were not associated with abnormal histopathology. The single incidence of thickening of the vaginal wall was associated with moderate, diffuse edema. (It should be noted that the vagina was not part of the battery of tissues examined in this study, and the previously mentioned tissue was examined based on the gross finding.) These findings are noted because of the treatment-related pathology noted in the uterus, vagina and mammary gland in the chronic study in rat.

There were no clearly toxicologically significant effects of treatment on organ weights, except the treatment, but not dose related decreases in absolute and relative thymus weights seen in males and females (with a smaller decrease than might be expected based on the frequency and severity of treatment-related clinical signs observed throughout the study in the HD groups and effect on body weight in the HDF (20% decreased compared to control)). Other changes were not associated with treatment-related pathology.

The histopathology examination was not peer reviewed and the sponsor did not preserve and examine an adequate battery of tissues for a chronic toxicity study in a non-rodent species. Notable omissions from sampling and examination include: bone marrow smear, eyes/optic nerves, vagina, cervix, lachrymal gland, larynx, nasal cavity, pharynx, skin, and tongue (the fallopian tube was not sampled; however, this is not unusual). CNS histopathologic examination consisted of the "brain (including substantia nigra)" and spinal cord (cervical, thoracic, and lumbar). Potentially treatment-related histopathology was confined to lymphoid depletion of the thymus in HDM (slight-moderate) and in one MDF (moderate/severe) and lymphohistiocytic infiltration of the prostate in one LDM (slight) and one HDM (moderate). There was surprising lack of thymic atrophy in HDF, considering the frequency and severity of the clinical observations and the effect on body weight (20% decreased compared to control). There were no treatment related findings in the brain.

Standard toxicokinetic parameters were assessed for TBZ, and the stereoisomeric metabolites α-HTBZ and β-HTBZ on Day 1, and Wk 13 and 39. Review of the data reveals highly variable TK. Exposure increased with increasing dose, with no obvious indication of saturation of absorption or metabolism. In general, at wks 13 and 39, exposure (based on AUC) to the three quantified drug-related components was greatest for β-HTBZ, then α-HTBZ, and then TBZ (except for LDF at wk 13 for which the order was [β-HTBZ > TBZ > α-HTBZ]).

In conclusion, this study is the only chronic toxicity study conducted in non-rodent and it has notable design flaws. Ophthalmologic examination, ECG and urinalysis were not conducted, and these are standard components of a definitive toxicity study. In addition, the following tissues, considered to be part of a standard toxicology study, were not examined microscopically: eyes/optic nerves, vagina, cervix, lachrymal gland, larynx, pharynx, skin, tongue, nasal cavity and bone marrow smear. The major treatment-related toxicity identified in this study was abnormal clinical observations. The NOEL for this finding was 1 mg/kg/day (LD) which is 0.32 times (on a mg/m^2 basis) the MRHD. The highest dose tested in the chronic study was 3.2 times (on a mg/m^2 basis) the MRHD. In the 2-week DRF study, a dose of 40 mg/kg/day (administered as 20 mg/kg bid) resulted in the unscheduled sacrifice of all four treated animal after 4 to 11 days of dosing due to treatment related clinical signs in all four dogs and possibly liver/gallbladder toxicity in one dog (based on clinical pathology findings; histopathology was not conducted). Finally, the sponsor should be asked to provide a better description of the microscopic examination of the brain, especially with regard to the substantia nigra, for the chronic study.

Summary for Reproductive Toxicology:

Fertility and early embryonic development: In the NDA package the sponsor notes that a fertility study is planned but has not yet been initiated. At the end-of-phase 2 meeting that took place on 30-Jun-04, it was agreed that a "segment 1" study could be provided as a Phase 4 commitment.

Embryo-fetal development in rabbit: The doses selected for this study were based on the results of a non-GLP oral dose range-finding (DRF) study in the same strain of rabbits. In the DRF study, pregnant does (6/gr) were treated by oral gavage from GD7-20 with TBZ doses of 0, 1.25, 2.5, 7.5 or 15 mg/kg/day. One doe treated with 7.5 mg/kg/day was found dead on GD10, with no clinical observations. Gross necropsy findings were supplied only for the uterus and the sponsor did not consider the death related to treatment. The sponsor conducted a TK assessment as part of this study and measured plasma levels of TBZ and the stereoisomeric metabolites α-dihydrotetrabnazine (αHTBZ) and β-dihyrotetrabenazine (βHTBZ) after the first and last day of dosing. Exposure to TBZ, αHTBZ, and βHTBZ increased in a greater than dose proportional fashion, for the doses of 7.5 and 15 mg/kg/day. αHTBZ was more prevalent in the plasma followed by βHTBZ and then TBZ.

Due to a lack of treatment-related toxicity seen with TBZ at a dose of 15 mg/kg/day, the sponsor took two non-pregnant stock rabbits and administered each a single oral gavage dose of 30 mg/kg TBZ. According to the sponsor, there were no clinical observations and no change in food consumption (assessed qualitatively). The animals were given a 3-day washout period and were then each administered TBZ at a dose of 60 mg/kg/day for 2 days. According to the sponsor, the animals were noted with constricted pupils, slight droop of the eyelids, a mild stupor that resolved within 3 hrs, and an estimated 30% decrease in food consumption (when compared to naïve animals). The animals were given an additional two day washout period after which they were administered single oral (gavage) doses of 120 mg/kg TBZ. According to the sponsor, the animals were noted with constricted pupils, recumbent for at least an hour post dose and were noted to have consumed little or no food (qualitative assessment) for at least 24 hrs after dosing. In two additional non-pregnant stock rabbits, TBZ was administered (via oral gavage) at a dose of 45 mg/kg/day for 5 consecutive days. The sponsor noted constricted pupils in both animals, rapid respiration (one day) in one animal, and decreased food consumption and an associated body weight loss in one animal.

The definitive study was conducted in premated female Hra: (NZW)SPF rabbits treated once daily, via oral gavage, from gestation day (GD) 7-20 with TBZ at doses of 0, 10, 30 or 60 mg/kg/day. TK assessments were not carried out as part of this study. Three animals (one in each of the TBZ treated groups) were sacrificed after abortion of the litters. In the LD animal (sacrificed on GD23) and the MD animal (sacrificed on GD19), body weights and food consumption were unremarkable compared to the rest of the group. No clinical signs were noted in the LD doe and the MD doe was noted only with constricted pupils on GD11. Neither animal had any remarkable findings on necropsy and the sponsor considered the abortions to be unrelated to treatment, a reasonable conclusion considering the lack of increase in incidence with increasing dose. The HD doe was sacrificed on GD27. This animal was noted with thin appearance (GD27), recumbent (GD19), constricted pupils (GD8-12, 15-17, 19-20), squinted eyes (GD14), rapid respiration (GD17, 19-20), and few or no feces (GD-11, 27). The animal was noted with decreased food consumption and body weight gain. The necropsy finding for this animal consisted of dark material found in the stomach, i.e., one placenta found in stomach. The sponsor attributed this abortion to treatment.

