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APPLICATION NUMBER:

022567Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 22-567

Agency receipt date: 3/22/2010

Drug: VIIBRYD (vilazodone hydrochloride)

Applicant: PGxHealth, LLC

Indication: treatment of major depressive disorder

Reviewing Division: Division of Psychiatry Products

Background:

The pharm/tox reviewer and team leader concluded that the nonclinical data support approval of vilazodone hydrochloride for the indication listed above.

Reproductive and Developmental Toxicity:

Reproductive and developmental toxicity studies in rats and rabbits revealed no evidence of teratogenicity although there was some evidence of reduced fetal body weight and delayed ossification in both species. These effects were not seen at doses up to 10 times the MRHD in rats or 4 times the MRHD in rabbits. The reviewer recommended pregnancy category C.

Carcinogenicity:

Vilazodone was tested in 2 year rat and mouse carcinogenicity studies. These studies were reviewed by the division and the executive carcinogenicity assessment committee. The committee concluded that the studies were acceptable. There were no drug-related neoplasms in rat. The Committee concluded that there was an increased incidence of mammary adenocarcinomas and adenoacanthomas (combined) in female mice at the high dose (135 mg/kg) and hepatocellular neoplasms (adenomas, carcinomas, and adenomas or carcinomas, combined), in male mice at the high dose (135 mg/kg). The division has also noted that the mammary adenocarcinomas in female mice appear to be increased at the mid dose of 45 mg/kg even though the incidence did not meet usual CDER standards for statistical significance for pairwise comparison. The reviews also note that vilazodone increased prolactin levels in mice.

Established Pharmacologic Class:

The pharm/tox reviewer, team leader and supervisor have carefully considered the data on the pharmacologic activity of vilazodone. Vilazodone appears to have both SSRI and 5-HT1A partial agonist activity. ^{(b) (4)}



Impurities and metabolites:

Several genotoxic or potentially genotoxic impurities were identified during review of the NDA. This issue is discussed in detail in the team leader memo.

Specifications for each of these impurities have been limited so humans will be exposed to not more than ^{(b) (4)} µg of each per day at the MRHD of 40 mg.

Two major human metabolites of vilazodone were identified. The nonclinical assessment of these is discussed in detail in the team leader memo. Both metabolites have been adequately assessed for all toxicity endpoints except it is not clear that one metabolite (M17) was adequately assessed for embryofetal toxicity because its presence was not confirmed in rats or rabbits. The division is recommending that M17 be assessed in an embryofetal study or that data be provided demonstrating that M17 was adequately assessed in one of the already conducted embryofetal studies. The division is recommending this as a post marketing requirement.

Pediatric assessment:

The division is recommending that the efficacy and safety of vilazodone be assessed in pediatric patients with major depressive disorder as part of the deferred pediatric requirements. Prior to dosing children less than 13 years of age, the division is recommending that the applicant complete a juvenile animal study in rats and include evaluation of neurological/behavioral development and reproductive development in this study.

Conclusions:

I agree with the division pharm/tox conclusion that this application can be approved from a pharm/tox perspective. The proposed post marketing requirements are acceptable.

I have discussed the proposed carcinogenicity labeling with the team leader. The proposed wording states that mammary adenocarcinomas in female mice were numerically increased at the mid dose in addition to describing the statistically significant increase in tumors. This is a true statement and is acceptable. Proposed labeling includes wording noting the potential role of elevated prolactin in these tumor findings.

Other labeling as proposed by the reviewer and team leader is acceptable.

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/s/

PAUL C BROWN
01/14/2011

SUPERVISORY PHARMACOLOGY/TOXICOLOGY MEMO TO THE FILE

NDA 22-567.

Submissions: N-000, original submission, letter-dated 3/22/2010, received 3/22/2010.

Drug: vilazodone hydrochloride, as 10-, 20-, and 40-mg oral tablets [VIIBRYD™].

Sponsor: PGxHealth, LLC (original sponsor).

Indication: treatment of major depressive disorder.

Reviewer: Linda H. Fossom, Ph.D., Pharmacologist, Team Leader.

Division of Psychiatry Products, HFD-130.

I. BACKGROUND:

Vilazodone is a new molecular entity submitted under the current NDA for treatment of major depressive disorder. The pharmacology/toxicology studies have been reviewed in detail by Violetta Klimek, Ph.D., Pharmacologist (review finalized 12/22/2010). In her review, Dr. Klimek has thoroughly and critically evaluated the non-clinical information provided in support of this NDA. In general, these studies provided adequate assessment of pharmacology, general toxicity (including chronic studies in rats and dogs), genotoxicity, carcinogenicity (2-year studies in rats and mice), and reproductive toxicity. I agree with Dr. Klimek that vilazodone did not demonstrate any particular concerns based on these studies; the findings for genotoxicity, reproductive toxicity, and carcinogenicity will be included in labeling.

However, late in the review cycle, it became apparent that 2 major human metabolites, one of which had not been identified as circulating in human plasma at the time of NDA submission, might not have been adequately assessed for toxicity in animals. We contacted the Sponsor to obtain their explanation of this issue (on 12/14/10 and again on 12/20/10); however, their full response was not available at the time Dr. Klimek's review was finalized. That information is now available and is discussed below.

This memo contains additional comments on 4 issues:

- (b) (4)
- The carcinogenicity findings presented in labeling;
- The control of genotoxic (and potentially genotoxic) impurities;
- Toxicological assessment of the 2 major circulating human metabolites.

[Of these issues, only the toxicological assessment of one of the major human metabolites (M17) needs further study by the Sponsor and this will be required as a post-marketing commitment.]

II. LABELING RELATED TO ESTABLISHED PHARMACOLOGIC CLASS:

(b) (4)



(b) (4)



[It should be noted that the Warnings and Precautions Section of labeling already includes serotonin syndrome or neuroleptic malignant-like syndrome for vilazodone.]

(b) (4)



However, the Mechanism of Action section will include 5-HT_{1A} partial agonist activity, as well as SSRI activity, but will indicate that the role of 5-HT_{1A} partial agonism in the antidepressant effect is not as well accepted (or demonstrated) as the role of SSRI activity, as recommended by Dr. Rosloff.

III. THE CARCINOGENICITY AND EMBRYO-FETAL TOXICITY FINDINGS PRESENTED IN LABELING:

Regarding carcinogenicity findings, we have included in labeling only the findings that the Executive Carcinogenicity Assessment Committee (E-CAC) found statistically significant (based on the statistical analysis provided in the Statistical Review and Evaluation, by Mohammad Nagem, Ph.D., 11/9/2010) and biologically relevant, with one exception. For mammary tumor findings in female mice, the E-CAC found only the incidence of adenocarcinomas and adenoacanthomas combined (but not either tumor type alone) to be increased and only at the high dose of 135 mg/kg, based on statistical significance in both the trend test and pair-wise comparison with the vehicle control group. [Note that the Sponsor had identified significantly increased incidence of adenocarcinomas at both the mid dose (of 45 mg/kg) and the high dose.] The statistical analysis of these findings is provided below:

Table 1. Statistical analysis of incidence of mammary tumors (adenocarcinomas and adenoacanthomas) in female mice (excerpted and reformatted from the table on page 22 of Dr. Nagem's statistical review, 11/09/2010). An asterisk (*) indicates statistical significance.

TUMORS	DOSE, mg/kg				P-VALUE		
	0 (C)	15 (LD)	45 (MD)	135 (HD)	C vs MD ¹	C vs HD ¹	Trend ²
adenocarcinomas	3/120	1/60	6/60	7/60	0.0482	0.0166	0.0050*
adenocarcinomas + adenoacanthomas	3/120	1/60	6/60	8/60*	0.0482	0.0069*	0.0017*

¹: $p \leq 0.01$, indicates statistical significance for pair-wise comparison (for common tumors).

²: $p \leq 0.005$, indicates statistical significance for dose-response by trend test.

Although only total malignant mammary tumors (adenocarcinomas plus adenoacanthomas) were statistically significantly increased and only at the high dose, the data seem to indicate that adenocarcinomas were increased at both mid and high doses. [Note that there was only a single mouse (at high dose) with an adenoacanthoma, compared with 6 and 7 mice with adenocarcinomas at the mid dose and high dose, respectively.] The incidences for adenocarcinomas alone or combined with adenoacanthomas show significant dose-responses (i.e., they are both positive by trend test) and the incidences at both mid and high doses are clearly higher than at the low dose and for controls, so the significant dose-response for adenocarcinomas depends on the findings at the mid dose, as well as the high dose. It would seem more reasonable to consider the increased incidence for total malignant mammary tumors (without specifying which ones contributed to the statistical significance). The incidence of malignant mammary tumors was increased in a dose-related manner (statistically significant trend tests for adenocarcinomas alone and when combined with adenoacanthomas) and this increase was evident at the mid dose (but not statistically significant by pair-wise comparison versus control) and at the high dose (statistically significant by pair-wise comparison versus control).

It should also be noted that the incidence of acinar hyperplasia was higher at the mid dose (11%) and high dose (14%), compared with the low dose (7%) and controls (7.6%) in this study; and the incidences of adenocarcinomas at the mid and high doses were above the historical range for mammary carcinomas based on the NTP studies in this strain of mice [see Dr. Klimek's review].

Additionally, prolactin was shown to be increased in mice at the high dose of 135 mg/kg (but was not assessed at the lower doses of 15 and 45 mg/kg) at week 13 in the carcinogenicity study; and at doses of 45 mg/kg (the mid dose used in the carcinogenicity study) and 270 mg/kg in a separate 2-week study. The possibility that the increase in mammary tumors in mice was due to this increase in prolactin, a mechanism that has unclear relevance of humans, is suggested in labeling. The fact that increased prolactin was demonstrated at 45 mg/kg adds biological plausibility to a true drug effect on tumors at this dose.

The findings for malignant mammary gland tumors in female mice are described in labeling (along with the findings for hepatocellular carcinomas in male mice) as:

In mice, the incidence of hepatocellular carcinomas was increased in males at 16.5 times the MRHD; this finding was not observed at 5.5 times the MRHD. The incidence of malignant mammary gland tumors was numerically increased in females at 5.5 and 16.5 times the MRHD, with statistical significance at 16.5 times the MRHD; this finding was not observed at 1.8 times the MRHD. Elevated prolactin levels were observed in a 2-week study of vilazodone administered at 5.5 and 33 times the MRHD. Increases in prolactin levels are known to cause mammary tumors in rodents.

(b) (4)



IV. GENOTOXIC IMPURITIES:

During the review of this NDA, 7 genotoxic or potentially genotoxic impurities were determined to share a common structural alert: they are all (b) (4). There was considerable internal discussion (by Pharmacology/Toxicology and Chemistry disciplines) about whether 1) the limit for each genotoxic impurity should be lowered so that the daily clinical exposure to each would be no more than (b) (4) µg (at the maximum recommended human dose of 40 mg) or 2) the limit for the total all 7 genotoxic impurities sharing this structural alert should be lowered so that the daily clinical exposure to all would be no more than (b) (4) µg. We communicated our concerns to the Sponsor (10/15/10), as excerpted below:

...
6. In addition to 6 potential genotoxic (b) (4) impurities (b) (4) (b) (4) (b) (4) to be genotoxic,
based on the positive in vitro findings for mutagenicity (Ames test) and clastogenicity (chromosomal aberration test in V79 Chinese hamster cells). Consequently, your proposed limit for (b) (4) of not more than (b) (4) as an unspecified impurity, is not acceptable. Therefore, update your proposed in-process and/or drug substance specifications to control this impurity at a level of not more than (b) (4) µg/day clinical exposure, as you have done for each of the other 6 impurities. You need to provide an updated analytical method and validation data for (b) (4) with an appropriate lower detection limit to meet the total daily exposure requirement, as well as updated batch results to show that the exposure to this impurity is not more than (b) (4) µg/day.
Additionally, we have some concern because these 7 impurities share a common structural alert for genotoxicity (they are all (b) (4)). Therefore, we recommend that you further evaluate whether these 7 impurities can be controlled at levels lower than the currently proposed levels to reduce the overall patient exposure to this class of potentially genotoxic impurities. We also recommend that you provide any information that is available on the levels of each of these 7 impurities in the batches of drug substance used for the carcinogenicity studies in rats and mice, as well as any other information that might be relevant regarding the carcinogenic potential of these impurities.
...

In response (11/04/2010), the Sponsor agreed to do the following (as summarized by this Reviewer):

- Regarding impurity (b) (4), they tightened the specification from not more than (NMT) (b) (4) ppm (i.e., NMT (b) (4), as an unspecified impurity) to NMT (b) (4) ppm (i.e., NMT (b) (4) µg at the maximum recommended human dose of 40 mg [which is considered close enough to the (b) (4) µg/day limit for human exposure to a genotoxic impurity, set according to current policy and Guidances]).

- Regarding the other 6 (b) (4) impurities, they lowered the specifications for (b) (4) and (b) (4) each from NMT (b) (4) ppm to NMT (b) (4) ppm; and will evaluate whether the 4 others (which are currently limited to NMT (b) (4) ppm) can be further lowered to reduce the overall patient exposure (to be submitted in their 1st annual report).

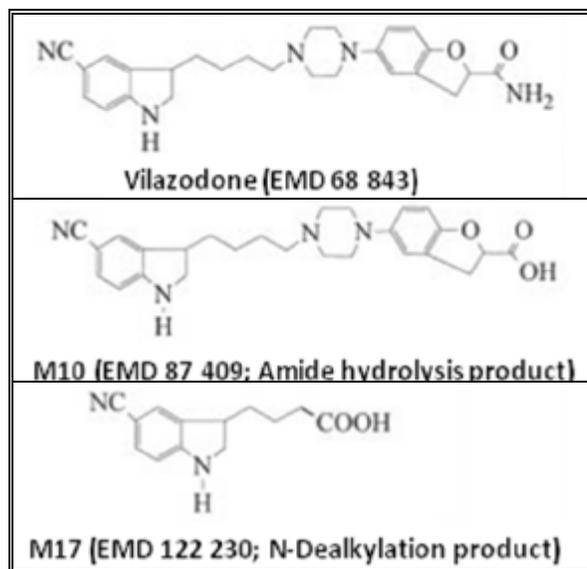
The Sponsor also noted that the batches used in the 2-year carcinogenicity studies in mice and rats were manufactured using Process 1 (not the process currently used for the clinical substance/product), which resulted in the formation of (b) (4) at levels (i.e., present in each batch at amounts between (b) (4) ppm [the LOQ] and (b) (4) ppm) that are higher than the currently-proposed limit of NMT (b) (4) ppm, but did not result in the formation of any of the other potentially-genotoxic impurities. The presence of (b) (4) (which was mutagenic and clastogenic when tested directly in in vitro tests) in the batches of vilazodone that were used in the carcinogenicity studies at 2.5 (or more) times the limit in the clinical batches also might give some small assurance that the findings in the carcinogenicity studies also cover this impurity.

Conclusions: The Sponsor's actions regarding the 7 (b) (4) impurities are considered adequate from a Pharmacology/Toxicology perspective, as described here and in Dr. Klimek's review (and from a Chemistry perspective, see Chemistry review by Pei-I Chu, Ph.D., Chemist, 1/03/2010). The specification for each of these impurities has been limited so that human will be exposed to not more than (b) (4) µg of each per day at the MRHD of 40 mg. The Sponsor will attempt to further lower the specifications, to reduce the overall patient exposure.

