Days 1-6): Male and Female"; however, the only listings are "lethargy", "subdued", and "agitated". Based on this table, no other clinical signs

occurred between days 1-6. Table 39 is listed as a "Summary of Weekly Detailed Clinical Signs (Weeks 1 – 26): Males and Females". This table lists the number of animals affected per group for a variety of clinical signs. This table also appears to be inaccurate. For example, this table does not include listing for three treatment-related clinical signs that were listed in Table 37 and Appendix 38 (i.e., excessive grooming, excessive rearing and burrowing). Also, there are duplicate listings for several observations with different listed incidences, and no explanation for the discrepancies. The following three examples occurred in males:

	group	CM	LDM	MDM	HDM
	dose level (mg/kg, bid)	0	2.5	7.5	15
	animal count	30	30	30	30
1st listing	Abnormal vocalization in hand				1
	after handling				1
	continuously	1			
	intermittently			5	
2nd listing	Behavior				
	abnormal vocalization in hand			1	
	intermittent				4

	group	CM	LDM	MDM	HDM
	dose level (mg/kg, bid)	0	2.5	7.5	15
	animal count	30	30	30	30
1st listing	Agitated			3	6
2nd listing	Agitated	3		7	15

	group	CM	LDM	MDM	HDM
	dose level (mg/kg, bid)	0	2.5	7.5	15
	animal count	30	30	30	30
1st listing	Subdued	30			
2nd listing	Subdued	1		1	3
	markedly				1

In addition, there are some listings that are just unclear (e.g., from the summary in males, "dose related signs", "posture", and from the summary in females, "coat", and "feet abnormal locomotion" versus "locomotion abnormal").

Conclusion: The sponsor has not provided an accurate characterization of the treatment-related clinical observations in the chronic toxicity study.

The sponsor's discussion of clinical signs focused on lethargy, hyperactivity and aggressive behavior. Treatment-induced convulsions were not discussed (nor were several other potentially CNS-related signs listed in the (incomplete) clinical observation individual line listings). From the limited data submitted, convulsions occur in the later part of the study, with Day 133 the earliest day on which convulsions were noted. A reliable set of line listings for each animal might have provided further insight into the progression of the CNS toxicity.

The high dose in this study, which is associated with potentially treatment-related convulsions, morbidity, and death, is only approximately three-times the maximum recommended human dose on a mg/m^2 basis. The highest dose not associated with unexplained morbidity/mortality is the mid dose which is approximately 1.5 times the maximum recommended human dose on a mg/m^2 basis. It is not possible to

establish a no-effect dose for the more serious treatment-related clinical observations, because, to date, the sponsor has not submitted comprehensive, accurate line listings for individual animals, or an accurate summary of occurrence per group.

Original Approvable Issue #2b – Pathology Reports for the Chronic Toxicity Study in Rat:

- **Refers to 26 Week Toxicity Study in Rats with Twice Daily Dosing Administration by Gavage and 13 Week Interim Kill —— Project # 244738)**

Background: The original study report did not contain a separate pathology report, and the integrated summary report did not contain the signature of the study pathologist (—— , BSc BVSc MAnimSc FRIPHH MRCVS). Therefore, there was no assurance that the discussion of the pathology findings accurately reflected the views of the pathologist.

As noted in the 24-March-06 Approvable Letter, submission of a copy of the pathology report was needed prior to approval:

> "The study report did not include a signed Pathologist's Report. In order to document the gross pathology and histopathology findings in the chronic study, you need to provide a copy of this report."
>
> In Amendment 30 (dated 09-February-07), the sponsor stated that they had submitted a copy of the signed Pathologist's Report in this response to the Approvable letter. The signed pathology report to which they referred was limited to additional brain histopathology for the 26-week study in rat. This deficiency was conveyed to the sponsor in the Incomplete Response letter that issued 16-March-07.

The sponsor submitted copies of the pathology reports for the 26-week toxicity study in rat and the 13-week interim kill in Amendment # 32 (dated 04-April-07).

Evaluation of the sponsor's response: Review of the pathology reports did not result in any changes in the interpretation or discussion of gross pathology and organ weight from those in the original NDA review. Review of the pathology reports did change the description of the histopathology evaluation and the interpretation of the results; therefore, the discussion of the histopathology evaluation below has been revised to reflect this.