In general, treatment related clinical observations were noted in the MD and HD groups and consisted of constricted pupils, squinted/closed eyes, rapid respiration, few or no feces, and recumbency (HD). There was a very slight treatment-related decrease in mean body weight in the HD group (maximum of 5% when compared to control). There was a significant decrease in body weight gain GD 7-21 for HD

animals; however this was predominantly noted on GD7-13. There was an increase in mean body weight change in the LD and HD from GD21-27. There were significant decreases in food consumption GD 7-21 for HD animals. The food consumption in the HD animals was not significantly different from control on GD21-29.

In addition to the three abortions (one in each LD, MD and HD), one MD and one HD female were determined to not have been pregnant and one MD dose was noted to have been pregnant, but with no viable fetuses. According to the sponsor, mean post-implantation loss was slightly increased in the MD group due to animal #F61252 (noted with no viable fetuses, 3 corpora lutea, 2 implantation sites and 2 early resorptions). Examination of the data reveals that this doe did contribute to the finding; however, was not the major contributing factor. If the data from this doe were eliminated the total number of early resorptions would still be increased compared to control (5 resorptions from 5 litters compared to 2 resorptions from 2 liters). Relationship to treatment is questionable because a similar effect was not seen in the HD. There were no notable effects of treatment on mean fetal weights even when covariate adjustment was made.

There were no external findings in any fetus based on examination of 183, 173, 144 and 165 fetuses from the C, LD, MD and HD, respectively. There were no treatment-related soft tissue malformations. There was an apparent treatment-related decrease in the total fetal soft tissue variations (based on the decreased fetal incidence and litter incidence of variations of the major vessels in LD, MD and HD groups, and decreased fetal incidence (LD, MD and HD) and litter incidence (HD) of small or missing intermediate lobe of the lung). There were no skeletal malformations in the HD group and no clear evidence of a treatment-related effect on skeletal malformations. There were no clear treatment-related effects on skeletal variations.

In conclusion, the NOEL for maternal toxicity was 10 mg/kg/day (approximately 1.9 time the MRHD on a mg/m^2 basis), based on treatment-related clinical observations seen at the MD and HD and changes in body weigh and food consumption seen at the HD. The NOEL for embryo fetal development is 60 mg/kg/day (the highest dose tested) (approximately 11.6 times the MRHD on a mg/m^2 basis).

Embryo-fetal development in rat: According to the sponsor, the choice of the high dose was based on the previously conducted 4- and 26-week toxicity studies in rat. It should be noted that 4- and 26-week toxicity studies in rat utilized the same total daily doses; however, administered as divided doses (BID); whereas this study utilized QD dosing. Premated female — CD®(SD)IGS BR rats were treated once daily, via oral gavage, from gestation day (GD) 6-17 with TBZ doses of 0, 5, 15 or 30 mg/kg. TK assessments were not conducted.

There were no unscheduled deaths. Clinical signs in the dams consisted of hypoactivity and squinted or closed eyes in MD and HD throughout the dosing period. The incidence of these signs increased with increasing dose. The duration of the clinical signs on a daily basis was not provided. There were no significant differences in the group mean body weights and the maximum decrease seen in the HD group was 3%. There were statistically significant decreases in mean group body weight changes; however, the differences were small. The mean food consumption was decreased in the MD and HD groups (the decrease over the duration of treatment was 8% for the MD and 9% for the HD). There were no remarkable findings at necropsy for any dam in any group. There was no effect of treatment on group mean gravid uterine weight. The mean corrected body weight (terminal body weight minus the gravid uterine weight) was slightly (4%), but not significantly, decreased in the HD group. There was a dose-related non-significant decrease in the mean change in body weight (corrected by subtracting the gravid uterine weight) from Day 0 until sacrifice for the LD (3%), MD (9%) and HD (16%) groups.

Pregnancy rate was similar across groups. No dam aborted or had an early delivery, and all dams had litters with viable fetuses. There were no differences among groups for the group mean number of

corpora lutea, implantation sites or preimplantation loss. Although not acknowledged by the sponsor, there appears to be a treatment-related increase in total resorptions and early resorptions in the HD group, and an increase in post-implantation loss in the HD group. The mean number of live fetuses per litter was similar across groups. There was also a slight (nonsignificant) dose-related increase in mean fetal weight, and covariate adjusted (for number of fetuses per litter) fetal weight for total fetuses, male fetuses and females fetuses.

It should be noted that historical control data were not submitted for fetal variations and malformations. There were no external findings in any of the 319, 327, 345 and 323 fetuses from the control, LD, MD and HD groups, respectively. There were no head malformations in any fetus and no evidence of a treatment-related effect on variations of the head. There was no effect of treatment on soft tissue malformations and variations. No skeletal malformations were noted in the study. This seems unusual, and the sponsor has not provided historical control data from the contract laboratory for interpretation. In addition, there were no clear effects of treatment on skeletal variations.

In conclusion, the NOEL for maternal toxicity is 5 mg/kg/day (approximately 0.48 times the MRHD on a mg/m^2 basis), based on the occurrence of treatment related clinical signs at the MD and HD. The NOEL for embryo-fetal viability is a 15 mg/kg/day (approximately 1.5 times the MRHD on a mg/m^2 basis), based on an increase in post implantation loss and a slight increase in early resorptions in the HD group. The NOEL for fetal development is 30 mg/kg/day (the highest dose tested) (approximately 3 times the MRHD on a mg/m^2 basis).

Prenatal and post natal development: According to the sponsor, the choice of the high-dose level was based on the previously conducted 4- and 26-week toxicity studies in rat. It should be noted that 4- and 26-week toxicity studies in rat utilized the same total daily doses; however, administered as divided doses (BID); whereas this segment III study utilized QD dosing. Premated female — CD®(SD)IGS BR rats were treated once daily, via oral gavage, from gestation day (GD) 6 – lactation day (LD) 20 with TBZ doses of 0, 5, 15 or 30 mg/kg. TK assessments were not conducted.

There were no unscheduled deaths; however, a control female that had not delivered a litter by GD30 was sacrificed (as per protocol) and found not to be pregnant. Treatment related clinical observations were noted in the MD and HD groups and consisted of hypoactivity, closed eyes and squinted eyes throughout the gestation and lactation periods. The incidences of these clinical observations increased to some extent with increasing dose. During the lactation period, MD and HD dams were subjectively rated as not tending to their litters. There was a treatment-related slight decrease in group mean maternal body weight during gestation in the MD and HD groups (in the HD group the maximum decrease was 6%) and during lactation (in the HD group the maximum decrease was 11% on LD4, returning to a 7% decrease by LD14). The decreases in mean body weight noted during lactation may be a carry over from the gestation period since the mean body weight changes noted during the lactation period did not demonstrate treatment-related decreases. There were treatment related decreases in food consumption during the gestation period and lactation period (through LD14 the last data available) in the MD and HD.