V. MAJOR HUMAN METABOLITE(S):

The non-clinical sections of the original NDA submission provided no indication that there were any metabolites circulating in humans that were not also circulating in animal species. In fact, the Cross-Species Metabolite Comparison summary table (Table 3 in the Non-clinical Overview section of this NDA), showing the presence (or by implication absence) of 15 metabolites in the urine, feces, or plasma of humans, rats, and dogs, indicated that only metabolite M10 was present in human plasma and that metabolite was also present in plasma of rat and dog. It should be noted that metabolite M10, which had been early recognized as a significant metabolite circulating in humans, had also been quantified in plasma of animals in the toxicity studies. However, in the table referred to above, metabolite M17 was indicated to be present in urine of humans, rats, and dogs, but not in plasma of any of these species [with a footnote that “In nonclinical species, only metabolites present as $\geq 3.0\%$ of dose or $\geq 5.0\%$ of sample radioactivity are listed, where information is available.”].

Figure 1. Structures of vilazodone and major human metabolites M10 and M17 (structures excerpted from the Sponsor’s Figure 7, on page 52 of the Non-clinical Overview section).



Although the report for the mass-balance study (PGX-08-P1-07) was provided with the original submission of this NDA, the quantitative metabolite data for humans was submitted late in the review cycle (08/31/2010) and indicated that 2 metabolites, namely M17 in addition to M10, were each present in plasma at greater than 10% of total drug-related species (see Clinical Pharmacology review by Bei Yu, Ph.D., 12/08/2010). The data provided in that submission are summarized in the table below and indicate that M10 and M17 each represented more than 10% of total circulating vilazodone (M16)-related species after a single 20-mg dose to healthy male subjects. The amount of M10 represented more the 10% of total radioactivity at 5-8 hr (~12%), and 10-24 hr (~14%).

The amount of M17 represented more the 10% of total radioactivity at 0.5-4 hr (~11%), 5-8 hr (~18%), and 10-24 hr (~22%), as shown in the table below.

Table 2. Human plasma exposure to vilazodone (M16) and major human metabolites M10 and M17 in healthy males administered 20 mg (radiolabeled) vilazodone in the mass-balance study (PGX-08-P1-07). [Relevant sections were excerpted directly from Table 2 of the study report, page 15; high-lighting added.]

Table 2. Summary of Concentrations of Vilazodone Related Substances in Plasma											
Subject ID	Time Range (h)	Concentrations in ng-eq/mL of Vilazodone free base, and as % of Total Vilazodone-derived Material in the Pooled Sample									
		M16		M10		M11		M17		Sum of 4 Analytes Conc Percent of Total	Mean ¹⁴ C Concentration in Pooled Plasma Sample
Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total		
...											
Mean	0.5-4	12.75	(28.4%)	2.41	(5.3%)	0.57	(1.3%)	4.82	(10.7%)	20.55 (45.7%)	46.9 (100%)
Mean	5-8	22.62	(33.5%)	7.77	(11.8%)	0.58	(0.9%)	11.85	(17.9%)	42.82 (64.1%)	68.0 (100%)
Mean	10-24	16.58	(41.6%)	5.71	(14.2%)	0.38	(0.9%)	8.65	(21.7%)	31.32 (78.9%)	40.7 (100%)

When this new information came to our attention, we contacted the Sponsor (12/14/10), asking them to address the safety and toxicity of both metabolites M10 and M17, as assessed in non-clinical studies, and referring them to the ICH Guidance: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (2010); and the CDER Guidance: Safety Testing of Drug Metabolites (2008).

In response to the Sponsor's reply (12/20/2010 email) and to clarify our concerns, we immediately contacted the Sponsor for a second time (12/20/10):

"We appreciate your (12/20/10) response addressing the presence of M17 as a metabolite in the plasma of vilazodone-treated dogs. We agree that you should submit your response to the NDA and that you should correct Table 3 (Cross Species Comparison) to reflect the presence of M17 in plasma of dogs and humans.

However, qualification of major human metabolites requires studies other than those that assess chronic toxicity, as in the 52-week study in dogs that you cite.

Specifically, the potentials for reproductive (embryo-fetal) toxicity, genotoxicity, and carcinogenicity need to be addressed. We have the following questions:

- Regarding embryo-fetal toxicity, were M10 and M17 detected and quantified in plasma of rats or rabbits and, if so, at what levels? This would determine whether the embryo-toxicity studies for vilazodone in rats and rabbits would also assess the embryo-fetal toxicity of the metabolites.
- Regarding genotoxicity, does either M10 or M17 have structural alerts?
- Regarding carcinogenicity, were M10 and M17 detected and quantified in plasma of rats or mice and, if so, at what levels? This would determine

whether the carcinogenicity studies for vilazodone in rats and mice would also assess the carcinogenicity of the metabolites.

”

The Sponsor (12/22/10 email; 12/29/10 official submission) provided their response, which I have summarized and reviewed below.

The Sponsor had provided (12/20/2010 email) the following table summarizing the plasma exposures to vilazodone (M16) and major metabolites M10 and M17 in humans, based on the human mass-balance study at a single 20-mg dose in healthy males.

Table 1: Absolute and Relative Exposures, Based on AUCs, for M10 and M17 in Humans Following a Single 20 mg Dose of Vilazodone HCl

Collection Times of Pooled Samples	Parent Drug ^a (ng/mL)	M10 (ng eq/mL) ^b	M17 (ng eq/mL) ^b
0.5, 1, 2, 3 and 4 hours	12.75	2.41	4.82
5, 6 and 8 hours	22.62	7.77	11.85
10, 12, 16 and 24 hours	16.58	5.71	8.65
AUC ₀₋₂₄	407 ng•h/mL	132 ng eq•h/mL	205 ng eq•h/mL
Metabolite/Parent AUC ₀₋₂₄ Ratio	Not Applicable	0.325	0.504

Data Source: [PGX-08-P1-07-metabolite-report](#) (SN 0014 submitted on 30 Aug 2010)

^a termed M16 in [PGX-08-P1-07](#)

^b expressed as ng equivalents of vilazodone free base /mL

The Sponsor calculated the projected exposures to M10 and M17 at steady-state at the MRHD of 40 mg, based on the AUC for 40 mg of vilazodone at steady-state of 1645 ng•h/mL (based on study PGX-08-P1-06) and the metabolite/parent AUC ratios from the mass-balance study (see table, above); the estimated AUC for M10 was 534 ng•h/mL per day and for M17 was 829 ng•h/mL per day at steady-state.

Both M10 and M17 have been adequately assessed for general toxicity in dogs: The Sponsor had also provided (12/20/2010) data on systemic exposure to both M10 and M17 in dogs. They had calculated (estimated) steady-state exposures for M10 and M17 and determined that: 1) human AUC exposure to M10 at the MRHD of 40 mg was 6.3% of that for dogs at the NOAEL (mid dose) of 10 mg/kg in the 52-week toxicity study and 2) human exposure to M17 at the MRHD of 40 mg was ~26% of that for dogs at the NOAEL (mid dose) of 10 mg/kg in the 52-week toxicity study. Thus, the NOAEL of 10 mg/kg in the 52-week study in dogs provided human safety margins of 16-fold for M10 and 4-fold for M17.

Regarding metabolite M10, the Sponsor provided (12/29/10 submission) their evidence that M10 was present at adequate levels in plasma of rats and rabbits: the AUC in rats at the high dose of 75 mg/kg in the 26-week study was 3-5 times the estimated AUC at steady-state in humans at the MRHD of 40 mg [in the rat embryo-fetal toxicity study, although the mid dose of 40 mg/kg was the NOEL for embryo-fetal toxicity, only decreased fetal weights and delayed skeletal ossification were seen at the high dose of 200 mg/kg; there was no increases in malformations] and the AUC in rabbits at the high dose of 50 mg/kg in the embryo-fetal study was 22 times that in humans at the MRHD. This confirmed our previous conclusion that the toxicity of M10 has been adequately assessed for general toxicity, reproductive (specifically embryo-fetal) toxicity, and carcinogenicity in the studies conducted with vilazodone. [The Sponsor also found no structural alerts for genotoxicity or carcinogenicity, based on Derek and Multicase analyses.]

However, metabolite M17 was only present in plasma of dogs and mice (and not demonstrated in the plasma of rats or rabbits), so the reproductive toxicity of this metabolite cannot be considered to have been adequately assessed. Regarding genotoxicity/carcinogenicity, it should be noted that this metabolite did not have any structural alerts for genotoxicity or carcinogenicity (based on Derek and Multicase analyses, as stated by the Sponsor). Additionally, M17 appears to have been detectable in plasma of mice (at 1-2% of total radioactivity after an intravenous dose of 1 mg/kg; however, M17 was only identified by retention time, not by identification and specific analysis). Thus, the mouse carcinogenicity study for vilazodone might also provide some assessment of the carcinogenicity of M17. Finally, although not mentioned by the Sponsor, no preneoplastic changes were noted by the pathologist for the 52-week dog study. [It should be noted that fibroepithelial hyperplasia in mammary gland was found in several drug-treated female dogs, but not in controls. However, the study pathologist did not comment on this finding; and it is not standard nomenclature for dogs, whereas for cats it is recognized as a benign, hormone-responsive proliferation. Consequently, this finding seems unlikely to indicate an additional concern for carcinogenicity with M17. It should also be noted that mammary gland tumors in mice, that are presumed to be prolactin-dependent, are included in the labeling for vilazodone.]

The Sponsor also noted that metabolite M17 was generated “robustly” by hepatocytes from rabbits (mice, monkeys, and humans), citing study GPP-007-NCD-PKM-2002-034; however, it should also be noted that in that study, M17 was only identified by retention time in rabbits (and monkeys).

Conclusions regarding the toxicological assessment of major human metabolites M10 and M17: Metabolite M10, which is present at significant levels in plasma of rats, dogs, and rabbits, is considered to have been adequately assessed for toxicity (general, embryo-fetal, and carcinogenicity) in the animal studies with vilazodone.

Metabolite M17, which is present in plasma of dogs and (probably) mice, is considered to have been adequately assessed for general toxicity (in dogs) and for

genotoxicity/carcinogenicity, based on the absence of structural alerts and also supported by the carcinogenicity study in mice and the 52-week study in dogs. However, since M17 was not demonstrated to be present in plasma of either rats or rabbits, the original embryo-fetal toxicity studies with vilazodone did not adequately assess the toxicity of M17. Nonetheless, M17 is present in plasma of dogs and there were no indications of treatment-related histopathology in sex organs of female (or male) dogs in the 52-week study up to the high dose of 40 mg/kg (as assessed by Dr. Klimek in her review and verified by this Reviewer). Although the AUC for M17 at this dose in dogs was not calculated, it would be expected to be ~16 times that for humans at the MRHD (estimated from the safety margin of 4 afforded by the 10 mg/kg dose in dogs, as provided in the Sponsor's 12/20/2010 email submission, see above).

The Sponsor will need to assess the reproductive toxicity of metabolite M17 in an embryo-fetal study. However, this will be allowed as a post-marketing requirement, rather than being required for approval, based on lack of effects on sex organs in dogs in the 52-week study (at estimated steady-state systemic exposures ~16 times those estimated for humans at the MRHD of 40 mg) and the lack of other specific concerns, such as structural alerts for genotoxicity.

Based on the formation of M17 by hepatocytes from rabbits, it is possible that M17 may be present at adequate amounts in plasma of rabbits treated with vilazodone (i.e., at exposures equal to or greater than those in humans at the MRHD). If this is demonstrated for the doses of vilazodone that were used in the original embryo-fetal study, that study would be considered to have adequately assessed M17. Otherwise, a study administering M17 directly to rats or rabbits, using a route that will produce adequate systemic exposure, will be needed.

The following wording was sent to the Sponsor (1/4/2011) to explain the PMR that will be required (the Sponsor agreed to this PMR on 1/4/11):

Because the major human metabolite M17 was not demonstrated to be present in plasma of either rats or rabbits, the embryo-fetal reproductive toxicity studies with vilazodone did not adequately assess the toxicity of M17. Consequently, you will need to commit to assessing the reproductive toxicity of metabolite M17 in an embryo-fetal study.

However, because M17 was formed by hepatocytes isolated from rabbits, it is possible that M17 is present in plasma of rabbits treated with vilazodone. If the systemic exposure to M17 at the doses of vilazodone that were used in the original embryo-fetal study is demonstrated to be equal to or greater than the exposure in humans at the MRHD, the original rabbit study would be considered to have adequately assessed the embryo-fetal toxicity for M17.

Otherwise, an embryo-fetal study in either rats or rabbits where M17 is administered by a route that will produce systemic exposure equal to or greater than the exposure in humans at the MRHD will be required.

VI. OVERALL CONCLUSIONS:

There are no pharmacology/toxicology issues that would prevent the approval of this NDA.

Some labeling is still being negotiated, however, the wording for the carcinogenicity findings of malignant mammary tumors in female mice has been agreed upon. Additionally, the genotoxic impurities have been adequately limited in the drug substance/product.

Regarding the 2 major human metabolites: 1) M10, which is present at significant levels in plasma of rats, dogs, and rabbits, is considered to have been adequately assessed for toxicity (general, embryo-fetal, and carcinogenicity) in the animal studies with vilazodone; and 2) M17, which is present at significant levels in plasma of dogs and (possibly) mice, is considered to have been adequately assessed for general toxicity (in dogs) and for genotoxicity/carcinogenicity, based on the absence of structural alerts and possibly supported by the carcinogenicity study in mice. However, since M17 was not demonstrated to be present in plasma of either rats or rabbits, the original embryo-fetal toxicity studies with vilazodone did not adequately assess the toxicity of M17. Consequently, the Sponsor will need to assess the reproductive toxicity of M17 in an embryo-fetal study. However, this will be allowed as a post-marketing requirement, rather than being required for approval; and the Sponsor has agreed to this PMR.

Additionally, to support use of vilazodone in children under 13 years of age (the deferred pediatric studies under PREA), the Sponsor will need to conduct a study to assess the safety/toxicity of vilazodone in juvenile rats. This study must include evaluation of neurological/behavioral development and reproductive development. The Sponsor has agreed to this PMR.

VII. RECOMMENDATIONS:

From a Pharmacology/Toxicology perspective, because the Sponsor has agreed to conduct a reproductive (embryo-fetal) toxicity assessment for the major human metabolite M17 as a post-marketing requirement, there are no issues that would prevent the approval of this NDA, assuming that labeling issues can be negotiated.

Linda H. Fossom, Ph.D., Pharmacologist, Team Leader *{see appended electronic signature page}*

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/s/

LINDA H FOSSOM
01/12/2011

Barry N. Rosloff, Ph.D.
12/29/10

**NDA 22-567 (VILAZODONE)—RECOMMENDATIONS CONCERNING
PHARMACOLOGIC CLASS AND MECHANISM OF ACTION
STATEMENTS IN LABELING**

BACKGROUND

The purpose of this memo is to address various portions of the proposed vilazodone labeling which concern the proposed mechanism of action of this drug. Briefly, the sponsor is claiming that vilazodone has two mechanisms of action as an antidepressant: (1) It acts as a serotonin-specific reuptake inhibitor (SSRI) and (2) [REDACTED] (b) (4)

[REDACTED] There is a considerable amount of evidence that depression can be caused by a deficiency in brain serotonergic activity and that SSRIs are effective by virtue of blocking the reuptake of serotonin into the presynaptic nerve terminal (since reuptake is the primary mechanism for terminating the action of serotonin at the postsynaptic receptor). It is known that SSRIs (and other currently marketed antidepressants) require several weeks of administration before an adequate antidepressant response is achieved. One theory for this delay is that serotonin released into the synapse, in addition to activating postsynaptic 5HT receptors, activates presynaptic inhibitory autoreceptors (which are of the 5HT1A subtype), which in turn decreases serotonin release, thus counteracting the increased synaptic levels of serotonin caused by reuptake blockade. There is some evidence that repeated dosing with SSRIs results in a downregulation of these inhibitory autoreceptors, thereby resulting in increasing levels of synaptic serotonin over time which may then result in antidepressant efficacy. For many years it has been hypothesized that combining an SSRI with a 5HT1A receptor antagonist (or partial agonist, which in the presence of high levels of serotonin would act as an antagonist) should hasten the onset of antidepressant activity, although this strategy has met with inconclusive results. There is also theoretical reason to believe that stimulation of *postsynaptic* 5HT1A receptors might have antidepressant activity in its own right.