Histopathology: The tissues listed in the original histopathology tissue inventory 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) MD 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); tissues missing from sampling and analysis include the lachrymal gland, larynx, nasal cavity, pharynx, harderian gland and zymbal gland. Only one eye and optic nerve examined per animal.

- Peer review: yes (x). The identity of the peer review pathologist was not provided. The report represents the consensus opinion. Peer review was limited to the following:
    - From the 13 week interim sacrifice groups, all tissues were peer reviewed from two HDF (#213 and #215) and two HDM (#95 and premature sacrifice #93).
    - From the 26 week portion of the study, the following were peer reviewed: (1) all tissues from eight animals [CM #11, CF #134, HDM #103, HDM #120, HDF #221, HDF #222, HDF #239,and HDF #240], (2) brains from 16 animals [CM's #11-#14, CF's #133-#136, HDM's #102-#105, HDF's #221-#224] [CF #133 was a premature sacrifice wk 14], (3) vaginal tissue from all CF, MDF and HDF, and (4) mammary glands from C and HD animals.

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 or the pathology 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 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 neuropathology, 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).

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 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.

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
skin/subcutis	dermatitis, chronic	total	0/20	3/20	0/20	0/20
		minimal		1		
		mild		1		
		moderate		1		

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 pathology report described a minimal grade finding as have one or two foci, while a mild grade finding as having at least three foci. 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 no clear relation to treatment in the females where there appeared to be a chance distribution and so could not be attributed 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 ($p<0.001$). The report stated 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 original report did not define or describe the term "physiological hyperplasia". The pathology report provided the following definition:

> "Physiological Hyperplasia, mammary gland: where there were increased numbers of lobules with regular eosinophilic epithelium the finding was graded as minimal. When the lobules appeared more basophilic, with epithelial cells of variable size due to the extent of secretory activity and number of lipid vacuoles, and there were increased numbers of ducts, with milk present, the finding was graded as mild or moderate."

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. 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. *Ann Neurol* 12(3):257-62, 1982). 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 determined as part of [redacted] Study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats. The results of the prolactin evaluation were not submitted to the NDA in time for the first review cycle. The final study report was submitted (Amendment #30, Module 4/volume 3) and demonstrated that daily oral administration of TBZ to rats at the same dose as the current HD (15 mg/kg bid [30 mg/kg/day]) for 14 days did not result in sustained increased serum prolactin levels.

3. Vaginal cycle in proestrus in 100% of HDF and minimal vaginal epithelial degeneration in 2/20 HDF. According to the pathologist, "All Group 4 [HD] females showed vaginal mucification, with epithelial thinning and occasional epithelial degeneration, and appeared to be in pro-estrus." The pathologist notes that this is statistically significant ($p<0.001$), and the report 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 during wk 14, was noted with the vaginal cycle in proestrus (and with "vaginal

mucification and epithelial degeneration). The histopathology findings for 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. The submission of the pathologist's report provided clarification with a working definition of pro-estrus that suggests that the term was being used in an unexpected way:

> "Pro-oestrus: was used to describe the oestrus stage of all rats showing vaginal mucification, regardless of the number of and stage of follicles/corpora lutea in the ovary and any uterine findings. In the Group 4 [HD] females, the vaginal epithelium was thin and degenerate in occasional animals, indicating that this was not a normal proestrus in this group."

Treatment-related vaginal mucification was also attributed to TBZ-induced increases in serum prolactin, (as with the mammary hyperplasia). The sponsor references Batten and Ingleton (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 Vertebrae Endocrinology*. Chester-Jones, I., Ingelton, PM. Phillips, JG (Eds). 1st Edition. Plenum Press, New York. Pgs 285-409). As stated above, the sponsor has not provided evidence that the doses and dosing regimen utilized in the pivotal toxicity study result in increased serum prolactin levels.

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. There was 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. As demonstrated in the mass balance study in humans, the majority of the drug-related compound is 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 chromic 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.

Conclusion: Review of the pathology reports did change the description of the histopathology evaluation and the interpretation of the results; therefore, the current discussion of the histopathology evaluation supersedes that filed with the original NDA review.