One control F_0 female was not pregnant. All pregnant F_0 dams delivered litters with some live fetuses. Although not acknowledged by the sponsor, there was a slight increase in the duration of the gestation period in the MD and HD groups. The mean number of pups delivered was similar across groups; however, the number of stillborn pups (as well as the number of affected litters) was increased at the MD and HD. There is a notable discrepancy between the summary table and the individual animal line listings with regard to stillborn pups. The individual line listings for each dam indicated that there were no stillborn pups in any litter; however, the summary table lists 1, 1, 15 and 32 for the C, LD, MD and HD groups, respectively. The sponsor assessed the pup deaths on postpartum day 0 (LD0) as soon as possible after birth, and determined whether the dead pup met one of two criteria for designation as a stillborn pup, i.e., (1) the pup has no milk in its stomach (indicating that the pup has never nursed), or (2)

the lungs do not float (indicating that the pup has never breathed). There was also a treatment-related increase in the number of dead, missing, or cannibalized pups in the MD and HD groups between birth and LD4. There were entire litter losses in 1-MD dam and in 3-HD dams. Thus, the live-birth index and the viability index were decreased in the MD and HD groups. There was no effect of treatment on weaning index.

It is not clear that lack of milk in a pup's stomach should be used as a criterion establishing a stillbirth. The live born pups could be affected by their in-utero exposure to TBZ or its metabolites (a new born pup may not be able to metabolize and/or excrete the TBZ and/or its metabolites) resulting in their inability to nurse and, thus their death. Since the pup status was not assessed immediately after birth, the definitive designation of some of the perinatal deaths as stillbirths can be problematic. The sponsor should address the discrepancies in the reporting of stillborn pups. Since the drug is sedating, it may be worth asking the sponsor to provide a reanalysis of the data for the stillborn pups to indicate those in which the only criteria for the designation as stillborn was a lack of milk in the stomach at necropsy.

The sponsor discussed some possible causes of treatment-related increase in stillbirths and perinatal deaths including: (1) a difficult or prolonged delivery resulting in pup suffocation (the sponsor noted that there was no evidence of this in this study), (2) lack of maternal care at birth (e.g., pups not clean quickly or adequately resulting in suffocation), or (3) pup defects that are incompatible with viability (the sponsor noted that there was no data to support this based on the result of this study or the segment II study in rats). There were treatment-related increases in observations of pups noted as cold, weak or thin in the MD groups, and pups noted as cold, weak, thin or pale or lacking milk in the stomach in HD group.

F_0 maternal pup retrieval was assessed on LD3 (approximately 1 hr post dose) by distributing the pups around the cage, with the dam in the center. The number of pups not retrieved by the dam was recorded at 10, 30 and 60 minutes post distribution. The purpose of this assessment was to help determine whether pup death was secondary to the lack of maternal care (particularly, lack of maternal or litter group body heat). There was decrease in pup retrieval in the LD, MD and HD treated groups. The sponsor concluded that lack of maternal care may have contributed to an increase in stillborn pups and deaths in the early post natal period. It is reasonable to postulate that that the increase in perinatal pup deaths noted in the MD and HD groups might be due, in part, to maternal neglect (due to the treatment-induced hypoactivity in dams); however, with the available data it is not possible to quantify its contribution to the perinatal pup deaths. The possibility of a treatment-related effect on the pups cannot be dismissed.

There were no treatment related effects on the F_0 dams at necropsy.

Pup weights were slightly decreased in the MD and HD group at all time points during lactation. Necropsies were conducted on F_1 pups that were culled from the litters, stillborn pups or pups that were found dead. There were disparities in number of pups that were supposed to be examined in the LD, MD and HD groups and the number that were actually examined that was not mentioned by the sponsor. Conclusions drawn from this data are somewhat limited by the increased incidence of autolysis (abdominal region, or entire fetus) in the HD group (2/129-control, 0/146-low dose, 5/147-mid dose and 28/173-HD). The only possible treatment related finding noted was an increased incidence of pups with no milk in the stomach in the MD and HD groups.

F_1 pups were weaned on LD21, and 1 pup/sex/litter (supplemented randomly from appropriate groups, if needed, to achieve 20 rats/sex/group) were randomly selected for the maturation phase (7 weeks duration) and subsequent reproductive assessments. The physical development and behavior of the F_1 pups was assessed according to the contract laboratories SOPs. Examination of the individual pup data suggests (1) a slight treatment-related delay in pinna unfolding in the HD group (not noted by the sponsor), (2) a dose related delay in hair growth in the LD (slight), MD and HD (the sponsor only acknowledges the effect in the HD, and attributes it to decreased pup weight), (3) a treatment-related slight delay in eye opening in

the HD pups (not noted by the sponsor), (4) a treatment-related slight delay in vaginal opening in the LD, MD and HD group (not acknowledged by the sponsor), and (5) a treatment-related delay in preputial separation in the MD and the HD (acknowledged by the sponsor only in the HD, and attributed to decreased pup weight).

There were no effects of treatment on (1) incisor eruption, (2) auditory startle*, (3) pupil reflex*, (4) surface righting reflex, (5) open field testing , and (6) water M maze testing *. The asterisk refers to procedural problems. The auditory startle test was conducted only on a single day when all offspring had a positive response; therefore, the choice of date may have been inappropriate to demonstrate an effect of treatment. All animals had a positive response on the first day of testing for pupil response; therefore, the choice of dates may have been inappropriate to demonstrate an effect of treatment. The sponsor did not provide a description of the protocol for the water M-maze test and, therefore, the appropriateness of the study cannot be assessed and interpretation of the data is limited. Furthermore, there were some discrepancies in the cumulative pup incidence for the following developmental signs that should be resolved: (1) for vaginal opening in the control and mid dose group, (2) for preputial separation in the control and mid dose group, (3) surface righting reflex in the control and low dose groups. While these discrepancies do not affect the interpretation of the study results, they still should be resolved.

There was one unscheduled death in the F_1 generation post weaning. According to the sponsor, MD F_1 female (#B73526) was found dead on Day 89 and that prior to death this animal had no remarkable clinical observations. The cause of death is unknown and the only necropsy findings were a pale (light red) spleen and the entire glandular mucosa of the stomach appeared dark brown. Similar findings were not seen in other animals. The sponsor refers to Day 89 as part of the post-gestation resting phase. This is inconsistent with the study protocol and needs to be addressed by the sponsor. There were other discrepancies in the report for this animal that will be discussed in the section on the reproductive potential of the F_1 animals.

In general, the clinical observations during maturation and resting phases were mostly unremarkable and isolated, with the exception of rough haircoat in 3-HDM on days 119 and/or 120. Clinical observations during the gestation period for F_1 females were unremarkable. Weekly mean body weights were slightly decreased (although not significantly) in a dose-related way in males from the MD and HD groups from maturation day 0 (corresponds to approximately post partum day 28) through the end of the study (maturation day 120). The decreases in the HD varied from 3% to 9%. The mean body weight change duration the 120 day post-weaning maturation and resting phases was less than 3% for the HDM. For F_1 females, the weekly group mean body weights were slightly decreased in MD and HD groups from maturation day 0 through maturation day 28 (2-9% decreases in the HD). Group mean weekly body weights in females from day 35 through day 56 were not notably different from control. The mean body weights for pregnant females were not notably different from control throughout gestation or on lactation day 0 (last lactation date reported).