Vilazodone is a potent SSRI as well as a potent partial agonist at 5HT1A receptors. The sponsor's proposed labeling contains [REDACTED] (b) (4)

[REDACTED] (The SSRI mechanism is not in question here; although the mechanism of any psychiatric drug is not conclusively known, labeling for marketed drugs contains the generally accepted theory). Theoretically, since vilazodone is a partial agonist at 5HT1A receptors, it might be expected to act as an agonist in the presence of low concentrations of endogenous agonist (serotonin), and as an antagonist in the presence of high concentrations of endogenous agonist. Indeed, both agonistic and antagonistic effects have been seen depending on the experimental paradigm and brain region examined. It is also possible that effects at presynaptic and postsynaptic 5HT1A receptors could cancel each other out; e.g. an antagonist effect at the

presynaptic autoreceptor would theoretically be good for treating depression (due to block of the negative feedback inhibition of serotonin release), but a similar antagonistic action at the postsynaptic receptor might counteract this effect. (In fact, one published paper, noting the relatively limited activity of vilazodone in an animal model of depression, suggested that this might be due to antagonism of serotonin at the postsynaptic 5 HT1A receptor [Page, et. al., JPET 302:1220-1228, 2002]. Another author stated that “It may be extremely complicated to predict the net effect on 5-HTergic transmission induced by a particular dose of a ... 5HT1A receptor agonist” [DeVry, Psychopharmacology 121:1-26, 1995]). It is not known if the theoretically ideal combination—antagonism of presynaptic receptors and agonism at postsynaptic receptors—occurs at clinically effective doses of vilazodone.

Experimental support for a role of 5 HT1A agonists in treating depression or augmenting the effects of SSRIs is also inconclusive. In animals, administration of 5HT1A partial agonists does not consistently potentiate SSRI-induced increases in synaptic serotonin; in fact antagonism has also been observed. The sponsor claims that in animals (1) the increase in synaptic serotonin seen with vilazodone is greater than that seen with other SSRIs (b) (4)

Although anecdotal reports and open label clinical trials of partial 5HT1A agonists claiming to support antidepressant efficacy (both alone and adjunctive to standard antidepressant drugs) are present in the literature, it is not clear if this has been found in controlled trials. (b) (4)

Clinical trials of vilazodone, while not designed to be comparative with standard SSRI treatment, showed a degree of efficacy and time of onset similar to that usually seen with these drugs.

RECOMMENDATIONS

With these considerations in mind, the following recommendations are made regarding labeling:

1. Highlights section (Indications and Usage subsection)

(b) (4)

(b) (4)

(b) (4)

There is a CDER Guidance document which deals with determining an established pharmacologic class, which notes that such a class should be (1) “scientifically valid” and (2) “clinically meaningful”. For the first criterion it is stated that there should be “empiric” evidence that the class is “known, not theoretical”, and should be “relevant and specific to the indication”. Of course proposed mechanisms are never definitively “known”, but in this case I believe there is much more evidence to support the SSRI class (b) (4) regarding antidepressant effects. More importantly, the class should be “clinically meaningful” in that it “enhances the ability of professionals to understand physiologic effects related to the indication or to anticipate undesirable effects that may be associated with the drug or pharmacologic class”. (Beta-adrenergic blocker was given as an example of an established class based on mechanism of action in the Guidance). (b) (4)

(Note that it could be useful for a clinician to know that a drug has properties in addition to the established one; however the place for this [as we routinely do with other drugs having additional actions] is in section 12.2 [Pharmacodynamics, see below]. We are also recommending mention of the 5HT1A mechanism in the Mechanism of Action section [12.1], although in a more tentative form [see below]).

2. Indications and Usage (Section 1)

The Sponsor’s original proposal was to describe vilazodone as (b) (4)

In fact, the PLR rule says nothing about including such information in this section; therefore I agree that it can be omitted.

3. Description (Section 11)

The Sponsor’s original proposal was to describe vilazodone as above in Section 1 (i.e. (b) (4)). The PLR rule states that this section must contain the “pharmacological or therapeutic class of the drug”. For reasons discussed above, I recommend mentioning only the SSRI class; my second choice would be to mention neither.

4. Mechanism of Action (Section 12.1)

The Sponsor’s original version was (b) (4)

(b) (4) For reasons discussed above, my preference is to only include the SSRI mechanism. However, the Pharmacology reviewer of this NDA, Dr. Klimek, who is an expert in this area, believes that the overall evidence for the 5HT1A mechanism is adequate to warrant inclusion in this section. It was decided to include it in a more tentative form than the SSRI mechanism, as follows:

“The mechanism of the antidepressant effect of vilazodone is not fully understood but is thought to be related to its enhancement of serotonergic activity in the CNS through selective inhibition of serotonin reuptake. (b) (4)

5. Pharmacodynamics (Section 12.2)

The Sponsor’s original version included repetition of the (b) (4), along with other statements that were not substantiated (b) (4) (b) (4)) or unclear (b) (4)

POSTSCRIPT: NOTE TO DDMAC

I am concerned that there is a potential for unwarranted conclusions about the efficacy of vilazodone based on its pharmacology. For many years scientists have hypothesized that 5HT1A partial agonists should have antidepressant activity, as well as that adding a 5HT1A partial agonist to an SSRI should augment the magnitude of, and hasten the onset of, the antidepressant activity of the latter. Such theorizing is widespread throughout the open literature (b) (4); however in general results with 5HT1A partial agonists given alone or combined with an SSRI have been inconclusive, and certainly no advantage of vilazodone in these regards has been demonstrated. Another concern is that 5HT1A agonists are thought to be effective anti-anxiety agents; one such compound (buspirone) is marketed for this indication, but to my knowledge vilazodone has not been shown to be effective for anxiety.

Note that the original labeling as proposed by the sponsor (b) (4)

As noted above, I have recommended (b) (4) although we are recommending that it can be mentioned, in a more tentative statement than for SSRI effect, in the Mechanism of Action section (12.1). The proposed tradename, VIIBRYD, might also imply a (b) (4) and, by further implication, (b) (4)

Barry N. Rosloff
Supervisory Pharmacologist, DPP

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

/s/

BARRY N ROSLOFF
12/29/2010

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22-567
Supporting document/s:	N-0000
Applicant's letter date:	March 22, 2010
CDER stamp date:	March 23, 2010
Product:	VIIBRID™ (Vilazodone HCl)
Indication:	Major Depressive Disorder
Applicant:	PGx Health LLC
Review Division:	Division of Psychiatry Products (HFD-130)
Reviewer:	Violetta Klimek, Ph.D.
Supervisor/Team Leader:	Barry Rosloff Ph.D./Linda Fossom, Ph.D.
Division Director:	Thomas Laughren, M.D.
Project Manager:	William Bender, Pharm.D.

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12.1 TABLE 1: AFFINITY OF VILAZODONE, IPSAPIRONE, BUSPIRONE, 8-OH-DPAT AND FLUOXETINE FOR RECEPTOR AND ION CHANNEL BINDING SITES (IC₅₀, nM) 143

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: Approval, if the issue of assessment of the toxicity of 2 major human metabolites, M10 and M17, can be adequately addressed during this review cycle or as a post-marketing requirement.

1.1.2 Additional Non Clinical Recommendations: Not at this time (see above).

1.1.3 Labeling

Below are this Reviewer's recommendations for sections 8.1, 12.1, 12.2, and 13 of labeling. However, labeling is being negotiated.

Section 8: Use in specific populations

8.1 Pregnancy:

Pregnancy Category C

(b) (4)

Section 9: Drug abuse and dependence

9.2 Abuse and dependence: Vilazodone has been systematically studied in animals and did not demonstrate abuse or dependence potential.

Section 12: Clinical Pharmacology

12.2 Pharmacodynamics:

Vilazodone binds with high affinity to the serotonin reuptake site ($K_i = 0.1$ nM), but not to the norepinephrine ($K_i = 56$ nM) or dopamine ($K_i = 37$ nM) reuptake sites. Vilazodone potently and selectively inhibits reuptake of serotonin ($IC_{50} = 1.6$ nM). Vilazodone also binds selectively with high affinity to 5-HT_{1A} receptors ($IC_{50} = 2.1$ nM) and is a 5-HT_{1A} receptor partial agonist.

Section 13: Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of fertility

Carcinogenesis

Carcinogenicity studies were conducted in which B6C3F1 mice and Wistar rats were given oral doses of vilazodone up to 135 and 150 mg/kg/day, respectively, for 2 years. These doses are approximately 16.5 and 36 times the maximum recommended human dose (MRHD) of 40 mg, respectively, on a mg/m² basis.

In mice, the incidence of hepatocellular carcinomas was increased in males at 16.5 times the MRHD; this finding was not seen at 5.5 times the MRHD. The incidence of mammary gland adenocarcinomas was increased in females at 5.5 and 16.5 times the MRHD, but not at 1.8 times the MRHD. Elevated prolactin levels were observed in a 2-week study of vilazodone administered at 5.5 and 33 times the MRHD. Increases in prolactin levels are known to cause mammary tumors in rodents.

In the rat study, vilazodone was not carcinogenic in either sex at doses up to 36 times the MRHD.

Mutagenesis

Vilazodone was not mutagenic in the in vitro bacterial reverse mutation assay (Ames test). Vilazodone was negative in the in vitro V79/HGRPT mammalian cell forward mutation assay. Vilazodone was clastogenic in two in vitro mammalian cell chromosome aberration assays. However, vilazodone was negative for clastogenic activity in both an in vivo rat bone marrow chromosome aberration assay and a micronucleus test. Vilazodone was also negative in an in vivo/in vitro unscheduled DNA synthesis assay in rats.

Impairment of Fertility

Treatment of rats with vilazodone at a dose of 125 mg/kg, which is 30 times the maximum recommended human dose (MRHD) of 40 mg, on a mg/m² basis, caused an impairment of male fertility with no effect on female fertility. Impaired male fertility was not seen at 6 times the MRHD.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology: Vilazodone binds with high affinity to the serotonin reuptake site ($K_i=0.1$ nM), but not to the norepinephrine ($K_i=56$ nM) or dopamine ($K_i=37$ nM) reuptake sites. Vilazodone inhibits reuptake of serotonin ($IC_{50} = 1.6$ nM and binds to 5HT_{1A} receptors with similar affinity ($IC_{50} = 2.1$ nM) and is 5HT_{1A} receptor partial agonist.

Vilazodone showed activity following oral administration (dose range of 1 – 10 mg/kg); its presence in the brain (*ex vivo* rodent studies) was demonstrated by occupancy of 5-HT reuptake binding sites and 5-HT_{1A} receptors (50% occupancy at 1-3 mg/kg; 90-100% occupancy at 10 mg/kg). Vilazodone was shown to increase level of serotonin in the brain (microdialysis study in freely moving rats) and to produce functional desensitization of presynaptic (inhibitory) 5-HT receptors in electrophysiological studies. Although the mechanism of vilazodone's antidepressant activity has not been fully understood, it is thought to be related to its role in enhancement of serotonergic neurotransmission in the CNS through selective inhibition of 5HT reuptake and partial agonist activity at 5-HT_{1A} receptors. Vilazodone demonstrated antidepressant-like activity in behavioral animal models using various routes of drug administration in forced swim test (in rats and mice) and in mouse tail suspension test.

Pharmacokinetics: Two major human metabolites, M10 and M17, each circulating at greater than 10% of total drug-related exposure, were identified late in this review cycle. These metabolites were found in urine of dogs and rats. M10 was found in plasma of rats and dogs; however, M17 was found in plasma of dogs, but was apparently not detected in plasma of rats to date. We have communicated to the Sponsor that they will need to address the safety of metabolites M10 and M17 as assessed in nonclinical studies; we referred them to ICH Guidance: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (2010); and the CDER Guidance: Safety Testing of Drug Metabolites (2008). The decision about whether additional studies will be needed to qualify these major human metabolites cannot be made at the time this review is being finalized.

General toxicology: Vilazodone was adequately tested in acute and repeat oral dosing toxicology studies in mice, rats, and dogs. Seizures (and seizure-related deaths) were seen in dogs at the high dose of 40 mg/kg/day in the 52-week study. No seizures or deaths were observed at this dose in the 26-week study. The NOAEL for seizures was 10 mg/kg (52-week study) or 40 mg/kg (26-week study), which provides a 3-fold or 9-fold margin of safety, respectively based on (AUC) exposure for the MRHD of 40 mg/day.

Genotoxicity: Vilazodone was not mutagenic without or with metabolic activation (S9 mix) in the Ames test or in the *in vitro* V79/HGRPT mammalian cell forward mutation assay. Vilazodone was clastogenic in V79 Chinese hamster lung cells (\pm S9 mix) and in human lymphocytes with S9 mix (but not without S9 mix) and only at cytotoxic concentrations in both of these *in vitro* assays. However, vilazodone was not clastogenic in two *in vivo* studies, rat bone marrow chromosome aberration assay and in micronucleus test in rats at single oral doses up to 2000 mg/kg. Vilazodone was also

negative in an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes.

Carcinogenicity: Carcinogenicity studies were conducted in rats and mice for 2 years. In rats, there were no biologically relevant, drug-related increases in incidences of neoplasms at doses up to the high dose of 150 mg/kg/day (36 times the MRHD of 40 mg/day).

In mice, biologically relevant, drug-related increases in incidences of neoplasms were limited to hepatocellular carcinomas in males at HD of 135 mg/kg/day and mammary gland adenocarcinomas in females at the mid dose of 45 mg/kg/day and the high dose of 135 mg/kg/day (doses: 15, 45, and 135 mg/kg/day; 1.8, 5.5, and 16.5 times the MRHD of 40 mg/day).

Reproductive toxicity: In fertility and early embryonic development studies, male rats treated with vilazodone administered orally at 5, 25, or 125 mg/kg/day for 4 weeks prior to pairing with untreated females, showed a reduced fertility index (76% versus 96% in controls) with no effect on mating, at 125 mg/kg (30 times the MRHD of 40 mg). Treatment of female rats with vilazodone (at doses up to 125 mg/kg/day for 2 weeks prior to pairing with untreated males) had no effect on their fertility or reproductive function. The NOAEL for decreased fertility in male rats was the mid dose of 25 mg/kg/day (6 times the MRHD of 40 mg/day).

In embryo-fetal development studies, vilazodone, administered orally during the period of organogenesis, was not teratogenic in rats at doses up to 200 mg/kg/day (48 times MRHD of 40 mg/day) or rabbits up to 50 mg/kg/day (35.8 mg/kg, actual dose; 17 times MRHD). [Although assessment of dosing usually confirms nominal doses, in this embryo-fetal study in rabbits the doses that were actually administered were considerably lower than the nominal doses. Consequently, in labeling, the doses and safety margins for rabbits will be provided for the actually administered doses, not the nominal doses.] Other findings in rabbits were limited to the maximum dose tested, and included maternal toxicity (death, spontaneous abortion and body weight loss), increased post implantation loss and reduced fetal weight at birth and delayed skeletal ossification. In rats maternal toxicity was limited to slight transient hyperemia of the furless skin and reduced body weight gain at the high dose. Fetal findings were also limited to the high dose; fetal weight was reduced and this finding correlated with observed delayed skeletal ossification.