Original Approvable Issue #3 – Submission of Prolactin Data from —— Study #7425-114:

Background: The sponsor attributed the treatment-related increases in physiological mammary hyperplasia and vaginal mucification demonstrated in the chronic toxicity study in rat to a treatment-related increase in serum prolactin or a change in the pattern of prolactin release. The sponsor had 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 evaluated in —— Study # 7425-114 (14-Day Oral Gavage Study

with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats). A draft report of the in-life portion of this study was submitted to the NDA during the original review cycle (original submission, module 4, volume 11), and revised a TK report was submitted to the NDA in Amendment # 5. However, serum prolactin data were not submitted in these reports.

As noted in the 24-March-06 Approvable Letter, submission of the data was needed prior to approval as stated below:

> You conducted a 14-day oral study of tetrabenazine to assess toxicokinetics and effects on serum prolactin in rats —— Study #7425-114). The toxicokinetics data have been provided, but the serum prolactin data have not. You need to submit a final report of the serum prolactin data. These data are important for the interpretation of the results of the chronic toxicity study in rats.

Evaluation of the sponsor's response: The sponsor responded in Amendment 30 (dated 09-February-07) by submitting a final report for —— study # 7425-114 (14-Day Oral Gavage with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats, which included the serum prolactin assessment.

Conclusion: There was no evidence of a treatment-induced increase in serum prolactin in rats treated for 13 days.

Original Approvable Issue #4 - Potential for Tetrabenazine-Induced Neuropathology:

Background: In the original NDA submission, a reference was cited describing TBZ-induced neurotoxicity and neuropathology in rat with repeat dosing (Satou et al. *Exp Toxic Pathol* 53: 303-308, 2001). 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 original 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 was 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.

Furthermore, Dr. Lois Freed noted the following in her Supervisory Memorandum dated 27-March-06.

> "In addition, Takahashi et al. (Takahashi N et al. *Proc Natl Acad Sci* 94:9938-9943, 1997) reported an enhanced sensitivity to MPTP in heterozygous VMAT2 knockout mice. MPTP (4 doses of 16 mg/kg i.p.) resulted in a 13% decrease in TH immunoreactive neurons in wild-type mice compared to a 30% decrease in heterozygous VMAT 2 knockout mice. Therefore, it is important to verify that the microscopic evaluation of brain in the rat and dog studies was adequate to address the neurotoxic potential of tetrabenazine. If, based on the sponsor's response, it is clear that the assessment was not adequate, additional nonclinical studies may be needed to address this concern."

As noted in the 24-March-06 Approvable letter, resolution of the following Pharmacology/Toxicology issue (as stated below) was needed prior to approval:

> "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)."

This was further discussed at the 25-May-06 End of Review Meeting, and was documented in meeting minutes that issued on 05-September-06):

> "Regarding the neurotoxic potential of tetrabenazine, the Sponsor needs to provide documentation, as requested in the approvable letter, that the histopathology techniques employed in the toxicology studies were adequate to rule out treatment-related neuropathology similar to that reported by Satou et al. (2001)."

Evaluation of the sponsor's response:

Subsequent Evaluation in Rat: In the 09-Feb-07 response to the approvable letter (Amendment 30), the sponsor provided the following statement from the study veterinary pathologist, ——— BSc, BVSc, MAnimSc, FRIPH, MRCVS, MRCPath (dated 18-April-06).

> "In the study (report No. 20730), the SNpc appears in our standard section 2 of brain (Paxinos atlas Bregma -4.80 to -6.30). This is a large area and no matter how sectioned, there will always be a good representative sample of SNpc on the slide. The slides were sectioned at 4-6 microns (Satou *et al.* did 50 micron brain slides for imagine analysis), stained with H&E and evaluated at x 10 magnification. All slides were peer reviewed by another veterinary pathologist. Based on the H&E examinations of the SNpc there was no evidence of neuronal loss or atrophy in rats receiving tetrabenazine at doses up to 30 mg/kg/day for 26 weeks. Special stains such as GFAP for post neuronal loss gliosis or FluoroJade for neuronal degeneration were not performed as there was no indication of neuropathology from H&E sections that warranted additional special staining.
>
> In conclusion, there was no evidence of neuronal loss or atrophy in 4-6 micron sections of the SNpc evaluated by H&E staining in rats administered tetrabenazine at doses up to 30 mg/kg/day for 26 consecutive weeks."