The breeding period for the F_1 animals began after a 7-week postweaning maturation period. The reproductive effects on the F_1 generation were not adequately described by the sponsor. There were discrepancies in the summary table and individual line listings that should be resolved. Corpora lutea counts and an evaluation of preimplantation loss do not appear to have been submitted for the F_1 females. The only conclusion that can be drawn, prior to the resolution of the discrepancies, is that pregnancy rate did not appear to be affected by treatment (96% for the control and 100% for the LD, MD and HD). According to the sponsor, there were no effects of treatment of reproductive parameters in the F_1 generation. The discrepancies in the reporting of the data preclude any further definitive conclusions.

- According to the report MD F_1 female #B73526 was found dead on day 89 during the rest phase (the period that follows LD1). The individual animal mating listing for this animal (pg 426) was as an unconfirmed pregnancy. According to the protocol, the earliest day of mating would have

been 74 (after the end of the 7-week maturation period that began on post partum day 28 ± 3). This would indicate that the animal should be no further along in pregnancy than GD12 when it died. The delivery results from this dam, suggest a full term pregnancy (17 pups delivered [16-live, 1-stillbirth] and all 16 pups still alive on post partum day 1, with pup weights ranging from 5.5 – 7.0 gr), and these data are incorporated into the group mean. Body weights for this animal during its gestation period are missing from the individual animal data and it would appear to be missing from the group mean summary table. In addition, the length of the gestation period for this animal is not provided. The sponsor needs to clarify the circumstances of this animal's pregnancy (e.g., age at mating, age at parturition, duration of gestation, date of death relative to date of parturition).

- LD F_1 female #B73509 was noted as pregnant in the individual animal mating listing (pg 425); however, data from this animal was eliminated from the individual litter and delivery table (pg 429) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 25g from gestation days 0 through 20). According to the protocol, any F_1 female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites. The individual parental necropsy observation table (pg 475) notes "No remarkable observations" at necropsy; however, no further information is provided about its reproductive status. All relevant data (including date of necropsy relative to date of mating and assessment of reproductive status) should be provided for this animal.

- HD F_1 female #B73557 was noted as pregnant on in the individual animal mating listings (pg 427); however, data from this animal was eliminated from the individual litter and delivery table (pg 431) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 27g from gestation days 0 through 20). According to the protocol, any F_1 female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites. The individual parental necropsy observation table (pg 479) notes "No remarkable observations" at necropsy; however, no further information is provided about its reproductive status. All relevant data (including date of necropsy relative to date of mating, and assessment of reproductive status) should be provided for this animal.

Gross necropsy of the F_1 males and females (post breeding, gestation and resting phases) did not reveal any treatment related effects. The one MD F_1 female that was found dead on D89 was noted with a pale (light red) spleen and dark brown coloration of the entire glandular mucosa of the stomach.

F_2 pups were observed only on LD0-1. There was not effect of treatment on F_2 covariate adjusted mean body weights for males or females. F_2 pup observations were noted only as part of the clinical observations of the dams and lacked detail (findings are listed by litter, with no indication of the number of pups affected) and are of limited use. The following observations are noteworthy, (1) one MD litter had at least one pup missing the proximal tail, (2) one HD litter that had at least one pup with a filamentous tail, (3) one LD litter was noted with at least one pup cold to touch on day 0 and two HD litters were noted with at least a single pup cold to touch. The sponsor noted not effect of treatment.

F_2 pups were killed on LD1. The pups were preserved in 10% neutral-buffered formalin, but necropsies were confined to stillborn pups and dead (originally liveborn) pups. There was no evidence of a treatment-related effect; however, the sample size is too small to make definitive statements. In the MD group one stillborn pup was not evaluated (the reason was not mentioned) and 2 could not be definitively evaluated due to the degree of autolysis of the entire fetus.

In conclusion, this study report has several discrepancies in the data that should be resolved before definitive conclusions can be made about the acceptability of the study, and the effects of treatment of the

F_0 generation on the subsequent (F_1) generation and the resulting offspring of that generation (F_2). A delineation of the requests for further information is provided in the overall conclusions and recommendations.

Based on examination of the data, the following conclusions can be made:

1. This study report has several discrepancies in the data that should be resolved before definitive conclusions can be made about the acceptability of the study, and the effects of treatment of the F_0 generation on the subsequent (F_1) generation and the resulting offspring of that generation (F_2).

2. Without further information, it can be stated that a NOEL for findings in the F_0 dams may not have been achieved, based on an effect on pup retrieval data at the lowest dose tested (despite lack of clinical observation in the dams). It should be noted that it is not possible to ascribe the effect to dam or pup, since it is very difficult to distinguish between dam and pup effects.

3. It appears that 5 mg/kg/day (approximately 0.48 times the MRHD on a mg/m^2 basis) was a NOEL for F_1 perinatal pup survival (The sponsor attributes the treatment related increase in still births and early pup deaths primarily to "a pharmacological effect on the dam affecting maternal behavior rather than a specific effect on the pups." While the pharmacological effects of TBZ on the dams may be contributory to the increase in perinatal pup deaths, a direct effect of treatment on the pup cannot be excluded.

4. There were treatment-related delays in the pinna unfolding (HD), hair growth (all doses), eye opening (HD), vaginal opening (all doses) and preputial separation (MD and HD). Therefore, a NOEL for development was not established.

5. Conclusions about an effect of treatment (of the F_0 dams) on the reproductive function in the F_1 generation cannot be made until the sponsor resolves the discrepancies in the data and provides, if available, corpora lutea counts and an evaluation of preimplantation loss for the F_1 females.

Summary for Genetic Toxicology:

Tetrabenazine (TBZ): TBZ was negative in the Ames test (in the presence and absence of metabolic activation). TBZ was positive in the *in vitro* chromosomal aberrations test in CHO in two independent tests (in the presence of metabolic activation) and it caused an increase in aneuploidy and/or endoreduplication. In the *in vivo* micronucleus assay in rat, TBZ was negative for males and produced equivocal results in females.

After the filing of the NDA, the sponsor submitted an *in vivo* micronucleus assay conducted in male mice. The results of this assay were negative; however, the assay should also have been conducted in females to serve as a valid *in vivo* assessment for chromosomal damage. According to the OECD guidelines for this assay, "If at the time of the study there are data available from studies in the same species and using the same route of exposure that demonstrate that there are no substantial differences between sexes in toxicity, then testing in a single sex will be sufficient." At the time of the conduct of the assay, female mice appeared to be the more than males to the CNS related toxicity, and the sponsor had not established that the *in vivo* metabolic profile was similar for male and female mice. Based on the limited toxicokinetic (TK) data available from the 90 day oral gavage toxicity study conducted in the same strain of mice, plasma exposures (based on AUC) to two of the major human metabolites of TBZ, α-HTBZ and β-HTBZ, were much greater in females than in males at 60 mg/kg/day, the highest dose evaluated. (In this study, plasma tetrabenazine could not be determined due to technical difficulties).

The sponsor also conducted genetic toxicology assays on the steroisomeric metabolites of TBZ, α-dihydrotetrabenezine (α-HTBZ) and β-dihydrotetrabenezine (β-HTBZ). α-HTBZ and β-HTBZ are two of the major circulating metabolites of TBZ detected in humans after oral administration of TBZ.