When pregnant rats were treated with vilazodone at doses 5, 25, and 125 mg/kg/d (30 times the MRHD of 40 mg/day) during the period of organogenesis and throughout pregnancy and lactation, the number of live-born pups was decreased. There was an increase in early postnatal pup mortality, and decreased body weight among surviving pups, delayed maturation, and decreased fertility in adulthood. The NOAEL for maternal toxicity was the low dose of 5 mg/kg/day based on reduced body weight and at higher doses. The NOAEL for offspring toxicities was also at 5 mg/kg/day based mainly on reduced body weight from birth to weaning and from weaning to sexual maturity.

Other toxicity-related issues: Several potentially genotoxic impurities were present or possibly present in the drug substance; the specifications for these impurities were lowered to acceptable limits.

It should be noted that corneal opacities were observed in dogs during the first few weeks of dosing with vilazodone and correlated with decreased tear production. However, the opacities resolved and cataracts were never observed for up to 52 weeks of dosing in dogs and never seen in other species.

2 Drug Information

2.1 Drug: VIIBRYD™

2.1.1 CAS Registry Number (Optional): 163521-08-2

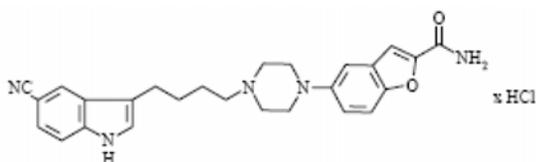
2.1.2 Generic Name: Vilazodone HCl

2.1.3 Code Name: EMD 68 843, SB 659746, SB 659746-A and GPP007

2.1.4 Chemical Name: 2-benzofurancarboxamide, 5-[4-[4-(5-cyano-1*H*-indol-3-yl)butyl]-1-piperazinyl]-, hydrochloride (1:1)

2.1.5 Molecular Formula/Molecular Weight: C₂₆ H₂₇ N₅ O₂ x HCl/477.99 g/mol

2.1.6 Structure:



2.1.7 Pharmacologic class: serotonin reuptake inhibitor and [REDACTED] (b) (4)

2.2 Relevant IND/s, NDA/s, and DMF/s: IND 54,613;

2.3 Clinical Formulation

2.3.1 Drug Formulation: Tablets (immediate release) of 10, 20, and 40 mg strength

2.3.2 Comments on Novel Excipients: only commonly used (generally acceptable) excipients such as Lactose Monohydrate NF, Microcrystalline Cellulose NF, Magnesium Stearate NF, and Colloidal Silicon Dioxide NF were used in vilazodone tablets.

2.3.3 Comments on Impurities/Degradants of Concern: Seven vilazodone HCl impurities were identified with structural alerts for genotoxicity which were not evaluated in non-clinical studies. The limits for these impurities were at the level of [REDACTED] ppm (except [REDACTED] (b) (4)) to allow a human exposure of [REDACTED] (b) (4) µg/day. An exposure of [REDACTED] (b) (4) µg/day equates to [REDACTED] (b) (4) ppm for vilazodone HCl at human dose of 40 mg/day.

[REDACTED] (b) (4) and [REDACTED] (b) (4) impurities were assessed for genotoxicity using standard test systems. [REDACTED] (b) (4) was found to be mutagenic in *Salmonella typhimurium* strain TA 1535 (Ames test – *in vitro*) and was clastogenic in V79 cells (*in*

vitro) without and with metabolic activation (S-9 mix) but was not genotoxic in an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay in rat liver and was negative for clastogenicity in mammalian micronucleus test system *in vivo*. (b) (4) was negative in the Ames test for mutagenicity and was considered not clastogenic in chromosome aberration assay with Chinese hamster lung cells *in vitro*.

The Sponsor considers (b) (4) impurity as having negligible potential for the risk of genotoxicity based on negative findings in two *in vivo* assays (bone marrow micronucleus assay and induction of hepatic unscheduled DNA synthesis in rats). Based on the positive *in vitro* findings for mutagenicity (Ames test) and clastogenicity (chromosomal aberration test in V79 Chinese hamster cells), (b) (4) impurity is considered to be genotoxic by this Reviewer. Therefore, (b) (4) needs to be controlled in the drug substance at the level of not more than (b) (4) µg/day of clinical exposure.

Following the communication with the Sponsor, the specification for (b) (4) was lowered from NMT (b) (4) ppm (i.e., NMT (b) (4), as an unspecified impurity) to NMT (b) (4) ppm (i.e., NMT (b) (4) µg/day human exposure). In addition the Sponsor lowered specifications for other (b) (4) impurities (for (b) (4) each from NMT (b) (4) ppm to NMT (b) (4) ppm) and agreed to evaluate whether the 4 others can also be lowered to reduce the overall patient exposure (to be submitted in their 1st annual report).

2.4 Proposed Clinical Population and Dosing Regimen

Treatment of Major Depressive Disorder (MDD), dosing at 40 mg QD with food after up titration starting with an initial dose of 10 mg QD for 7 days, followed by 20 mg QD for an additional 7 days than continue with 40 mg QD.

2.5 Regulatory Background

Vilazodone was developed through Phase 2, first by Merck KGaA, Darmstadt, Germany (Merck) and then GlaxoSmithKline plc, London, England (GSK). The reports of all studies conducted by these companies are included in this NDA submission. In 2004, PGxHealth, LLC acquired the license to vilazodone from Merck. Since that time, PGxHealth has conducted nonclinical and clinical studies to demonstrate safety, tolerability and efficacy of vilazodone.

3 Studies Submitted

3.1 Studies Reviewed

All submitted pivotal studies except for preliminary range-finding studies in various species and studies previously reviewed.

3.2 Studies Not Reviewed

Preliminary range-finding studies in various species (generally, non-GLP) were not reviewed in detail.

3.3 Previous Reviews Referenced

The following toxicology studies were previously reviewed by E.A. Gonzalez Barry, M.S. (original submission) and Linda Fossom, Ph.D. (13-week oral toxicity study in rats, 13-week oral toxicity study in mice and TSH immunohistochemistry study on pituitaries of mice) under IND 54, 613 and are cited or reproduced under the relevant study reviews in the present review.

4 Pharmacology

Vilazodone is an indolealkylamine derivative that shows activity as selective serotonin reuptake inhibitor (SSRI) and 5-HT_{1A} receptor partial agonist. Based on experimental data, vilazodone can cause an augmentation of serotonergic activity in brain areas thought to be deficient in depression. The active doses reported in animal models of depression and anxiety were generally in the 1-10 mg/kg range, the range in which vilazodone was shown in *ex vivo* studies to produce blockade of 5-HT reuptake as well as binding to 5-HT_{1A} receptors.

4.1 Primary Pharmacology

The pharmacology of vilazodone was studied to characterize its regulation of serotonin neurotransmission in the brain and effects in animal models of depression and anxiety. Its mechanism of action was evaluated in number of *in vitro*, *ex vivo* and *in vivo* tests which often included reference agents such as SSRIs (fluoxetine, paroxetine, citalopram, sertraline), tricyclic antidepressants (imipramine), monoamine oxidase A inhibitors (moclobemide), 5-HT_{1A} receptor agonists that are generally considered to be high efficacy (8-OH-DPAT) or low efficacy (buspirone, gepirone, ipsapirone, SB-2721830-A), and a 5-HT_{1A} receptor antagonist (WAY 100635). *In vitro* studies generally used the endogenous ligand 5-HT as the benchmark full 5-HT agonist.

5-HT reuptake site radioligand binding studies: [³H]-Paroxetine, [³H]-nisoxetine, and [³H]-nomifensine were used as radioligands for 5-HT, norepinephrine (NE), and dopamine (DA) reuptake sites, respectively.

In vitro studies:

Vilazodone binds *in vitro* with high affinity to the human (cloned) 5-HT reuptake site with a K_i = 0.1 nM and shows considerably lower affinity for NE (K_i = 56 nM; 560-fold) and DA (K_i = 37 nM; 370-fold) reuptake sites as summarized in the following table:

Reuptake site	IC ₅₀ (nM)	K _i (nM)
5-HT (serotonin)	0.25 (h);	0.1 (h)
NE (norepinephrine)	58 (h); 103 (rat)	56 (h); 40 (rat)
DA (dopamine)	48 (h); 35 (rat)	37 (h); 31 (rat)

Data source: Study: Gpp-007-NCD-PCL-2000-047and

Vilazodone was also evaluated for its ability to inhibit neurotransmitter reuptake *in vitro* in native tissue and in recombinant cell systems (IC₅₀, nM).

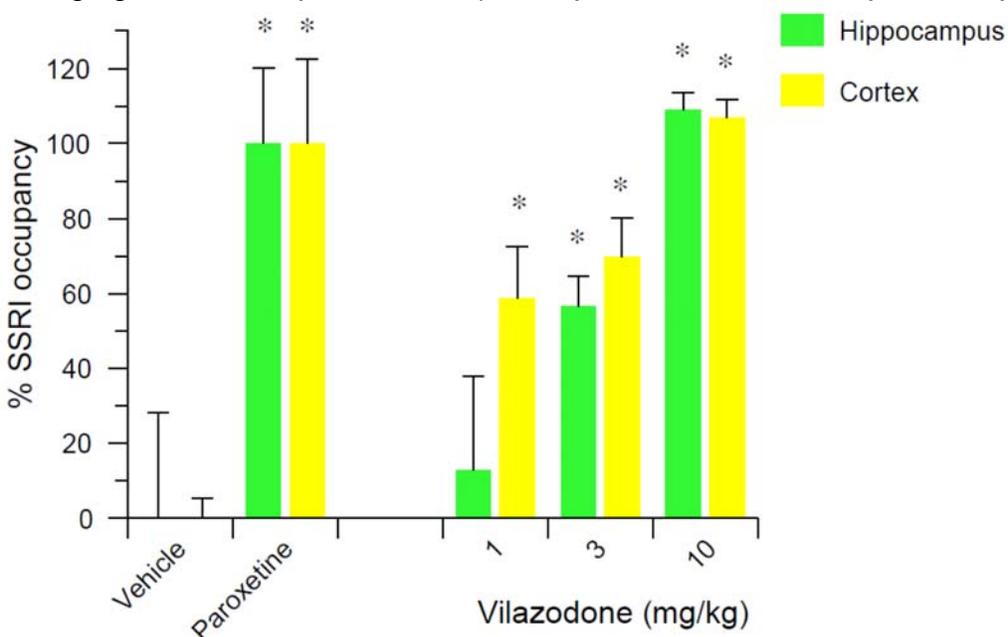
Reuptake	Vilazodone	Ipsapirone	Buspirone	8-OH-DPAT	Fluoxetine
<i>Rat brain synaptosomes</i>					
5-HT (serotonin)	0.2	6	>1000	>1000	>1000
NE (norepinephrine)	60	1000			
DA (dopamine)	90	3000			

Reuptake	Vilazodone	Ipsapirone	Buspirone	8-OH-DPAT	Fluoxetine
<i>Lewis-lung cancer porcine kidney recombinant cells transfected with rat or human (h) 5-HT transporter</i>					
5-TH	0.79	15.8			
5-HT (h)	1.58	9.12			

Data source: Study: GPP-007-NCD-PCL-1995-046 and GPP-007-NCDPCL- 2003-143

Ex vivo studies: 1) The following study design was based on the data from the positron emission tomography (PET) studies using [^{11}C] DASB which have reported that clinically efficacious doses of SSRIs occupy approximately 80% of the serotonin transporter (SERT) (Heyer et al.; *Am. J. Psychiatry*, 2001; 158, p.1843)

In this *ex vivo* study (GPP-007-NCD-PCL-2003-136), vilazodone (1, 3, 10 mg/kg) was dosed orally to rats, and 2 h later, hippocampus and cortex were dissected out and occupancy of brain 5-HT transporter sites was measured using displacement of [^3H]-DASB. As shown in the following Sponsor's figure, vilazodone produced a dose-dependent occupancy of 5-HT transporter sites, with 50% occupancy achieved at 1-3 mg/kg range and 100% occupancy at 10 mg/kg. The positive control SSRI, paroxetine (30 mg/kg, oral, 0.5 h pretreatment), also produced 100% receptor occupancy.



2) In hypothalamic synaptosomes preparations from mice dosed s.c. or orally with vilazodone (for 1 h), vilazodone demonstrated high potency as a 5-HT reuptake inhibitor with ED_{50} values of 0.45 mg/kg (s.c.) and 1.4 mg/kg (oral) dosing. Vilazodone was 5-fold more potent than fluoxetine by the s.c. route ($\text{ED}_{50} = 1.6$ mg/kg) and 1.6-fold more potent by the oral route ($\text{ED}_{50} = 2.3$ mg/kg). This study demonstrated that vilazodone following oral or subcutaneous administration readily enters the brain and achieves the targeted action of blocking 5-HT reuptake (Study: GPP-007-NCD-PCL-1995-050).

3) In rats orally administered vilazodone 3 times daily for 6 days, a subsequent acute oral challenge with vilazodone produced a dose-dependent inhibition of [³H]-5-HT reuptake into rat synaptosomes (ED₅₀ of ~1 mg/kg at the 2 h time point) that lasted for up to 6 h. The effect was comparable to that of orally administered paroxetine (10 mg/kg) and indicated that the tolerance was not developed to vilazodone's activity in inhibiting 5-HT reuptake after a subchronic oral dosing regimen (Study: GPP-007-NCD-PCL-2002-122).

5-HT Reuptake Inhibition: Mechanistic Bioassays: Para-chloroamphetamine (PCA) 5-HT depletion model: In the PCA-induced 5-HT depletion model, 5-HT reuptake inhibitors administered systemically to rats prevent a PCA-induced (and other agents such as fenfluramine) depletion of 5-HT in terminal projection areas such as the hypothalamus, striatum, and cortex of the brain.

In this *in vivo* functional assay of 5-HT reuptake blockade, vilazodone at oral or s.c. doses up to 10 mg/kg, administered 210 min prior to sacrifice and 180 min prior to PCA, potentially prevented PCA-induced depletion of hypothalamic 5-HT following either oral (ID₅₀ = 3.8 mg/kg) or subcutaneous (ED₅₀ = 0.65 mg/kg) administration as shown (oral) in the following Sponsor's table:

Vilazodone (mg/kg, oral)	5-HT (ng/g) mean ± s.e.m.	% Antagonism of PCA Effect
0 (vehicle controls)	1001 ± 10	-
0 (+ PCA)	399 ± 13	0
1 (+ PCA)	471 ± 27	11
3 (+ PCA)	579 ± 42 *	29
10 (+ PCA)	1258 ± 83 *	142
30 (+ PCA)	1400 ± 19*	166

* Statistically significant vs. PCA – treated group (Study: GPP-007-NCD-PCL-1995-051).

In this study vilazodone inhibition of PCA effect was equivalent to fluoxetine (ID₅₀ = 4.2 mg/kg) and the results of this study provided functional evidence that vilazodone blocks the 5-HT transporter *in vivo* in rats.

In another study, vilazodone was orally administered daily for 3 days, followed by an assessment of its activity in preventing PCA-mediated depletion. Vilazodone prevented the PCA-induced depletion of 5-HT with an ID₅₀ = 1.8 mg/kg (Study: GPP-007-NCD-PCL-1997-052), as compared to an ID₅₀ = 3.8 mg/kg in the single dosing study, demonstrating a lack of tolerance to its 5-HT reuptake inhibiting effect after subchronic oral dosing.