The sponsor also submitted an additional neuropathology study to address this issue, "Tetrabenazine Histological Evaluation Extension for the 26 Week Toxicity Study in Rats" (originally submitted as an unsigned draft in Amendment 30, module 4/volume 4, and resubmitted as a final report in Amendment 32, module 4/volume 1). This study was a re-evaluation of brain samples and slides from a subset (15/sex/group) of control and high dose animals that survived until the scheduled necropsy at week 27. This study was conducted as three sequential evaluations.

The first evaluation was conducted by the original study pathologist, ——— BSc, BVSc, MAnimSc, FRIPH, MRCVS, MRCPath, who had submitted the earlier statement reproduced above. The description of the methodology for this part of the evaluation was inadequate; however, it could be

reasonably constructed based on the subsequent initial peer review report (which will be discussed below). It appears that the standard three full coronal sections of brain and an additional section of the midbrain were stained with H&E, anti-GFAP, FluoroJade B, anti-tyrosine hydroxylase and Bielschowsky's silver, and were evaluated by light microscopy. Although not clearly stated, it appears that these sections may have been the same sections (re-stained) from the original assessment. For 21 of the 30 males, the sections did not contain sufficient SN for evaluation, because the section was taken too far caudally. Thus, the relevant tissue block (#20) was re-embedded, sectioned, stained and evaluated as above. According to the study pathologist, Dr. ——, no abnormalities were detected with any stain in any but one control male. There was no statement that either the SNpc or the SN was adequately assessed.

These already prepared slides were sent to Dr. —— DVM&S, MSc, DLAS, MRCVS, MRCPath for peer review.

According to the peer review pathologist, there were no abnormalities in any brain section, using any staining technique. Furthermore, the peer review pathologist stated that there were no morphologic differences between control and high dose animals with regard to substantia nigra, ventral tegmental area and striatal (caudate nucleus/putamen) areas, which are noted to be areas which contain TH positive neurons or projections of these neurons. According to Dr. —— the ventral tegmental area (VTA) "is closely aligned morphologically and functionally with substantia nigra". Sufficient evaluable SN (defined as having at least six TH positive neurons) was available for 13 of 14 control females, 15 of 15 high dose females, 6 of 15 control males and 7 of 15 high dose males.

Additional histopathologic evaluations were conducted by the peer review pathologist to increase the number of control males and high dose males from which substantia nigra could be evaluated.

Additional wet tissue and paraffin blocks were obtained from the animals that did not have sufficient evalauble SN in the previous portions of the neuropathology evaluation. Re-embedding the original paraffin block containing the midbrain did not yield any additional sections containing SN. Additional sections of the SN were obtained from the wet tissue from eight control males and five high dose males. Sections from these animals were stained with H&E, Fluoro-Jade B, GFAP, Bielschowsky's silver and TH; however, it is not clear if each stain was associated with a unique section, or if all four stains were tested on a single section.

The original study pathologist (Dr. —— did not review the supplemental slides generated during the second (supplemental) peer review. According to the peer review pathologist, there was no effect of treatment on brain pathology including the substantia nigra and striatal areas. The conclusion regarding the evaluation of the SN was based on 13 of 14 control females, 15 of 15 high dose females, 13 of 15 control males and 11 of 15 high dose males. Dr. —— noted that there was "considerable variability" in the number of neurons present in the SN among animals, and attributed this to the sectioning; however, the criteria used to determine adequacy of SN evaluation was the presence of at least six TH positive neurons.

With regard to the difference in histologic evaluation techniques used in this study versus those used in the referenced article published by Satou et al., the peer review pathologist states:

> "Morphometrics were not conducted on the current study. But TH staining was conducted, and this technique allowed for the specific identification of dopaminergic neurons in the substantia nigra. In the opinion of the RP [peer review pathologist], using hematoxylin and eosin stained sections to define neurons belonging to the substantia nigra would be a very subjective process. In

> the current study, based on light microscopy observations of the TH and GFAP stained sections, there were no differences between the control and test article treated animals."