α-Dihydrotetrabenezine: α-HTBZ was negative in the Ames test (in the presence and absence of metabolic activation). α-HTBZ was positive in the *in vitro* chromosomal aberrations test in CHL in the presence of metabolic activation in two independent assays, and in the absence of metabolic activation in the only assay conducted. An *in vivo* assessment for chromosomal damage using a rodent hematopoietic cell was not conducted with α-HTBZ.

β-Dihydrotetrabenezine: β-HTBZ was negative in the Ames test (in the presence and absence of metabolic activation). β-HTBZ was positive in the *in vitro* chromosomal aberrations test in CHL, in two independent tests, in the presence and absence of metabolic activation. An *in vivo* assessment for chromosomal damage using a rodent hematopoietic cell was not conducted with β-HTBZ.

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

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

2.6.6.10 Tables and Figures

n/a

2.6.7 TOXICOLOGY TABULATED SUMMARY

n/a

OVERALL CONCLUSIONS AND RECOMMENDATIONS

From a pharmacology/toxicology standpoint, the current package does not support approval for the following reasons:

1. We cannot ensure that the pivotal nonclinical studies adequately characterize the toxicity of the major drug-related circulating products in humans after oral administration of tetrabenazine (TBZ). According to Dr. Yasuda's OCBP review, 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. Dr. Yasuda concluded that the most abundant circulating component in humans in the mass balance study, P16, should be resolved and the extent to which the other individual metabolites (including the mono-and bis-dealkyltetrabenazine metabolites) should be clarified. The nonclinical development of TBZ was based on the belief that the two major circulating metabolites in humans after oral administration of TBZ were α-dihydrotetrabenazine (α-HTBZ) and β-dihyrotetrabenazine (β-HTBZ). Therefore, the TK assessments in the pivotal nonclinical toxicology studies were based on monitoring plasma levels of TBZ and the stereoisomeric metabolites α-HTBZ and β-HTBZ (measured in chiral assays), or dihydrotetrabenazine (HTBZ) (measured in a non-chiral assay in the earlier studies).

   The sponsor has 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 new *in vivo* mass balance study in humans.

   The sponsor must provide data that demonstrate that the major drug-related circulating products in humans have been adequately tested in the pivotal nonclinical studies including the pivotal test species/strains, and, when relevant, the metabolic activation systems used in the pivotal *in vitro* studies (e.g., *in vitro* genetic toxicity studies).

2. The report of the 26-week toxicity study in rat with a 13-week interim kill (⟶ Report # 20730) is inadequate to fully describe the results of the study. The original study report did not include a delineation of unscheduled deaths and moribund sacrifices, and in response to our request for this information, the sponsor supplied a delineation that was supposed to have included a discussion of the relevant information that preceded the death or unscheduled sacrifice for each animal. One of the high animals was sacrificed in moribund condition during week 23 of the study, and the sponsor listed the cause of moribund condition as chronic dermatitis (the dermatitis was listed as moderate in severity and appeared to be confined to the muzzle). In the description of this animal's condition there was no mention of convulsions; however, the line listings for this animal noted convulsions on days 133, 142 and 154 (and the animal was sacrificed during week 23). 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. Examination of the individual animal data suggested that the clinical observation data sets for each animal may not be complete. A request for further information regarding the conduct and reporting of the clinical observations was sent to the sponsor on 24-Jan-06, and to date, a response has not been submitted. Until the requested information has been submitted and reviewed, there is no assurance that the sponsor has submitted a full an accurate data set for each animal.

In addition, the study report did not contain a separate pathology report, and the integrated summary report does not contain the signature of the study pathologist. Therefore, there is no assurance that the discussion of the pathology findings accurately reflects the views of the pathologist.

In the discussion of the pathology findings there was no mention that 24 of 60 control animals and 17 of 60 high dose animals were infected with pinworm parasites. Only five animals (of 40) were noted as infected at the 13-week interim sacrifice; suggesting that the infestation occurred primarily between weeks 13 and 26. Since the intestines from low and mid dose animals were not microscopically examined, the number infected in those groups is unknown. Toxicology studies are supposed to be conducted in normal healthy animals, and clearly this was not the case for this study. The occurrence of the parasite infestation and implications (if any) on the validity of the study were not discussed, and the sponsor should provide this.

With regard to the conduct of the microscopic examination, the sponsor did not conduct histopathology on gross lesions unless they occurred in the control or high dose, or in low and mid dose animals that were unscheduled sacrifices. It is standard practice to examine gross lesions in all animals, and the rationale for this was not discussed. In general, the sponsor conducted microscopic examination on control and high dose animals as well as unscheduled deaths and using this paradigm the sponsor did not establish a no-effect dose for treatment-related pathology of the lung, i.e., minimal to mild multifocal accumulations of alveolar macrophages. The sponsor should have conducted a microscopic examination of the lower doses to establish a no effect level. The sponsor conducted microscopic examination of the vagina and mammary gland for the terminal sacrifice control, mid dose and high dose animals (plus unscheduled deaths from all groups). A no-effect dose was not established for the treatment-related physiological mammary hyperplasia.

With regard to the pathology assessment, the sponsor should provide (1) a copy of the pathologist's report, (2) a discussion of the parasite infection and implications (if any) on the validity of the study, and (3) establish no-effect dose levels for multifocal accumulations of alveolar macrophages and physiological mammary hyperplasia.

The sponsor attributes the treatment-related increase in physiological mammary hyperplasia to a treatment-related increase in serum prolactin or a change in the pattern of prolactin release. The sponsor has not provided data to support this contention. Although serum prolactin was not assessed in the 4-week or 13/26 week toxicity studies, it was supposed to have been evaluated in —— : Study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats). The prolactin data from this study do not appear to have been submitted to the NDA, and should be submitted.

There appears to be a notable lack of vigilance in the conduct and reporting of this study that should be further investigated.

3. In the study report for the chronic toxicity study in rat, a reference was cited describing TBZ-induced neurotoxicity and neuropathology in rat with repeat dosing (Satou *et al.* 2001. Repetitive administration of tetrabenazine induces irreversible changes in locomotion and morphology of the substantia nigra in rats, Exp Toxic Pathol 53: 303-308). (A copy of this reference was not included in the four volumes of literature references submitted to the pharmacology/toxicology section of this NDA). Literature searches on this topic did not identify any additional papers addressing this issue. In this study male Wistar rats were administered TBZ by intraperitoneal injection (1 mg/kg) either as a single injection or as daily injections for seven consecutive days. The results of the multiple dose portion of the study demonstrated (1) a statistically significant

treatment-related neuronal cell loss in the substantia nigra/pars compacta (SNpc), (2) a decrease in SNpc neuron area, and (3) a decrease in cell size. These findings progressed with increasing survival time (estimated [based on graphs] to be up to approximately 50% neuronal cell loss and approximately 30% decrease in area) at 15 days post dose (the last time point studied). An increase in staining for GFAP indicating glial proliferation was also noted in the SNpc. In addition these animals demonstrated a treatment-related decrease in locomotion that was not completely reversible, even after a 15 day recovery period.