The rat PCA depletion model was used to assess the duration of action of a single s.c. dose of vilazodone and reference SSRIs by varying the pretreatment time (Study: GPP-007-NCD-PCL-2000-053). Vilazodone displayed potent activity up to 8 h, with ID₅₀ values of 0.65 mg/kg, 0.21 mg/kg, and 0.55 mg/kg at 3.5 h, 5 h, and 8 h after injection, respectively. A substantial (> 100-fold) drop in vilazodone activity was seen at the next time point measured, 18 h after administration (ID₅₀ = 77 mg/kg), a profile similar to that seen for paroxetine and citalopram, while fluoxetine and sertraline were longer acting.

Consistent with the findings using PCA-induced depletion, s.c. dosing of vilazodone significantly and dose-dependently reversed the 5-HT depleting effect of fenfluramine (ID₅₀ values were 0.09 mg/kg, 0.085 mg/kg, and 0.11 mg/kg for striatum, hypothalamus, and frontal cortex respectively; Study: GPP-007-NCD-PCL-2002-111).

5-Hydroxytryptophan (5-HTP) Head Twitch Model: In mice, augmentation of central 5-HT levels by administration of the 5-HT precursor, 5-HTP, produces occasional head twitches. The frequency of head twitches in 5-HTP treated mice can be increased by pretreatment with antidepressants which increase 5-HT activity by 5-HT reuptake inhibition (e.g. fluoxetine) or by agents which inhibit monoamine oxidase A (e.g. moclobemide). Vilazodone (1 - 30 mg/kg, sc.) dose-dependently increased head twitches in mice pretreated with 5-HTP with significant effects seen at 10 mg/kg and 30 mg/kg, similarly to the reference agents moclobemide and fluoxetine, also significantly increased head twitches. The magnitude of the vilazodone effect was most similar to fluoxetine (Study: GPP-007- NCD-PCL-2001-115).

A second study assessed the effects of combinations of doses of vilazodone and moclobemide (s.c.), that by themselves did not significantly increase 5-HTP-induced head twitches. When administered with vilazodone (1 mg/kg), moclobemide at 3 mg/kg potentiated head twitches while doses of 0.3 mg/kg and 1 mg/kg decreased the number of head twitches (with 0.3 mg/kg moclobemide showing the more significant effect). When administered with vilazodone 3 mg/kg, all doses of moclobemide significantly potentiated head twitches in a dose-dependent manner (GPP-007-NCD-PCL-2001-116).

5-HT_{1A} receptor binding studies:

CNS 5-HT_{1A} receptors are present in the variety of brain regions, including its high density present in the dorsal raphe nucleus, cerebral cortex and hippocampus (pyramidal cell layer). These receptors in the raphe nucleus are largely somatodendritic autoreceptors however in other areas are postsynaptic receptors.

Standard *in vitro* and *ex vivo* receptor binding techniques were used to evaluate the potency and selectivity of vilazodone at the 5-HT_{1A} receptor binding site for animal and human receptors as summarized in the following sections. In addition human position emission tomography (PET) studies using [¹¹C] WAY 100635 have reported occupancy of pre-synaptic 5-HT_{1A} receptors (*in vivo*) by vilazodone up to 60% at 40 mg.

***In vitro* studies:** Vilazodone binds with high affinity to the rat 5-HT_{1A} receptor binding sites (IC₅₀ = 0.5 nM [hippocampus] and IC₅₀ = 1.6 nM [cerebral cortex]) and to the human 5HT_{1A} binding sites with IC₅₀ = 2.1 nM. Regarding selectivity, in over 50 additional binding assays performed, three activities with affinity ≤ 100 nM were observed: the sigma site, 5-HT_{2B} receptor and 5-HT₄ receptor as shown in the following table (IC₅₀, nM)*:

Receptor binding	Vilazodone	Ipsapirone	Buspirone	8-OH-DPAT	Fluoxetine
5-HT _{1A} serotonin	0.5; 1.6	6	30	0.9	>10000
5-HT _{1A} serotonin (h)	2.1			0.68	
5-HT _{2B} serotonin (h)	78; (Ki = 34 nM)				
5-HT ₄ serotonin	100; 196				

Receptor binding	Vilazodone	Ipsapirone	Buspirone	8-OH-DPAT	Fluoxetine
D ₃ dopamine (h)	140; 360				
(b) (4)	17	856	79	985	

Data from studies: GPP-007-NCD-PCL-1995-046 and GPP-007-NCD-PCL-1999-065).

* The Sponsor's table (Table 1) of affinity of vilazodone and its comparators to variety of receptor and ion channel binding sites is attached (see; Attachment);

Vilazodone exhibits potent affinity ($pK_i > 9.3$, $K_i < 0.5$ nM) for native tissue 5-HT_{1A} receptors from rat, guinea pig, mouse, and marmoset labeled with [³H] 8-OH-DPAT (Study: GPP-007-NCD-PCL-2002-130). Vilazodone had greater affinity (up to 2 log units) for the high-affinity agonist state (measured with [³H] 8-OH-DPAT) than for the low-affinity state (measured with [³H] WAY100635). In a similar fashion, vilazodone inhibited specific binding of [³H] WAY100635 and [³H] 8-OH-DPAT to recombinant human 5-HT_{1A} receptors with a pK_i of 7.35 ($K_i = 44.7$ nM) and 9.7 ($K_i = 0.2$ nM), respectively, at low and high agonist affinity states (Study: GPP-007-NCDPCL- 2003-123).

Ex vivo studies: The ability of orally administered vilazodone (2 h pretreatment) to occupy rat cortical 5-HT_{1A} receptors (postsynaptic) was measured by displacement of [³H]-8-OHDPAT (Study: GPP-007-NCD-PCL-2002-129). An oral dose of 3 mg/kg was the estimated ED₅₀ (the dose that produced 50% occupation of cortical 5-HT_{1A} receptors) whereas a dose of 10 mg/kg was estimated to produce ~90% receptor occupancy. By varying the pretreatment time at 10 mg/kg, it was demonstrated that ~90% receptor occupancy lasted up to 5.5 h.

Another study (GPP-007-NCD-PCL-2003-137) evaluated 5-HT_{1A} receptor occupancy in three regions of the rat brain (hippocampus, dorsal raphe and cerebellum) at 2 h post oral administration of vilazodone (1, 3, and 10 mg/kg) but was inconclusive given high variability in the data.

5-HT_{1A} receptor functional studies:

The majority of studies with vilazodone measured its ability to stimulate the binding of [³⁵S]-GTPγS (GTP analog) to G-proteins in membranes. When tested on recombinant human 5-HT_{1A} receptors stably expressed in membranes of CHO cells, vilazodone acted as a high efficacy partial agonist with an EC₅₀ of 1.4 nM and E_{max} of 87% relative to that of 5-HT as a ligand (Study: GPP-007-NCD-PCL-2000-088). In this assay, the 5-HT_{1A} receptor agonist 8-OH-DPAT was 14-fold less potent than vilazodone (EC₅₀ = 19.3 nM) but exhibited an equivalent level of partial agonism (E_{max} = 83%, relative to 5-HT).

In human recombinant Sf9 cells, vilazodone again profiled as a high efficacy partial agonist at 5-HT_{1A} receptors, increasing [³⁵S]-GTPγS binding to 69% of the magnitude of the full 5-HT_{1A} receptor agonist 8-OH-PIPAT (Study: GPP- 007-NCD-PCL-2002-148).

When tested on human recombinant 5-HT_{1A} receptors expressed in HEK 293 cells, vilazodone acted as a full agonist (intrinsic activity of 99%) equivalent to the ligand 5-HT in comparison to the reference 5-HT_{1A} partial agonist SB-272183-A, which demonstrated an intrinsic activity of 0.38 (38% of 5-HT) (Study: GPP-007-NCD-PCL-2002-128).

Summary of vilazodone intrinsic activity relative to 5-HT (reference agonist) in 5-HT_{1A} receptor functional assays *in vitro*:

In Vitro Assay	Relative Intrinsic Activities (% of full agonist response)
Chinese hamster ovary cells	5-HT (100%) > Vilazodone (87%) > 8-OH-DPAT (83%)
SF9 cells	8-OH-PIPAT (100%) > Vilazodone (69%)
HEK 293 cells	5-HT (100%) > Vilazodone (99%) > SB-272183-A (38%)
Rat hippocampus	5-HT (100%) > Vilazodone (61%) > 8-OH-DPAT (45%) > Buspirone (19%)
Rat lateral septum	5-HT (100% stimulation) > 8-OH-DPAT (93%) > Vilazodone (87%) > Buspirone (45%) > Gepirone (34%)
Hippocampus	5-HT (100%) > 8-OH-DPAT (85%) > Vilazodone (45%) > Buspirone (34%) > Gepirone (32%)
Dorsal raphe	5-HT (100%) > 8-OH-DPAT (78%) > Buspirone (51%) > Gepirone (49%) > Vilazodone (43%)
Forskolin stimulated cAMP	5-HT (100%) > Vilazodone (98%) = 8-OH-DPAT (98%)

Stimulation of 5-HT_{1A} receptors *in vitro* with agonists such as 8-OH-DPAT inhibits electrically evoked twitch response in isolated guinea pig ileum, by decreasing transmitter release from the enteric cholinergic neurons. In this assay, vilazodone produced inhibition of electrically-evoked twitch response (EC₂₀ = 34 nM) with a 3-fold greater potency than 8-OH-DPAT (EC₂₀ = 100 nM). The 5-HT_{1A} antagonist WAY 100635 prevented vilazodone dependent inhibition, consistent with the conclusion that vilazodone's effect is produced by an agonist action at 5-HT_{1A} receptors (Study: GPP-007-NCD-PCL-1997-089).

5-HT_{1A} receptor functional studies; pre- and post-synaptic actions:

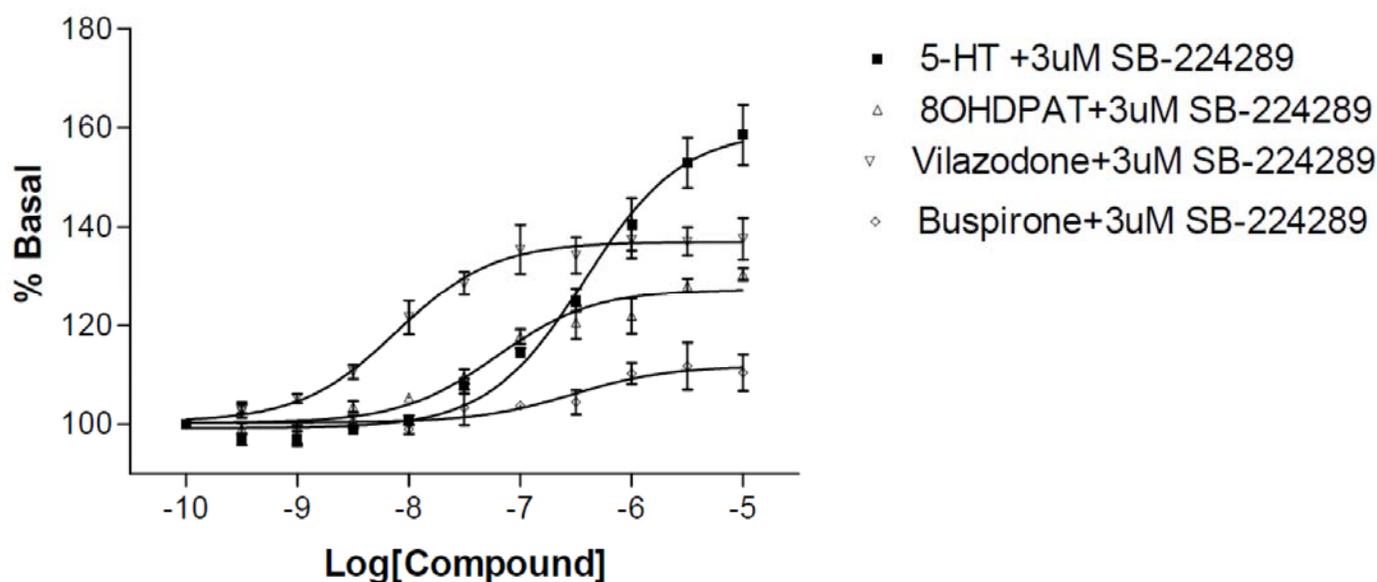
Pre- and post-synaptic mechanism: In the study GPP-007-NCD-PCL-2003-121 guanosine-5'-O-(3-thio)triphosphate ([³⁵S]GTPγS) autoradiography was used to examine the effect of vilazodone on 5-HT_{1A} receptor coupling in the rat lateral septum and hippocampus (regions where 5-HT terminals project and synapse onto postsynaptic 5-HT_{1A} and other 5-HT receptors) as well as in dorsal raphe (a region containing 5-HT cell bodies where presynaptic (somato-dendritic) 5-HT_{1A} receptors exert an inhibitory influence on 5-HT cell firing and 5-HT release) *in vitro* in treatment naïve tissue and *ex vivo* following chronic (21 day) administration. Positive controls, buspirone, gepirone and 8-OH-DPAT (all at 10 μM) and fluoxetine (10 mg/kg i. p.) were used *in vitro* and *ex vivo*, respectively.

Results show that in the dorsal raphe from treatment-naïve rats, acute vilazodone (10 μM) treatment had the profile of a low efficacy partial agonist in that it produced a level of 5-HT_{1A} receptor activation (43%, of 5-HT) less than 8-OH-DPAT (78%), buspirone (51%) and gepirone (49%). In the hippocampus, vilazodone stimulated [³⁵S]GTPγS

binding with an efficacy (45% of 5-HT) lower than 8-OH-DPAT (85%) but slightly greater than that of buspirone (34%) and gepirone (32%); whereas in the lateral septum, vilazodone's efficacy (87% of 5-HT) was more similar to that of 8-OH-DPAT (10 μ M; 93%) than that of buspirone (45%) or gepirone (34%).

Following chronic dosing with vilazodone (3 and 10 mg/kg p.o.) and the positive control fluoxetine (10 mg/kg i. p.), [35 S]GTP γ S binding in the dorsal raphe, lateral septum and hippocampus was indistinguishable from vehicle treated controls. Therefore it can be concluded that desensitization at the cellular level did not develop following chronic administration of vilazodone or fluoxetine. It is not known what chronically-administered 8-OH-DPAT would do under these conditions as this compound was not run as a concurrent reference control.

Post-synaptic mechanism: A partial agonist activity of vilazodone at the 5-HT $_{1A}$ receptor was demonstrated in the study of 5HT-induced stimulation of [35 S]-GTP γ S binding in treatment naive rat hippocampus. In this study an intrinsic activities relative to 5-HT (1.0) were 0.61 (vilazodone with pEC $_{50}$ =8.1), 0.45 (8-OH-DPAT with pEC $_{50}$ =7.2) and 0.19 (buspirone with pEC $_{50}$ =6.5) (Study: GPP-007-NCDPCL-2003-124) as illustrated in the following figure: (SB-224289 blocked 5HT $_{1B}$ - mediated component of 5HT response)



In addition, in this system, chronic administration of vilazodone to rats for 21 days did not alter the sensitivity of post-synaptic hippocampal 5-HT $_{1A}$ receptors as was determined by using 5-HT or 8-OH-DPAT-induced stimulation of [35 S]-GTP γ S binding.