Evaluation in dog: Amendment #30 (dated 09-February-06), which was the sponsor's initial response to the approvable letter did not address the potential for treatment-related neuropathology in the chronic toxicity study in dog. In the letter of 16-March-07 the Division informed the sponsor that the 09-February-07 submission was not a complete response to the approvable letter, in part, because this issue had not been addressed. The relevant section of this letter is reproduced below.

> "You have not addressed the potential for treatment-related neuropathology in the chronic toxicity study in dog, you need to document that the microscopic examination of brain was conducted using techniques sensitive enough to have detected, if present, neuropathological findings similar to those reported by Satou et al. (2001) in rat."

In response the sponsor submitted "Neuropathology Extension of 9-Month Toxicity Study in Dogs" (Amendment #37). This report provided the results of an additional examination of brain tissue from all control and TBZ treated animals (4/sex/group) from the original 9-month toxicity study in dogs. This evaluation was conducted by Dr ——— , DVM&S, MSc, DLAS, MRCVS, MRCPath.

For each animal Dr. — took the three original tissue blocks and re-sectioned them, the resulting serial sections were processed with the following stains: anti-tyrosine hydroxylase (TH), Bielschowsky's silver, anti-GFAP and Fluoro-Jade B (H&E stained sections were already available). In addition, the remaining wet tissue was re-trimmed and embedded to produce an additional eight blocks per animal, which were then sectioned. The resulting serial sections were also processed with H&E, anti-tyrosine hydroxylase (TH), Bielschowsky's silver, anti-GFAP and Fluoro-Jade B.

All of the sections described above were evaluated by light microscopy. The pathologist stated that "The extensive sectioning and staining scheme allowed for a very comprehensive morphologic evaluation of the brain including the frontal cortex, cerebral cortex, caudate/putamen, thalamus, midbrain (including the substantia nigra and pons), cerebellum, medulla oblongata and other structures." There was no indication of treatment related pathology.

Conclusion: It is reassuring that treatment related pathology was not demonstrated in brain from the chronic toxicity studies in rat and dog using a variety of staining techniques, in addition to the standard H&E. However, it is not clear that the techniques employed address our pivotal question, i.e., were the techniques sensitive enough to have detected, if present, neuropathological findings similar to those reported by Satou et al. (2001).

The differences in the processing of brain between Satou et al. (2001) and the sponsor are notable and expected, considering the different primary purposes of the studies, and the additional evaluations of the existing brain slides and additional sections, do not address this concern.

Satou et al. demonstrated a decrease in neuronal count (up to approximately 50%), neuronal area (up to approximately 20-30%) and neuronal size in the SNpc, using a predefined, controlled stereotaxic section. The sponsor's reevaluation of the brains from the 26-week study in rat was based on a notably less controlled level of sectioning and potentially inadequate sampling of the target region. In the sponsor's evaluations there was no indication that the pars compacta was specifically evaluated, rather, the report referred to the evaluation of the broader regional designation of the substantia nigra. Furthermore, the sponsor considered the substantia nigra to be adequately assessed if a minimum of six dopaminergic neruons were evaluated. The sponsor noted the variability in the amount of substantia nigra present for evaluation and noted that it was due to the variability of the sectioning of the brain. It would not appear

that the sponsor would be able to detect a similar type of neurotoxic effect with the variability of the sectioning and the limited evaluation conducted.

A similar reasoning applies to the expanded evaluation of the brain from the chronic toxicity study in dog, except that somewhat more extensive sectioning was conducted due to the larger size of the brain. Again, it is not clear from the report that the pars compacta was specifically evaluated and the sponsor did not state the criteria for adequate evaluation.

Furthermore, there are significant differences in the type of behavioral evaluation conducted as part of the chronic toxicity study in rats and the study conducted by Satou et al. (2001). The chronic toxicity study employed standard intermittent cage side observation and weekly detailed physical examination. The chronic study clearly demonstrated treatment-related lethargy as well as several other notable CNS effects. The study conducted by Satou et al. (2001) demonstrated a treatment related decrease in spontaneous locomotion following seven days of i.p. dosing that did not fully reverse after a 15 day recovery period. This was based on data generated by an automated activity sensor system that measured spontaneous activity over the 24 hour period prior to sacrifice for each animal. Equivalently powered data were not available from the chronic toxicity study in rats.