Based on the findings of Satou *et al.* (2001), the CNS histopathology in the chronic toxicity studies in rat and dog was expanded to include an examination of the pons in rats and the substantia nigra in dog. The extent of the histopathological examination of the brain was not specified, nor the inclusion of any special techniques (if any) in the examination of the brain. Without further information, it is not possible to preclude treatment-induced neuropathology. It should be noted that the sponsor states that TBZ "has been reported to cause parkinsonism" due to depletion of striatal dopamine. The sponsor also states that in clinical study TBZ 103,004, three (of 54) patients were noted with parkinsonism as a dose-limiting adverse event. The sponsor further states, "In these patients, dose adjustment resulted in maintained efficacy with complete reversal of the AE in 2 patients, and a partial resolution in one patient." (section 2.5.6 of the NDA (module 2, volume1, page 19).

The sponsor should provide a detailed description of the histopathologic examination of the brains from the chronic toxicity studies in rat and dog, with an emphasis on the techniques used, and the extent of the examination, especially with regard to the substantia nigra.

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

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

Phase 4 commitments:

1. The sponsor has not submitted carcinogenicity assessments as part of the NDA, and "...commits to conduct and report the findings of two rodent carcinogenicity studies upon NDA approval." According to the sponsor, the symptoms of Huntington's disease generally manifest between the ages of 35 and 40 years and death usually occurs after approximately 15 years from the onset of symptoms. The sponsor is seeking approval fo[redacted] chorea associated with

Huntington's disease, and not for an alteration of the course of the disease. If TBZ is shown to have a clinically meaningful effect on the lives of Huntington's disease patients, there would be a rationale for deferring carcinogenicity assessment until phase 4; however, the sponsor should commit to starting the carcinogenicity studies at or near the time of approval (i.e., the protocols should be completed and the contract laboratories reserved), with an agreement in place for starting dates for the studies at the time of approval.

2. The sponsor must commit to conducting nonclinical assessments of fertility and early embryonic development with agreements in place for a starting date(s) at the time of approval.

3. The adequacy of the segment III reproductive toxicity study in rat (Oral Developmental Toxicity Study and Pre- and Postnatal Development Study with Tetrabenazine, in the Rat (Segment III Study), ——— Study # 7425-106) cannot be determined until the discrepancies delineated below are resolved and the requested information is submitted for review. It should be noted that the study is not a negative study (producing treatment-related perinatal pup death and developmental delays in the pups); however, the requested information may provide more accurate labeling. In addition, there appears to be a notable lack of vigilance in the conduct and reporting of this study that should be further investigated.

   a. The summary table of the natural delivery data and litter data (pg 39) lists 1, 1, 15 and 32 stillborn pups in the C, LD, MD and HD groups, respectively. The individual line listings for each dam (pg 204 - 207) indicate that there were no stillborn pups in any litter, in any group. The sponsor should address this discrepancy. In addition, the sponsor should provide a delineation of which pups were determined to be stillborn based solely on a lack of milk in the stomach at necropsy.

   b. According to the report MD F_1 female #B73526 was found dead on day 89 during the rest phase (the period that follows LD1). The individual animal mating listing for this animal (pg 426) was an unconfirmed pregnancy. According to the protocol, the earliest day of mating would have been 74 (after the end of the 7-week maturation period that began on post partum day 28 ± 3). This would indicate that the animal should be no further along in pregnancy than GD12 when it died. The delivery results from this dam, suggest a full term pregnancy (17 pups delivered [16-live, 1-stillbirth] and all 16 pups still alive on post partum day 1, with pup weights ranging from 5.5 – 7.0 gr), and these data are incorporated into the group mean. Body weights for this animal during its gestation period are missing from the individual animal data and it would appear to be missing from the group mean summary table. In addition, the length of the gestation period for this animal is not provided. The sponsor needs to clarify the circumstances of this animal's pregnancy (e.g., age at mating, age at parturition, duration of gestation, date of death relative to date of parturition).

   c. Low dose F_1 female #B73509 was noted as pregnant in the individual animal mating listing (pg 425); however, data from this animal was eliminated from the individual litter and delivery table (pg 429) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 25g from gestation days 0 through 20). According to the protocol, any F_1 female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites. The individual parental necropsy observation table (pg 475) notes "No remarkable observations" at necropsy; however, no further information is provided about its reproductive status. All relevant data (including date of necropsy relative to date of mating and assessment of reproductive status) should be provided for this animal.

d. High dose F_1 female #B73557 was noted as pregnant on in the individual animal mating listings (pg 427); however, data from this animal was eliminated from the individual litter and delivery table (pg 431) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 27g from gestation days 0 through 20). According to the protocol, any F_1 female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites. The individual parental necropsy observation table (pg 479) notes "No remarkable observations" at necropsy; however, no further information is provided about its reproductive status. All relevant data (including date of necropsy relative to date of mating, and assessment of reproductive status) should be provided for this animal.

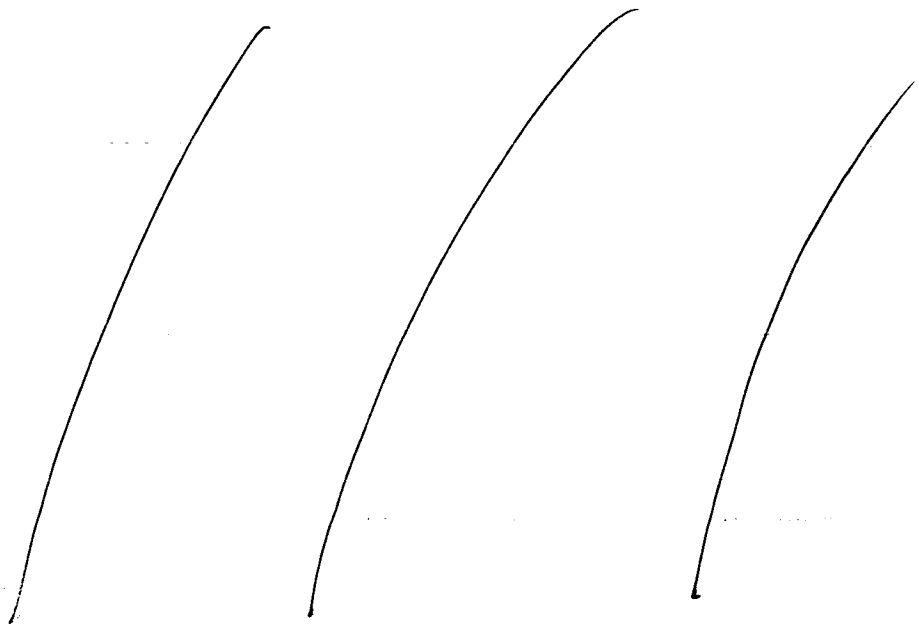
e. Corpora lutea counts and an evaluation of preimplantation loss do not appear to have been submitted for the F_1 females. If these data are available, they should be submitted.