5-HT $_{1A}$ receptor agonism/antagonism: mechanistic bioassays

Route	Expected Agonist Effect in Assay	Vilazodone Profile	Reversed by WAY 100635?	Comparison to Reference Compound
In vitro	Decreases electrically evoked twitch- guinea pig ileum	Agonist	Yes	Like 8-OH-DPAT

Route	Expected Agonist Effect in Assay	Vilazodone Profile	Reversed by WAY 100635?	Comparison to Reference Compound
In vitro	Increases 5-HT release in dorsal raphe brain slice	Agonist	Yes	Unlike SSRI
In vitro	Increases 5-HT release in rat hippocampal brain slice	Agonist	Not done	Like 8-OH-DPAT; Unlike SSRI
In vitro	Increases 5-HT release in guinea pig cortical brain slice	Agonist	Not done	Lower magnitude than fenfluramine
Intra-venous	Decreases dorsal raphe 5-HT neuron firing in anesthetized rat	Agonist	Yes	Like 8-OH-DPAT; Like SSRI
Oral	Decrease 5-HTP accumulation in brain	Agonist	Not done	Not done
Oral	Decrease body temperature	Not an agonist	Not applicable	Not done
IP	Produce "5-HT Syndrome"	Not an agonist ^b	Not applicable	Not done
IP	Substitutes for 8-OH-DPAT in rat drug discrimination model	Agonist	Not done	

Neurochemical Studies: 5-HT Synthesis: Treatment of rats with an aromatic decarboxylase inhibitor NSD-1015 inhibits the conversion of the precursors, 5-HTP and DOPA, to 5-HT and DA, respectively, leading to an accumulation of 5-HTP and DOPA in various brain areas. Via feedback regulation, agonists at the 5-HT_{1A} receptor (but not 5-HT reuptake inhibitors) decrease the accumulation of 5-HTP, and similarly, DA receptor antagonists increase DOPA accumulation. In one study (Study: GPP-007-NCD-PCL-1995-051), accumulation of 5-HTP by NSD-1015 was blocked by orally administered vilazodone (1 mg/kg, 3 mg/kg, 10 mg/kg; 70 min pretreatment), consistent with an agonist action on 5-HT_{1A} receptors. No significant effects on DOPA accumulation were observed, suggesting that vilazodone lacks DA receptor antagonist effects.

In a second study (Study: GPP-007-NCD-PCL-1998-067), orally administered vilazodone (3 mg/kg and 10 mg/kg given once daily for 3 days) induced small but significant decreases of 5-hydroxyindole- acetic acid (5-HIAA, a major metabolite of 5-HT) and increases of 5-HT in various brain areas. These findings of decreased 5-HT turnover are consistent with a 5-HT_{1A} receptor agonist and/or partial agonist action of vilazodone.

Behavior: 5-HT syndrome; 8-OH-DPAT administered to rats produces a constellation of behavioral effects (flat body posture, hind limb abduction, lateral head weaving, resting tremor, forepaw treading, and Straub tail) known as "5-HT Syndrome" which can be blocked by the 5-HT_{1A} antagonist WAY100635 and therefore attributable to stimulation of 5-HT_{1A} receptors. In this study (GPP-007-NCD-PCL-2002-148; actually it is a published article by Page M.E., et al.; *JPET*, 2002; 302:1220), unlike the 5-HT_{1A} full agonist 8-OH-

DPAT, vilazodone administered i. p. alone did not produce a 5-HT syndrome in rats. Pretreatment with vilazodone at 1 and 3 mg/kg, however, dose dependently blocked expression of the 5-HT behavioral syndrome induced by 8-OH-DPAT as shown in the following table excerpted from this article.

EMD 68843 prevents the serotonin syndrome induced by 8-OH-DPAT							
Rats were observed for 15 min after drug administration and scored for the intensity, appearance, and degree of expression of the six behavioral components of the serotonin syndrome on a 0 to 3 scale. Values shown are mean intensity scores on 0 to 3 scale for each symptom. Rats that displayed a score of 2 or higher on four or more signs were considered to display the serotonin syndrome (<i>n</i> = 6 rats for each group).							
Treatment (mg/kg)	Posture	Hind Limb Abduction	Head Weaving	Tremor	Forepaw Treading	Straub Tail	5-HT Syndrome (%)
DPAT (1.0)	2.2	2.3	2.0	1.7	2.3	2.3	100
EMD (3.0)	0.0	0.0	0.0	0.0	0.0	0.0	0
EMD (3.0)/DPAT (1.0)	0.9	1.2	0.3	0.5	0.3	0.3	0
EMD (1.0)/DPAT (1.0)	1.5	1.5	0.8	0.3	1.0	1.0	33
EMD (0.3)/DPAT (1.0)	1.9	2	1.7	1.8	2.0	2.0	88

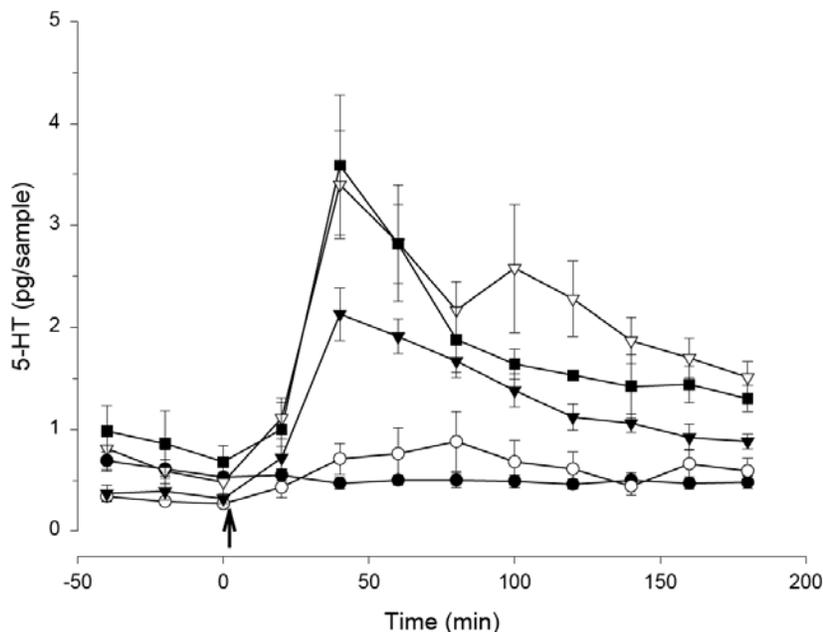
This profile (no 5-HT syndrome, blockade of 8-OH-DPAT syndrome) is similar to 5-HT_{1A} partial agonists such as buspirone, based on published literature. The SSRI fluoxetine was also tested in this assay and did not produce a 5-HT syndrome, nor did it block the effects of 8-OH-DPAT. Therefore it can be concluded that vilazodone's profile in the 5-HT syndrome model is consistent with 5-HT_{1A} receptor partial agonism

Behavior: drug discrimination model: When rats were trained to discriminate 8-OH-DPAT (0.4 mg/kg, i. p.) from saline with 95% accuracy then vilazodone was substituted for 8-OH-DPAT; vilazodone (0.5, 1, 2, and 4 mg/kg, i. p.) dose dependently generalized to the 8-OH-DPAT cue with a maximal generalization response of 54% at 4 mg/kg. These results are consistent with the conclusion that the compound has agonist activity at 5-HT_{1A} receptors (Study: GPP-007-NCD-PCL-1997-091).

Effects on Regional Brain Levels of 5-HT:

In Vivo Microdialysis: A series of studies were performed using *in vivo* microdialysis in freely moving animals to measure the effects of vilazodone on 5-HT release in the rat frontal cortex or hippocampus (post synaptic sites).

Vilazodone at dose range 0.3 - 10 mg/kg i.p. increased extracellular 5-HT levels in rat frontal cortex in a dose-dependent manner as shown in the following graph (Study: GPP-007-NCD-PCL-2001-057).



The lowest dose of vilazodone 0.3 mg/kg (O) had no effect while 1 mg/kg (▼) caused a ~3-fold increase over baseline and both 3 mg/kg (Δ) and 10 mg/kg (■) caused a ~4-5-fold increase. In this study, the SSRIs fluoxetine (3 - 20 mg/kg), citalopram (3 - 20 mg/kg), and paroxetine (1 - 10 mg/kg) also caused significant dose related increases in 5-HT levels but in all cases the increases were significantly lower than that caused by vilazodone.

Another study reported that acute vilazodone (3 mg/kg i. p.) in freely moving rats produced a larger maximal increase of serotonin than did the SSRI fluoxetine (10 mg/kg i. p.) in the frontal cortex (5.3-fold versus 1.6-fold, respectively) as well as in a second serotonin projection area, the ventral hippocampus (5.6-fold versus 2.7-fold). When challenged with the 5-HT_{1A} receptor agonist 8-OH-DPAT, the vilazodone-induced increases in serotonin in the frontal cortex were reduced to a lesser extent than were fluoxetine-induced increases, a difference attributable to an “interfering” effect of vilazodone on presynaptic 5-HT_{1A} autoreceptor inhibition. A different pattern of activity was observed in the ventral hippocampus in that vilazodone- and fluoxetine-induced increases in serotonin were reduced equivalently by 8-OH-DPAT, a finding which may be attributable to a lesser influence of presynaptic 5-HT_{1A} receptors in this region (Study: GPP-007-NCD-PCL-2002-148).

In summary, the microdialysis studies demonstrated that vilazodone augments extracellular 5-HT levels in the frontal cortex and hippocampus with the frontal cortical effects generally greater than those produced by SSRIs. Also, relative to an SSRI, the vilazodone-induced increase in 5-HT release in frontal cortex was suppressed to a lesser extent by co-administration of 8-OH-DPAT, indicating that vilazodone can attenuate presynaptic feedback inhibition resulting from stimulation of 5-HT_{1A} autoreceptors. Vilazodone decreased 5-HT levels in a dorsal raphe brain slice preparation, whereas an SSRI increased them, suggestive of less potential presynaptic feedback inhibition by vilazodone.

Animal Models of Antidepressant Like- Activity

Behavioral despair test (Porsolt or forced swim test): Oral administration of vilazodone to mice (10, 30, 55, or 100 mg/kg) 2 h before testing resulted in significant reductions in immobility at all doses (Study: GPP-007-NCD-PCL-1997-068). Pretreatment time may be an important variable as orally administered vilazodone (1, 10 mg/kg) administered with a shorter pretreatment time (0.5 h) before testing was not active (GPP-007-NCD-PCL-1995-069). However, the positive comparator imipramine given orally at 0.5 h pretreatment was active in this study. In contrast, the 5-HT_{1A} partial agonist ipsapirone (1-30 mg/kg) and high doses of the SSRI fluoxetine (30, 55 mg/kg) orally administered 0.5 h before testing were not active.

In contrast, significant antidepressant effects were seen in rats following administration of 3 mg/kg (but not 1 mg/kg) vilazodone given 3 times in 24 h, or when vilazodone was administered once daily for 7 or 14 days. Antidepressant effects were also seen with 10 mg/kg given for 7 days. Higher doses of vilazodone were generally inactive or increased immobility duration. The reference agent imipramine was active at 10 mg/kg and 30 mg/kg only after repeated acute and subchronic oral treatment and was inactive at 3 mg/kg, regardless of the treatment schedule (Study: GPP-007-NCD-PCL-1996-070).

Tail suspension test: The mouse tail suspension test was less sensitive, Vilazodone administered orally (1-100 mg/kg; 2 h pretreatment) did not significantly reduce immobility time (Study: GPP-007-NCD-PCL-1997-072). However, in the same dose range administered orally three times (24, 5, and 1 h) before testing, vilazodone produced a significant reduction in immobility time at 30 and 55 mg/kg by 65% and 58%, respectively. In this study, vilazodone was more potent than the reference tricyclic antidepressant imipramine, which decreased immobility time by 82% following repeated oral administration at 55 mg/kg but not at 30 mg/kg.

Learned helplessness test: In this study, rats were placed in a shuttle-box apparatus divided into two compartments to which they had free access. Two days after being exposed to a series of inescapable footshocks (“helpless” condition) or no footshocks (controls), the rats were returned to the shuttle-box for conditioned avoidance training for 3 consecutive days.

Vilazodone, vehicle, or the reference antidepressant (imipramine administered by the oral or i.p. routes; fluoxetine, administered orally) were then administered daily for 5 consecutive days. Escape failure demonstrated by rats exposed to inescapable shock was reduced by vilazodone (60, 100 mg/kg) on days 4 and 3, respectively, of the 5 day treatment period. Fluoxetine was effective after oral 100 mg/kg on day 5. Escape deficits were also reversed by imipramine (30 mg/kg, i.p.) on days 4 and 5, whereas the drug was not active by the oral route of administration (Study: GPP-007-NCD-PCL-1997-074).

Chronic mild stress model: In this test, rats are trained to consume a 1% sucrose solution and are then exposed to various forms of unpredictable mild stress (e.g. tilted

floor cage, intermittent illumination, food deprivation, etc.) for a period of 8 weeks. Cumulative daily exposure to these unpredictable stressors results in a progressive decline in sucrose consumption (presumably reflecting a state of progressive “anhedonia”) and a drug-induced reversal of this decline is interpreted as indicative of an antidepressant action. Using this model, after 3 weeks of exposure to chronic mild stress, rats received once daily administrations of vehicle or vilazodone (3, 10, 30, 55 mg/kg, p.o.). Stressed animals in the vehicle group consumed about 30% less sucrose than unstressed controls. Unfortunately, vilazodone decreased sucrose consumption in both the stressed and unstressed controls causing the results not interpretable (Study: GPP-007-NCD-PCL-1997-075).

Animal Models Assessing Anti-Anxiety-Like Activity

In brief: Vilazodone administered by oral, subcutaneous and/or intraperitoneal routes was evaluated for anti-anxiety activity in a wide range of animal models in different species utilizing exposure to acute stressors after both acute and chronic administration. Following acute administration, vilazodone inhibited ultrasonic vocalizations in the shock-induced ultrasonic vocalization model in adult rats and in the maternal separation model in rat pups, and inhibited defensive burying in the shock-probe test in rats. As noted for the antidepressant animal studies, pretreatment time may be a critical factor, as demonstrated by the footshock-induced vocalization test in rats in which orally administered vilazodone demonstrated consistent anti-anxiety activity using a 2 h, but not 0.5 h, pretreatment time. The majority of acute administration studies were performed with a 1 h pretreatment time (and a number of these did not demonstrate significant anti-anxiety activity); further testing using longer pretreatment times may reveal anti-anxiety activity in these models. Following chronic oral administration, vilazodone (like SSRIs) demonstrated significant anti-anxiety activity in the social interaction test in rats. Vilazodone showed anti-anxiety activity in the human threat test in a non-human primate (marmoset).

An analysis of the oral dosing studies performed indicated that the active dose ranges reported in animal models of depression and anxiety were generally in the 1-10 mg/kg range, which paralleled the range in which vilazodone was shown in *ex vivo* studies to produce blockade of 5-HT reuptake as well as binding to 5-HT_{1A} receptors.