Informal discussions with an Agency neurotoxicologist provided additional support for this concern. Degenerated neurons are detectable for a finite period of time and that period of time can vary, based on the nature of the insult and the type of cell affected. Thus, if the vulnerable neurons were affected earlier in the course of the 26-week treatment, the ability to detect the effect could be diminished or absent at the time of evaluation (at least 26 weeks, or nine moths after the initiation of dosing for rat and dog, respectively).

Therefore, to address the concern that TBZ may induce neuropathologic changes in the SNpc, the sponsor should conduct a short-term neurotoxicity study in rat to determine whether the tetrabenazine induced-neuropathology demonstrated in rat by Satou et al. (2001) can be replicated, and whether oral administration of tetrabenazine to rats results in similar histopathology in the brain. This study should include a group treated according to the multi-dose paradigm used by Satou et al. (i.e., repeated administration of tetrabenazine at a dose of 1 mg/kg/day, i.p. for seven days), several groups treated with oral tetrabenazine using a range of doses up to a maximum tolerated dose, and appropriate control groups. Appropriate sections of the substantia nigra should be stained and evaluated for neurodegeneration. It is recommended that the sponsor submit the protocol for review by the Division, prior to the conduct of the study. The conduct of this study should be a Phase 4 commitment.

Original Approvable Issue #5 - The need for further investigation of the equivocal positive response in the in vivo micronucleus study in rat

- "Tetrabenazine Micronucleus Test in Bone Marrow of Rats 0 h + 24 h Dosing and 48 h Sampling" (___ : Report Number 19434)

Background: Tetrabenazine and two of its metabolites, α- and β-dihydrotetrabenazine, were not mutagenic in the Ames test. Tetrabenazine was clastogenic in the in vitro chromosome aberration assay in Chinese hamster ovary cells, only in the presence of metabolic activation. The metabolites α- and β-dihydrotetrabenazine were clastogenic in the in vitro chromosome aberration assay in Chinese hamster lung cells in the presence and absence of metabolic activation. There was no evidence of clastogenicity in the in vivo micronucleus assay in male mice (female mice were not tested). In the in vivo micronucleus test in rats, there was no evidence of clastogenicity in males and an equivocal clastogenic response in

females. Thus, the one in vivo genetic toxicity test in which female animals were evaluated produced equivocal results.

With regard to the in vivo micronucleus assay in rats, it should be noted that the evaluation in females was somewhat unusual. This study, as proposed, was a standard design, with concurrent vehicle and positive control, three dose groups for males and females evaluated only at the high dose (as well as vehicle control). Treatment groups were 5/sex/group, except for the inclusion of an additional 5/sex in the high dose group to serve as contingency animals "in case of unscheduled deaths or potential sex difference." TBZ was administered as two doses 24 hrs apart and sampling was conducted 24 hrs after the last dose. The doses were selected based on DRF studies and the doses were adequate; higher doses would not have been tolerated. No relevant TK data are available from this study or any other study; the highest dose for which TK is available is 15 mg/kg BID, and the high dose used in this study is 100 mg/kg/day (administered as a QD dose). Furthermore, this study was conducted in CD rat (with no further strain designation); all toxicity studies and TK assessments were conducted in SD rat.

The data are provided in a sponsor-generated summary table and in a reviewer-generated (individual animal data) table that follow. Comparisons of the PCE/NCE ratio among groups did not indicate treatment-induced bone marrow toxicity in males or females. There was no evidence of a treatment-related increase in MN-PCEs in males; however, the original five high dose females demonstrated an increase in the MN-PCEs compared to the vehicle control. The sponsor chose to assess the bone marrow of the five "spare" high dose females because the increase in MN-PCEs seen in the original five high dose females was "greater than 10% above the maximum range of the historical control data for a negative response." According to the sponsor two vehicle control slides and two positive control slides that were previously evaluated were selected for reevaluation concurrently with the 'spare' high dose females in order to preserve the blinded evaluation. There was no evidence of a treatment-related increase in MN-PCE in the "spare" or contingency females when compared to the negative control or the historical control. When the results of the evaluation of the original and "spare" high dose females were combined, the combined frequency of MN-PCE was increased compared to the vehicle control and was outside of the historical control range (0.12 versus 0.09), although not statistically significant. The sponsor concluded that the TBZ did not induce a treatment-related increase in micronuclei in bone marrow at a maximally tolerated dose of 100 mg/kg/day.