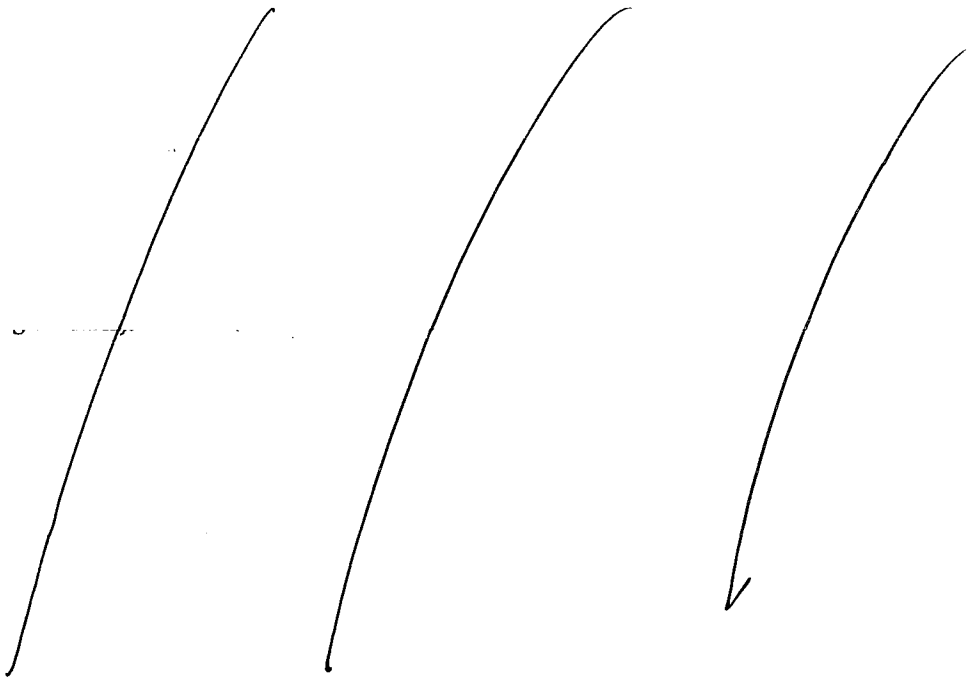
Conclusions: (1) From a pharmacology/toxicology standpoint, the current package does not support approval (the reasons are discussed above), (2) there were notable lacks of vigilance in the conduct and reporting of the 26-week toxicity study in rat with a 13-week interim kill (—— Report # 20730) and the pre- and postnatal development study in rat (—— udy # 7425-106) that should be further investigated.

Unresolved toxicology issues (if any): all issues have been discussed with reference to approvability issues and phase 4 commitments.

Recommendations: From a pharmacology/toxicology standpoint, the current package does not support approval.

Suggested labeling:





Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPEARS THIS WAY
ON ORIGINAL

APPENDIX/ATTACHMENTS

n/a

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

/s/

Andrea Powell
3/30/2006 12:29:57 PM
PHARMACOLOGIST

Lois Freed
3/30/2006 12:57:29 PM
PHARMACOLOGIST
Please see separate memo for comments/recommendations.

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 27, 2006

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

Subject: NDA 21-894 (Xenazine, tetrabenazine)

Tetrabenazine (Xenazine) is intended for the treatment of the chorea associated with Huntington's disease. The nonclinical data on tetrabenazine submitted in the NDA were reviewed in detail by Andrea M. Powell, Ph.D. (Pharmacology/Toxicology Review and Evaluation, v. 3/24/06). Based on this review, Dr. Powell has concluded that the NDA is not approvable from a nonclinical standpoint.

Dr. Powell has identified the following deficiencies that need to be addressed prior to approval:

1. The lack of sufficient 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 (Clinical Pharmacology and Biopharmaceutics Review, Sally U. Yasuda, MS, PharmD, 3/6/06).

2. Numerous deficiencies in the conduct and report of the 26-week oral toxicity study in rat.

3. The lack of a report for the serum prolactin data from a 14-day oral gavage study to assess toxicokinetics and serum prolactin levels in rat.

4. The lack of sufficient information regarding the microscopic evaluation of brain in the 26-week and 9-month oral toxicity studies in rat and dog, respectively.

5. Inadequate resolution of the equivocal findings in the in vivo micronucleus assay in rat.

Comments

1. I concur with Dr. Powell's recommendation to require additional in vivo metabolism data prior to approval. As noted by Dr. Powell, the information provided by the sponsor indicates extensive metabolism of tetrabenazine in animals and human. However, there is limited understanding of the major circulating drug-related compound in either animals or humans. The results of a recent mass balance study in humans (single 25 mg dose) indicate that the most abundant circulating drug-related component (P16) has not yet been identified or characterized.

Without sufficient data on the major circulating metabolites in animals and humans, it is not possible to determine the relevance of the nonclinical studies to an assessment of human risk.

2. Dr. Powell has identified numerous deficiencies in the conduct and report of the 26-week toxicity study in rat. According to Dr. Powell, the following issues need to be addressed prior to approval:

> (a) the sponsor's 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; however, instances of "lethargy" were noted in the summary table, but not in any individual animal line listing.
>
> (b) the lack of a signed Pathologist's Report.
>
> (c) the sponsor's lack of a discussion of the impact (if any) of the observed pinworm infestation on study results.
>
> (d) the lack of an identified NOEL for alveolar macrophage accumulation in lung (increased in HDM and, to a lesser extent, HDF) and physiological hyperplasia of the mammary gland (increased in MDF and HDF). Lung was not microscopically examined at the LD or MD and mammary gland was not microscopically examined at the LD.

In my opinion, the sponsor needs to address the first two of these issues prior to approval. At this point, the need to establish NOELs for the lung and mammary gland effects is arguable. If established to be NOELs, the LD (lung, mammary gland) or MD (mammary gland) would provide no safety margins compared to the maximum recommended human dose (MRHD) of 100 mg/day (based on mg/m^2); however, it would seem very unlikely that the MRHD would be lowered based on these microscopic findings.

The presence of pinworm infestation and the sponsor's apparent lack of an attempt to deal with the infestation is a clear violation of Good Laboratory Practice (GLP) (21CFR 58.90(c)); however, it is unclear what information the sponsor could provide that would lessen the concern regarding the infestation. One could argue that the study needs to be

repeated in healthy animals. However, the lack of signs of adverse biological effects associated with notable pinworm infestation (e.g., gastrointestinal effects (rectal prolapse, fecal impaction, perianal irritation), poor appearance (rough coat), liver granulomas) would suggest that the study results should not be dismissed out-of-hand. Also, additional information on the toxicity of tetrabenazine in rat will be obtained in the 2-year carcinogenicity. Questions regarding the adequacy of the 26-week study would warrant requiring the sponsor to begin the 2-year carcinogenicity study as soon as possible.

The sponsor needs to address the apparent discrepancies in the reporting of clinical signs. There is no excuse for these types of inconsistencies in a final, QA'd study report.

According to Dr. Viswanathan (Division of Scientific Investigations), the lack of a signed Contributory Scientist's report (e.g., Pathologist's Report) is a violation of GLP (21CFR 58.185(a)(12)). The sponsor needs to provide a signed Pathologist's Report (and a signed report of any other Contributing Scientist) for the 26-week study and any other definitive nonclinical study for which such reports were not provided.