Pharmacology of metabolites:

The following table shows the inhibition of monoamine reuptake into rat brain synaptosomes (IC₅₀, nM) by vilazodone, fluoxetine, and vilazodone metabolites *

Assay	Vilazodone	Fluoxetine	M11	M10	EMD	
					80 546	M17 e
5-HT (serotonin) reuptake	0.2	6	7	10	200	> 10000
NE (norepinephrine) reuptake	60	1000	82	>10000	200	>10000
DA (dopamine) reuptake	90	3000	78	>10000	400	>10000

Note: The Sponsor’s table (Table 2) of affinity of vilazodone and its metabolites in selected receptor binding assays is attached (see; Attachment);

Of four putative metabolites of vilazodone synthesized and evaluated for biological activity, M17 (EMD 122 230) is devoid of reuptake inhibiting activity ($IC_{50} > 10 \mu\text{M}$ at 5-HT, NE, and DA reuptake sites). The IC_{50} s for inhibition of reuptake at all three sites are $\geq 200 \text{ nM}$ for EMD 80 546 (no "M" designation). The IC_{50} s for M10 for inhibiting reuptake were 10 nM (5-HT) and $>10 \mu\text{M}$ (norepinephrine and dopamine). M11 (EMD 113 084) selectively inhibited 5-HT reuptake ($IC_{50}=7 \text{ nM}$) relative to NE ($IC_{50}=82 \text{ nM}$) and DA ($IC_{50}=78 \text{ nM}$). IC_{50} s at the rat 5-HT_{1A} binding site were 35 nM for M11 and $\geq 200 \text{ nM}$ (M10 and M17). For EMD 80 546 the IC_{50} at the human 5-HT_{1A} receptor was $>1 \mu\text{M}$ and 20 nM at the rat 5-HT_{1A} receptor. Vilazodone was at least 9.5- to 40-fold (IC_{50} s = 0.5-2.1 nM) more potent than EMD 80 546 ($IC_{50} = 20 \text{ nM}$), the most potent among metabolites identified as a selective 5-HT_{1A} receptor binding metabolite. Vilazodone was at least 35-fold ($IC_{50} = 0.2 \text{ nM}$) more potent than M11 ($IC_{50} = 7 \text{ nM}$), the most potent among metabolites identified with 5-HT reuptake properties when assessed in the rat hippocampal membranes.

In summary, the primary pharmacological profile of vilazodone is inhibition of reuptake of serotonin ($IC_{50} = 1.6 \text{ nM}$ and binds to 5HT_{1A} receptors with similar affinity ($IC_{50} = 2.1 \text{ nM}$) and is 5HT_{1A} receptor partial agonism. Vilazodone showed activity following oral administration (dose range of 1 – 10 mg/kg); its presence in the brain (*ex vivo* rodent studies) was demonstrated by occupancy of 5-HT reuptake binding sites and 5-HT_{1A} receptors (50% occupancy at 1-3 mg/kg; 90-100% occupancy at 10 mg/kg). Vilazodone was shown to increase level of serotonin in the brain (microdialysis study in freely moving rats) and to produce functional desensitization of presynaptic (inhibitory) 5-HT receptors in electrophysiological studies. Although, vilazodone's pharmacodynamic profile as 5HT reuptake inhibitor and 5HT_{1A} partial agonist activity was well documented, its *in vivo* mode of action in relation to antidepressant activity has not been fully understood.

4.2 Secondary Pharmacology

Vilazodone also binds *in vitro* with considerable affinity to the human NE reuptake site with $K_i = 56 \text{ nM}$ that is ~560-fold lower than its binding to 5-HT reuptake site ($K_i = 0.1 \text{ nM}$) and to DA reuptake site ($K_i = 37 \text{ nM}$; 370-fold lower than to 5-HT reuptake site).

In additional binding assays performed, vilazodone showed three activities with affinity $\leq 100 \text{ nM}$ that include: the sigma site ($IC_{50} = 17 \text{ nM}$), the 5-HT_{2B} receptor ($K_i = 34 \text{ nM}$; $IC_{50} = 78 \text{ nM}$), and the 5-HT₄ receptor ($IC_{50} = 100\text{-}196 \text{ nM}$).

Neuroprotection: Based on data indicating that 5-HT_{1A} agonists have potential as neuroprotective agents orally administered vilazodone was evaluated for potential activity as an anti-stroke agent in a focal permanent ischemia model in rats (GPP-007-NCD-PCL-2000-112). In this model, a high dose of vilazodone (120 mg/kg) reduced ischemic damage when administered 0.5 h before, or 1 or 2 h after, middle cerebral artery occlusion. The reduction in infarct size was similar at all 3 time points.

Food Consumption: In rats, oral administration of vilazodone at doses of 1 mg/kg, 3 mg/kg, or 30 mg/kg had no effect on food consumption measured 2.5 h and 5 h after drug administration. In comparison, oral administration of the 5-HT releasing agent fenfluramine (10 mg/kg) decreased food consumption (Study: GPP-007-NCD-PCL-2000-104). These data are consistent with the conclusion that vilazodone does not have a fenfluramine-like 5-HT releasing action.

Memory/Cognition: Orally administered vilazodone showed no memory-enhancing effects in two learning and memory paradigms in rats. In the first test, an animal model of Alzheimer's disease, deficits in passive avoidance learning induced by the cholinergic antagonist scopolamine were not attenuated by vilazodone (10, 30, or 55 mg/kg; 1 h pretreatment) (Study: GPP-007-NCD-PCL-2000-113).

In the second model, spatial learning deficits were measured with the Morris water maze task in aged rats and vilazodone given subchronically (1, 3, or 30 mg/kg; administered once a day for 6 days) produced no evidence for cognitive enhancement in this model (Study: GPP-007-NCDPCL-2001-114).

Analgesia: In the formalin test, intradermal injection of formalin into the paw of mice or rats results in a pain response (licking), consisting of a first phase (nociceptive pain) and a second phase (inflammatory pain). In mice, vilazodone at the single oral dose tested (10 mg/kg) reduced the licking time in the first phase by 59% and by 79% in the inflammatory phase (Study: GPP-007-NCDPCL-1995-069).

In a test of neuropathic pain, sciatic nerve ligation results in thermal hyperalgesia and tactile allodynia. Vilazodone administered orally at doses of 3 mg/kg, 10 mg/kg and 55 mg/kg, 60 min before the test, did not produce any analgesic activity in this test (Study: GPP-007-NCD-PCL-2000-098).

4.3 Safety Pharmacology

Safety pharmacology studies were performed *in vitro* and *in vivo* in several nonclinical species; some of which evaluated oral doses of vilazodone up to 360 mg/kg relative to the doses effective in animal models at 1 mg/kg to 10 mg/kg.

CNS Effects:

The general behavioral effects of vilazodone administered acutely or subchronically (once daily for four days) were measured in mice and rats using an oral doses from 36 to 360 mg/kg.

Irwin test (standard) in mice (study: GPP-007-NCD-PCL-1995-094), Vilazodone was administered orally at doses of 36, 120, and 360 mg/kg, either as a single administration or subchronically (once daily for 4 days). Measurements were made at 1 h, 4 h, and 24 h after the single dose or after the last subchronic administration.

The acute administration of vilazodone induced a dose-related stimulation during the first 4 hrs of the treatment which was still present 24 hrs later. There were no signs of muscular relaxation or reflex inhibition at any dose or at any point in time. The

stimulation was manifested in the home cage by increased motor activity of all animals during the first hr after treatment.

All mice treated with 360 mg/kg showed polyuria from 4 to 24 h after administration, and some of the animals exhibited jumping behavior at this dose. Rectal temperature was not affected at any dose.

Subchronic administration of vilazodone induced stimulation at all doses, with the greatest effect seen at 120 mg/kg. This effect was evident during the first 4 hrs post dose, but not at 24 h.

Except for a borderline muscular relaxation 1 hr at 360 mg/kg, there were no signs of muscular relaxation or reflex inhibition. Increased defecation and jumping behavior were observed in some animals during the first 4 hrs. Significant hyperthermia was noted at 120 and 360 mg/kg that lasted up to 24 h after the last administration.

The subchronic administration of vilazodone resulted in significantly lower body weights at various times during treatment, except of mice at 360 mg/kg that had significantly lower body weights at the end of the treatment.

Irvin test in rats: vilazodone (36, 120, and 360 mg/kg) administered either as a single administration or once daily for 4 days caused increases in exploratory activity, mainly during the first two hours; increased sensitivity of reflex responses, defecation, and vocalization induced by handling. Rectal temperature and body weight were not affected by the treatment.

Anticonvulsant Effects: Vilazodone (36, 120, 360 mg/kg) administered orally to mice produced no protective effects against pentylenetetrazole-induced convulsions or did not alter oxotremorine-induced tremor, but showed a dose-related tendency to protect against seizures induced by electroshock (statistically not significant), with effect of 20, 40, and 50% at 36, 120, and 360 mg/kg (Study: GPP-007-NCD-PCL-1995-094).

Drug Dependence: Vilazodone did not demonstrate abuse liability or dependence potential in 4 different models of behavioral dependence in mice or rats (Conditioned Place Preference; Saelens Test; Spontaneous Withdrawal Following Chronic Administration and Heroin Substitution)

Cardiovascular Effects

hERG channel assay: Vilazodone (0.01 – 10.0 μ M) was tested at hERG K⁺ channels stably expressed in CHO cells (Study: GPP-007-NCD-PCL-2000-100). In this study vilazodone had no effect at any concentration tested (< 20% inhibition at all doses), indicating low potential for producing increases in QTc interval duration and related arrhythmias in vivo.

Papillary Muscles of Guinea Pig Heart: Proarrhythmogenic potential of vilazodone's side effects was investigated by examining the effect of vilazodone on action potentials in isolated right ventricular papillary muscles of the guinea pig heart (Study: GPP-007-NCD-PCL-2000-099). The results of this study demonstrated that resting membrane potential,

action potential amplitude, and action potential duration at 90% and 20% repolarization (APD₉₀ and APD₂₀, respectively) were affected by vilazodone, at concentrations up to 3 µM, relative to vehicle. Due to the limited solubility of vilazodone, effects could not be studied at concentrations higher than 3 µM. Thus, a pronounced prolonging effect of vilazodone was not found, indicating that proarrhythmogenic properties are unlikely.

Blood Pressure: Orally administered vilazodone (30, 100 mg/kg) was tested for effects on blood pressure in conscious, spontaneously hypertensive rats. Vilazodone did not affect mean arterial pressure or heart rate relative to vehicle during the 210 min post-dosing test session (Study: GPP-007-NCD-PCL- 995-101).

The effects of slow intravenous administration (5 min infusion) of 0.1 mg/kg, 1 mg/kg and 3 mg/kg vilazodone in propanediol solvent on hemodynamic parameters and blood-gas values in anesthetized normotensive pigs were investigated (Study: GPP-007-NCD-PCL-1995-102). During a test period of 3 h and at doses up to 3 mg/kg, vilazodone produced no changes in the measured and calculated cardiovascular and blood-gas values in anesthetized pigs.

Gastrointestinal Effects

In a series of studies, the potential gastrointestinal effects of orally administered vilazodone (36, 120, and 360 mg/kg, acute or subchronic for 4 days) were assessed in mice and rats (Study: GPP-007-NCD-PCL-1995-094). The effect of vilazodone on gastrointestinal transport was assessed by measuring the distance in the intestine traversed by charcoal during a given time. Vilazodone was administered orally 0.5 h before charcoal loading and 0.25 h (rats) or 0.5 h (mice) later, the animals were sacrificed and the distance the charcoal traveled in the intestine was measured. This study results revealed that vilazodone did not change the rate of the gastrointestinal transit in mice or rats. Effects on gastric emptying were also measured as transit of a bolus of carboxymethylcellulose and phenol red out of the stomach 20 min after its administration. Orally administered vilazodone (0.5 h pretreatment) did not affect the rate of stomach emptying in rats. However, the tendency to reduced gastric volume was observed.

Overall, the safety pharmacology studies conducted with vilazodone did not reveal any significant areas of concern.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption, distribution, metabolism, and excretion (ADME) were examined in mice, rats, dogs, and/or cynomolgus monkeys. With the exception of cynomolgus monkeys, all other species were used in safety pharmacology and/or toxicology studies. The formulations used in non-clinical PK studies were vilazodone in 0.25% hydroxypropyl methylcellulose (HPMC) or in 60% polyethylene glycol (PEG) 200.

Absorption: Vilazodone was absorbed after oral administration in all nonclinical species and humans. Peak plasma concentration generally occurred within 4 h postdose. Single oral dose PK data for vilazodone (radiolabeled with ^{14}C) in laboratory animals and in humans are summarized in the following Sponsor's table:

Species	Sex	Dose (mg/kg)	Formulation	Tmax (h)	Cmax (ng/ml)	AUC _(0-∞) (ng·h/ml)	F (%)
Mouse	M	1	PEG b	0.5 ^c	49.6 ^c	ND	ND
Mouse	F	1	PEG	0.5 ^c	81.4 ^c	ND	ND
Rat	M	1	PEG	1.0	6.34	17.2 ^e	5.2 ^f
Rat	F	1	PEG	4.0	20.4	99.3 ^e	28.4 ^f
Dog	F	1	PEG	3	10.3	66.9	15.6
Monkey	F	1	PEG	2.5	46.9	187	27.4
Human	M/F	20mg	Aqueous solution	4.3	92.3	2071	72.3

b) 60% PEG200, c) total radioactivity, e) AUC_{0-t}; t = 4 h, f) bioavailability determined from AUC_{0-t} (recalculated from original data)

Low oral bioavailability was observed in rats (~5% in males and ~28% in females), dogs (< 16%), and monkey (~27%) however in humans, oral bioavailability of vilazodone was 72% - 81%. Systemic availability of vilazodone-related radioactivity following oral administration of ^{14}C -vilazodone ranged from 40%-59% in mice, rats and dogs, but was 72% in monkeys. The exposure of rats treated with single dose of vilazodone (lower dose range) was several times higher in females than in males. However, at repeat dose paradigm, the exposure in males approached that of females. No sex difference was observed in dogs and exposure trends were similar after single and repeat dosing, generally increasing in a dose proportional manner at lower doses (up to 10 mg/kg) and less than dose proportional at > 10 mg/kg (more details in general toxicology section). No changes in exposure over time were observed in dogs. In general, low oral bioavailability of vilazodone seen in rats and dogs was considered to be partly due to poor absorption and partly due to extensive first-pass metabolism of absorbed drug.

PK data of repeat oral dosing of vilazodone in rats (reviewed in the toxicology section) are summarized in the following table:

Dose mg/kg	Day	C _{max} (ng/ml)		T _{max} (h)		AUC ₀₋₆ (ng•h/ml)		AUC ₀₋₂₄ (ng•h/ml)	
		M	F	M	F	M	F	M	F
4	1	34	81.3	3	3	141	292	NC	NC
	28	38.2	134	1-3	1-3	155	508	NC	NC
20	1	154	450	3	3	564	1660	NC	NC
	28	326	827	3	3	1140	3070	NC	5070
100	1	415	751	3	3-6	1800	3380	4180	8760
	28	1270	1960	3-6	3	4970	7610	10300	15300
100	1	540	989	5	3	2140	4220	5740	9930
	28	924	2860	5	3	410	11600	10000	24400
300	91	2940	3650	3	3	12600	14600	27600	34400
	1	852	2850	4	24	3370	4950	11200	40100
	28	2940	4100	4	3	12800	17800	32800	45800
1000	91	2810	2930	5	3	12600	13500	39600	45100
	1	1510	3890	11	24	4380	7830	23200	57300
	28	3510	4840	3	4	16000	23400	50400	82600
	91	3720	3760	10	17	17400	19700	67800	77500

The PK data of repeat dosing in rats show that exposure increased less than dose proportionally and increased over time.