Treatment	Dose (h)	Sex	No. of Rats Scored	Erythrocytes				
				Normochromatic Cells (NCE)	Polychromatic Cells (PCE)			PCE/NCE
				No. of MN-NCE	PCE Analysed	No. of MN-PCE	% MN-PCE	Mean ± S.D.
10 ml 0.5% carboxymethyl cellulose $kg^{-1}.day^{-1}$	0 + 24	♂	5	4	10013	9	0.09	0.95 ± 0.07
		♀	5	3	10048	7	0.07	0.92 ± 0.03
		♂♀	10	7	20061	16	0.08	0.94 ± 0.05
25 mg Tetrabenazine $kg^{-1}.day^{-1}$	0 + 24	♂	5	4	10022	9	0.09	0.93 ± 0.06
50 mg Tetrabenazine $kg^{-1}.day^{-1}$	0 + 24	♂	5	6	10032	3	0.03	0.92 ± 0.08
100 mg Tetrabenazine $kg^{-1}.day^{-1}$	0 + 24	♂	5	11	10028	8	0.08	0.89 ± 0.09
		♀ a	5	4	10020	15	0.15 β	0.91 ± 0.05
		♀ b	5	9	10018	8	0.08	0.90 ± 0.07
		♀ a+b	10	13	20038	23	0.12 β	0.91 ± 0.06
50 mg Cyclophosphamide. $kg^{-1}.day^{-1}$	0 + 24	♂	5	78 α	10017	352	3.51Φ	0.56 ± 0.10

PCE = Polychromatic erythrocytes
MN-PCE = Micronucleated PCE
NCE = Normochromatic erythrocytes
MN-NCE = Micronucleated NCE
Φ = Positive response in PCE
α = Evident response in NCE
β = Outside negative historical control range, but not statistically significant
a = core group
b = contingency group

Percentage of MN-PCE for each animal			
sex	group	%MN-PCE for each animal	% MN-PCE (mean ± SD)
males	vehicle control	0.05, 0.05, 0.05, 0.15, 0.15	0.09 ± 0.055
	low dose (25 mg/kg/day)	0.00, 0.05, 0.10, 0.10, 0.20	0.09 ± 0.074
	mid dose (50 mg/kg/day)	0.00, 0.00, 0.05, 0.05, 0.05	0.03 ± 0.027
	high dose (100 mg/kg/day)	0.00, 0.05, 0.10, 0.10, 0.15	0.08 ± 0.057
	positive control	2.35, 2.75, 3.59, 3.64, 5.23	3.51 ± 1.11
females	vehicle control	0.00, 0.00, 0.10, 0.10, 0.15	0.070 ± 0.067
	high dose (100 mg/kg/day)	0.10, 0.10, 0.15, 0.20, 0.20	0.150 ± 0.050
	contingency-high dose	0.00, 0.05, 0.05, 0.15, 0.15	0.080 ± 0.067
	original high dose + contingency high dose	0.00, 0.05, 0.05, 0.10, 0.10, 0.15, 0.15, 0.15, 0.20, 0.20	0.115 ± 0.067

Two vehicle control animals and two positive control animals were "re-evaluated" with the contingency animals to help preserve the blind. The original and subsequent values for % MN-PCE are presented for these retested animals:

- vehicle controls: 0.00% → 0.10% and 0.15% → 0.15%
- positive controls: 2.75% → 3.39% and 5.23% → 3.10%

Historical control for negative control:

- mean = 0.06% ± 0.06
- range for individual animal = 0.00% – 0.20%
- range for 5-6 animals = 0.01% - 0.12%
- range for 10-12 animals = 0.04% - 0.09%

During the original review cycle for the NDA, the Division concluded that the genotoxic potential of the test compound and the relevant major circulating drug-related compounds (in humans) should be adequately characterized prior to approval, especially considering the strong signal for in vitro clastogenicity and the agreement that carcinogenicity studies could be conducted as phase 4 commitment.