3. I concur with Dr. Powell's recommendation that serum prolactin data from the 14-day toxicokinetic and serum prolactin study in rat needs to be submitted. It is reasonable to expect the sponsor to provide all relevant nonclinical data to the NDA; it should have been provided in the original NDA submission. Serum prolactin data are important in interpreting the results of the nonclinical studies.

Done if Required

4. I concur with Dr. Powell's recommendation that the sponsor needs to provide additional detail regarding the conduct of the microscopic evaluation of brain in the 26-week and 9-month toxicity studies in rat and dog, respectively, prior to approval. As Dr. Powell notes, Satou et al. (Satou T et al. *Exp Toxic Pathol* 53:303-308, 2001) reported irreversible behavioral changes (up to 15 days following the last dose) and neurotoxicity ("...significant decreases in SNpc neuron number, area, and average size...") in Wistar rats following 7 daily i.p. doses of tetrabenazine. Death of neurons in the substantia nigra pars compacta (SNpc) is known to be the cause of Parkinson's disease and symptoms of Parkinson's disease have been observed in Huntington's disease patients treated with tetrabenazine. 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 VMAT2 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.

5. I concur with Dr. Powell's recommendation that the sponsor needs address the equivocal findings in the in vivo micronucleus assay in rat prior to approval. Tetrabenazine was negative in male rats and mice (in separate studies), but positive in one of two groups of female rat at the high dose of 100 mg/kg. In mice, plasma exposure

data indicate that circulating levels of metabolites, α- and β-dihydrotetrabenazine, are higher in females than males. The negative results in males do not necessarily lessen the concern regarding the positive finding in females.

Tetrabenazine, α-dihydrotetrabenazine, and β-dihydrotetrabenazine were positive in the in vitro chromosomal aberration assay in CHO cells. (Tetrabenazine was positive only in the presence of metabolic activation, whereas the metabolites were positive both in the absence and presence of metabolic activation.) Therefore, these three compounds are considered clastogenic. However, considering the relative insensitivity of the in vivo micronucleus assay, a positive in this assay is a notable finding.

It is unclear how best to address this concern. Since tetrabenazine is clastogenic in vitro only in the presence of metabolic activation (suggesting that one or more metabolites are responsible for the equivocal finding), one could argue that direct testing of α-dihydrotetrabenazine and β-dihydrotetrabenazine would be preferable. In that case, a repeat study or studies should include testing of both males and females. In addition, once P16 (the major circulating drug-related material in humans) is identified, direct testing of P16 in both in vitro and in vivo assays may be warranted.

Adequate genotoxicity testing of tetrabenazine and major (human) circulating metabolites is particularly important considering the possibility that tetrabenazine may be approved prior to completion of carcinogenicity studies. Unfortunately, exactly what comprises adequate testing is unclear due to the lack of in vivo metabolism data in humans.

Dr. Powell has recommended that the following nonclinical issues may be addressed post-approval (i.e., as Phase 4 commitments):

1. conduct of carcinogenicity studies, provided clinical efficacy data are sufficiently robust to warrant approval without these data.

2. conduct of a fertility and early embryonic development (to implantation) study.

3. numerous deficiencies in the conduct and report of the pre- and postnatal development (including maternal function) study in rat (study #7425-106).

Comments

1. Clearly, carcinogenicity studies are required for tetrabenazine considering the chronic nature of Huntington's disease. A waiver of this requirement to Phase 4 would, as noted by Dr. Powell, be based on the fact that there is no approved treatment for Huntington's disease. I agree with Dr. Powell that the results of carcinogenicity studies would not be needed prior to approval if the clinical efficacy data were determined to be sufficiently robust. However, considering the available genotoxicity data on tetrabenazine and the α- and β-dihydrotetrabenazine metabolites, I would recommend that the sponsor be required to initiate carcinogenicity studies as soon as possible (i.e., not wait until after approval). Dr. Powell notes that the sponsor's protocol for a 26-week p53 transgenic mouse assay

has been reviewed and discussed by the Executive CAC; minutes of the Executive CAC were sent to the sponsor on October 27, 2005. Therefore, the p53 assay may have already been initiated, and, if not, could be in the near future. The sponsor has recently submitted a protocol for a 2-year carcinogenicity study in rats that is currently under review. The sponsor should be asked to commit to a timeline for conduct of the studies and submission of final study reports for both the p53 transgenic assay in mice and a 2-year bioassay in rats.

2. I concur that a fertility and early embryonic development (to implantation) study may be conducted post-approval. The sponsor needs to commit to a timeline for conduct of the study and submission of the final study report.

3. Regarding the deficiencies/discrepancies in the pre- and postnatal development study in rat noted by Dr. Powell, the sponsor needs to address the following issues:

a. the lack of corpora lutea and preimplantation loss data in F1 females. The sponsor should submit those data if collected.

b. the number of stillbirths vs early postnatal deaths. The sponsor should 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, the sponsor should 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, LDF B73509, MDF B73526, and HDF B73557. The sponsor should provide all data (including pregnancy, litter, and final disposition) for these dams.

Although addressing these issues prior to approval is not necessary, the sponsor should be able to respond to them relatively quickly.

Language for the action letter (including labeling) follow:

<u>Information for the sponsor</u>

Prior to approval, you will need to address the following 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 metabolism data in humans. You need to provide additional data identifying and quantitating the major circulating metabolites in animals and humans. These data are needed in order to determine the relevance (and adequacy) of the nonclinical studies to an assessment of human risk. In

particular, there is concern that the potential toxicity of the major circulating drug-related material in humans (peak 16) may not have been adequately assessed in animals.

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 individual animal 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 conduct of the studies and submission of final reports of these studies. Final study reports would not be required prior to approval.

The following issues are to be addressed 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.

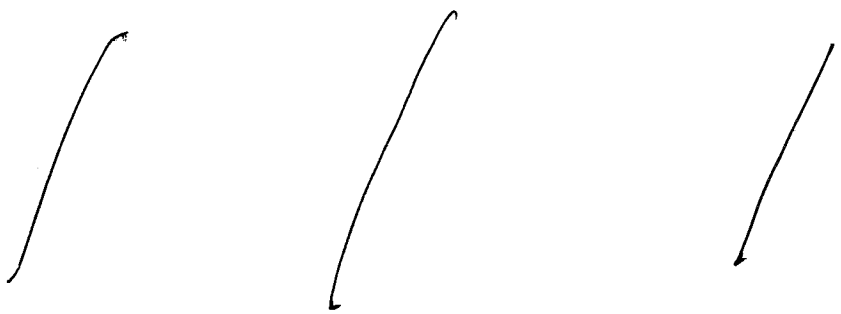
2. Conduct of a fertility and early embryonic development (to implantation) study. You should commit to a timeline for conduct of the study and submission of the final study report.

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.

Recommended labeling



2 Page(s) Withheld

✓ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

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

/s/

Lois Freed
3/27/2006 04:54:27 PM
PHARMACOLOGIST