As noted in the 24-March-06 Approvable Letter, resolution of the following Pharmacology/Toxicology issue (as noted below) was needed prior to approval:

> "The equivocal finding in females in the in vivo micronucleus assay in rat needs to be further investigated, particularly considering the lack of carcinogenicity 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."

Evaluation of the sponsor's response: In Amendment # 30 (dated 09-February-07) the sponsor provided a response, based on the opinion of their consultant ——— PhD, JD.

According to Dr. ——— the results of the in vivo micronucleus assay in rats were negative for both males and females for the following reasons: (1) "the absence of statistical significance of any of the responses", (2) "the range of female responses being within the historical control range", (3) "the variability between the initial group of 5 animals and the second group of 5 that were all treated and processed at the same time", and (4) "the variability in responses between the first and second scoring of the coded control slides".

Furthermore, Dr ——— states "... the historical control is not used as a reference for determining the 'positivity' of the substance, only the acceptability for analysis of the data. The only published and generally accepted criteria for a positive or equivocal response is a statistically significant increase in MN measured either as a dose-related response or a single increased dose as compared with the concurrent control."

Although some of these points have merit; I do not agree with his conclusion that the results are negative. My conclusion that the results were equivocal in the original group of females (rather than the sponsor's assertion of negative), was based on the apparent treatment-related two-fold increase in MN-PCE over the concurrent control, in conjunction with the a group mean for the high dose females that was outside the historical vehicle control data. Dr ——— 's assertion that the response must be statistically significant in order to be considered equivocal or positive is not consistent with current guidelines (cf. OECD Guideline for the Testing of Chemicals" for the mammalian erythrocyte micronucleus assay, 21-July-97):

Neither Dr. ——— nor the sponsor discussed the validity of doubling the number of animals evaluated in the high dose female group only, to help ameliorate the concern for an equivocal response. On face, this approach seems problematic, and would possibly be more acceptable if the sample size were increased for the vehicle controls and the high dose groups (male and female).

Dr. ——— also states that "Tetrabenazine is metabolized by similar routes in both sexes, so that any differences between MN responses, if they exist, are expected to be quantitative." It is not clear that there are relevant metabolism data for CD rat; SD rat were used in the toxicity studies.

Feedback from the Genetic Toxicology Subcommittee of the Pharmacology and Toxicology Coordinating Committee provided support for our original conclusion of an equivocal response in female rats and request for follow-up.

Conclusion: Without new data, the results of this assay should still be interpreted as equivocal in females (and negative in males). The lack of the requested additional in vivo micronucleus assay in rats to clarify the equivocal results in female rats will not have an impact on the clinical use of this drug, especially since it has already been established as notably genotoxic in the in vitro chromosomal aberration assays. Therefore, this issue does not need to be further addressed, except in labeling. In the absence of an additional in vivo micronucleus assay in rats, the results of the in vivo micronucleus assays should be considered as negative in male rats and mice and equivocal in female rats (female mice were not evaluated).

Original Phase 4 Commitment #3a, b, and c

- Oral Developmental Toxicity Study and Pre- and Postnatal Development Study with Tetrabenazine, in the Rat (Segment III Study)" (——— Study # 7425-106))

Background: In my original review for this NDA, I had noted discrepancies in the report for the pre- and post-natal development study. The resolution of these discrepancies was made a Phase 4 commitment in the original approvable letter (24-March-06) as reproduced below:

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 F_1 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.

Evaluation of the sponsor's response: The sponsor has not yet addressed these issues.

Conclusion: With regard to issue #3b (stillbirths versus early postnatal deaths), further review of the original report indicated that it is possible to verify the number of stillbirths in each treatment group based on the data available in Appendix 15 (Individual Pup Necropsy Observations). Pups were designated as stillborn if either the pup had no milk in its stomach (indicating that the pup had never nursed), or the lungs did not float (indicating that the pup had never breathed). It is not clear that lack of milk in a pup's stomach should be used as a criterion establishing a stillbirth. The liveborn pups could be affected by their in-utero exposure to tetrabenazine or its metabolites resulting in their inability to nurse and thus their death. In addition, a newborn pup may not be able to metabolize and/or excrete the tetrabenazine and/or its metabolites. Using the sponsor's criteria for stillbirths without additional data (i.e., a delineation of which stillbirths were based solely on lack of milk in the stomach), it would be possible to overestimate