In the repeat dose study in humans, the exposure was generally dose proportional over the dose range studied and increase between Day 1 and Day 16 as shown in the following Sponsor's table (study: GPP-007-CLN-CP1-1998-230 under fasting condition)

Dose (mg/kg)	Day	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (ng•h/mL)	t _{1/2} (h)
10	1	14.0	5.0	161	25.3
	16	24.8	3.5	293	30.9
20	1	29.9	5.0	336	23.0
	16	45.5	4.5	528	29.5
40	1	39.8	5.0	488	24.5
	16	59.5	4.0	755	28.9

Administration of vilazodone with food increased exposure in humans, e.g. AUC_{0-∞} = 777 and 1645 ng•h/ml assessed at 20 and 40 mg, respectively (Study: PGX-08-P1-06)

Distribution:

The volume of distribution of vilazodone ranged from 4-7 l/kg across nonclinical species; in humans the mean volume of distribution after IV administration was approximately 8 l/kg. These values are greater than the volume of total body water, suggesting that vilazodone is distributed into tissues. It has than been determined that e.g. in rats after oral dosing 70%-90% of the radioactivity in tissues was present as unchanged

vilazodone. Moreover, substantial depositions of crystalline material, presumably the drug, were observed in several organ tissues in rat toxicology studies.

The following table (reproduced from the Sponsor) summarizes the distribution of unchanged vilazodone in selected tissues following repeat oral dosing in rats:

		Males (ng/ml or ng/g)							
Dose (mg/kg/day)	Day	Plasma	Liver	Kidney	Adrenals	Pancreas	Brain	Ref	
10	14	9.17	222	51.1	x	x	< 10	[a] b	
30	14	7.63	594	159	x	x	14.5		
100	14	55.3	1730	1500	x	x	50.0		
300	14	358	5430	2100	x	x	148		
4	28	NS	202	90.7	649	210	< 50	[c] b	
20	28	NS	327	148	1090	231	< 50		
100	28	NS	1070	673	2790	1370	57.0		
100	91	713	20900	NS	x	x	367	[d] e	
300	91	1500	37500	NS	x	x	688		
1000	91	3020	57300	NS	x	x	1490		
3	182	24.5	2510	739	x	x	72.5	[f] e	
15	182	353	12200	4520	x	x	221		
75	182	699	20600	11100	x	x	370		
		Females (ng/ml or ng/g)							
Dose (mg/kg/day)	Day	Plasma	Liver	Kidney	Adrenals	Pancreas	Brain	Ref	
10	14	4.75	564	88.1	x	x	< 10	[a] b	
30	14	9.84	1010	187	x	x	15.6		
100	14	60.4	2460	859	x	x	53.4		
300	14	1350	18700	8340	x	x	470		
4	28	NS	495	85.8	648	158	< 50	[c] b	
20	28	NS	1050	384	1790	763	33.4		
100	28	NS	2280	1030	3230	1490	92.1		
100	91	973	23800	NS	x	x	516	[d] e	
300	91	1920	41100	NS	x	x	883		
1000	91	3550	91800	NS	x	x	1450		
3	182	84.8	3720	897	x	x	55.8	[f] e	
15	182	286	9290	3540	x	x	155		
75	182	1110	25700	13900	x	x	553		

a) GPP-007-NCD-TOX-1995-172; b) at time of necropsy (24 -26 h after last dose); c) GPP-007-NCD-TOX-1995-173; d) GPP-007-NCD-TOX-1999-153 & GPP-007-NCD-PKM-1999-022; e) at time of necropsy (1 h after last dose); f) GPP-007-NCD-TOX-1997-176; x) sample not scheduled (protocol); NS – no sample.

The highest tissue concentrations of radioactivity were found in organs of absorption and elimination (GI tract, liver, kidney), and in adrenal, pancreas, and lungs.

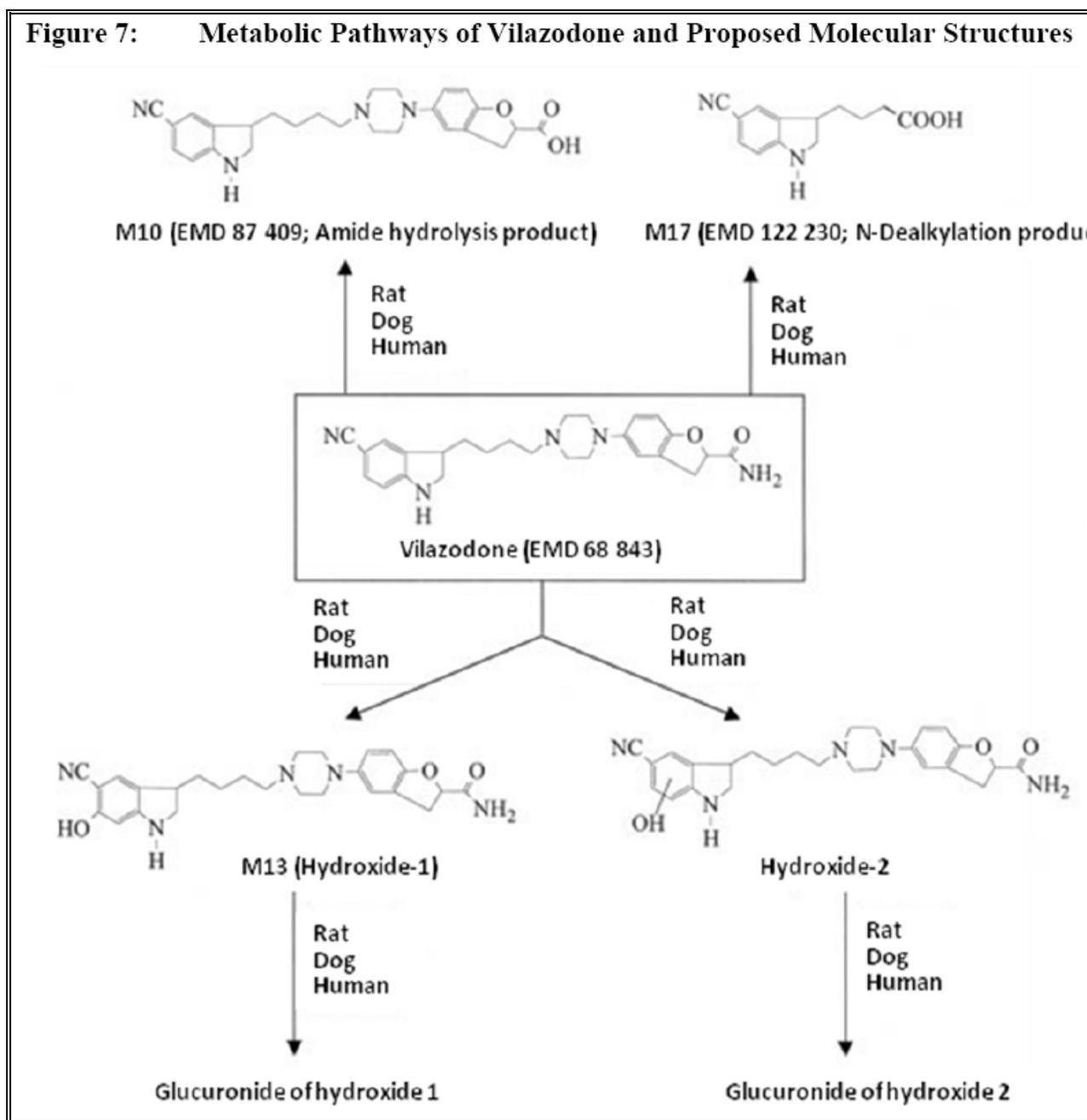
Concentrations in brain tissue were generally comparable to, or slightly lower than, plasma concentrations.

After administration of ^{14}C -vilazodone to pregnant or lactating rats, < 1% of radioactivity was found in fetuses or in suckling pups, indicating that very little vilazodone crossed the placental barrier of rats and that little was secreted into the milk of lactating rats (GPP-007-NCD-PKM-1999-013).

^{14}C -Vilazodone exhibited > **96% serum protein binding in all species tested**. Binding to serum proteins was independent of concentration. There was no preferential partitioning of ^{14}C -vilazodone into red blood cells in mice, rats, and dogs, with greater levels being seen in the plasma fraction.

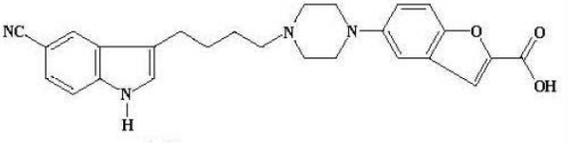
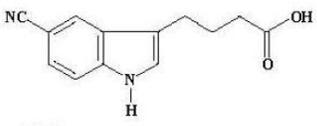
Metabolism: Vilazodone was extensively metabolized by all nonclinical species and humans (*in vivo*) and the metabolic profiles were similar between species. Twenty seven metabolites were identified and numbered from M1 to M27 by GSK during their drug development phase. The major route of metabolism of vilazodone was hydroxylation at various locations followed by glucuronidation (primary) or sulfation (secondary).

The sponsor provided (Nonclinical Overview section) the following figure showing the metabolic pathways in humans and the primary toxicology species, rat and dog.



Unchanged vilazodone and two metabolites, M10 and M17, have been identified in human urine; these metabolites were also present in the urine of dogs and rats. M10 was also identified as a major metabolite in the plasma of rats and dogs during toxicology testing.

Structures of two main metabolites identified in all nonclinical species and in humans (M10 and M17) are shown in the following table excerpted from the Sponsor's table:

GSK M#	EMD#	MW	Merck M#	Structure
M10	EMD 87 409	442.52	M442	
Vilazodone hydrolyzed to 5-{4-[4(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxylic acid				
M17	EMD 122 230	228.25	M228	
Vilazodone N-dealkylated and subsequently oxidized to 4-(5-cyano-3-indolyl)-butyric acid				

In vivo metabolism in animal species:

Mouse: Numerous metabolites (structures not identified) were noted in the plasma of mice treated with vilazodone, all had shorter retention times than the parent indicating a higher polarity. Less than 10% of plasma radioactivity remained as unchanged vilazodone by 2 h postdose

Rat: After oral dosing the metabolism was extensive, with unchanged vilazodone at 25 – 35% of plasma radioactivity (in contrast to 55 – 57% after IV administration) at 0.5 h postdose, indicating a significant first-pass metabolic effect. M10 metabolite was the most abundant metabolite in plasma (7.4 % at 1 h and 3.1% at 4 h) and tissues (in the liver – 10.8% at 1 h and 6.9% at 4 h) of total radioactivity in rats (it was also formed when incubated with rat plasma *in vitro*). M17 was not found in the rat plasma.

After a single oral dose of ¹⁴C-vilazodone, 12 mg/kg (GPP-007-NCD-PKM-2002-036), biliary excretion accounted for 56% of total radioactivity within 48 h, and included at least 10 identified metabolites comprising 88% of the biliary radioactivity. Metabolites M4 and M6, glucuronides of hydroxylated vilazodone, accounted for 44% and 12% of biliary radioactivity, respectively, and together accounted for 31% of total dose. Other metabolites each accounted for < 3% of total dose; unchanged vilazodone was not detected in bile. Fecal excretion, excluding the contribution from bile was about 40% of total dose, with 90% extraction recovery. Each of the other fecal metabolites accounted for < 1% of dose, with metabolites M10 and M11 being identified. Renal clearance was negligible in rats with only 2.7% of the oral dose of radioactivity with unchanged vilazodone at 0.2% of administered dose.

In another study (GPP-007-NCD-PKM-1997-018), the amounts of metabolites M10, M17 and of unchanged vilazodone found in the urine of male rats after an oral dose of 50 mg/kg were each < 0.4% of total dose. These urinary recoveries are similar to levels observed in female dogs at 10 mg/kg and in healthy humans at 80 mg (≤ 1.0% of dose for M17, M10 and unchanged vilazodone in all three species).

Dog: Numerous metabolites were noted in the plasma, all with shorter retention times than vilazodone, indicating higher polarity compared to the parent. A major metabolite found in dog plasma was M10. In a study of male beagle dogs administered a single oral dose of ¹⁴C-vilazodone at 10 mg/kg (*GPP-007-NCD-PKM-2002-037*), plasma contained mainly unchanged vilazodone and several metabolites, including O-glucuronides of hydroxylated vilazodone and hydroxylated M10 and non-glucuronidated M10 and M11; each comprising 9% - 19% of the radioactivity (up to 12 h).

After administration of a single oral dose of ¹⁴C-vilazodone (10 mg/kg) to male dogs (*GPP-007-NCD-PKM-2002-037*), oxidation and subsequent glucuronidation products were the primary metabolites seen in the bile. An O-glucuronide of hydroxylated vilazodone represented 68% of biliary radioactivity (M4 was 5% - 23% of dose). Other metabolites (M1, M5, M6, M10, M11, M20, and M21) accounted for 24% of biliary radioactivity (2% - 8% of the administered dose). Unchanged vilazodone was not present in dog bile; however, it was the major component of fecal radioactivity in bile-duct cannulated dogs (97% of fecal radioactivity; 58% - 91% of dose), and in intact dogs (88% of fecal radioactivity; 85% of dose). M10, M11, and M13 each accounted for ≤ 6% of the radioactivity recovered in feces. Since vilazodone bioavailability in dogs is low (<16%) and the percent of unchanged vilazodone in feces from bile cannulated dogs is high (97%), most of the vilazodone found in feces probably represents unabsorbed drug.

Renal clearance of vilazodone and its metabolites was negligible in (female) dogs dosed with 1 mg/kg; 3.7% of the radioactivity being excreted in the urine over 24 h (maximum 4.7% at 120 h); 5% of urinary radioactivity, or < 0.5% of the dose, was unchanged vilazodone (*GPP-007-NCD-PKM-1995-014*).

Metabolism in humans: The metabolic profile observed in humans was qualitatively similar to the profile observed in nonclinical species. Only 2 metabolites were identified in human urine, M10 and M17; these metabolites also were identified in urine in the rat and the dog. M10 was identified as a major circulating metabolite in toxicology studies in rat and dog (2-week and 4-week duration, respectively).

However, it should be noted that 2 major metabolites, M10 and M17, were found in humans in a mass-balance study (metabolite data provided [8/31/10; submission N-0014] during this review cycle). On average, vilazodone (M16) accounted for ~34% of total circulating drug species (i.e., of total radioactive equivalence of vilazodone). Metabolite M10 accounted for ~10% and metabolite M17 accounted for ~17% of total circulating drug species (see table below).

Major circulating human metabolites after a single oral dose of [¹⁴C]-vilazodone in healthy subjects (table excerpted from the report for clinical study PGX-08-P1-07; highlighting added by the Reviewer):

Table 2. Summary of Concentrations of Vilazodone Related Substances in Plasma

Subject ID	Time Range (h)	Concentrations in ng-eq/mL of Vilazodone free base, and as % of Total Vilazodone-derived Material in the Pooled Sample											
		M16		M10		M11		M17		Sum of 4 Analytes Conc Percent of Total	Mean ¹³ C Concentration in Pooled Plasma Sample		
		Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total				
0701-002	0.5-4	14.72	(43.3%)	2.05	(6.0%)	0.72	(2.1%)	N/A		17.50	(51.5%)	34.0	(100%)
0701-002	5-8	32.10	(54.8%)	5.40	(9.2%)	0.54	(0.9%)	6.82	(11.6%)	44.90	(76.5%)	58.6	(100%)
0701-002	10-24	13.23	(32.7%)	3.39	(8.4%)	0.76	(1.9%)	6.54	(16.2%)	23.90	(59.1%)	40.5	(100%)
0701-003	0.5-4	14.63	(20.4%)	3.94	(5.5%)	0.97	(1.3%)	3.83	(5.3%)	23.40	(32.6%)	71.7	(100%)
0701-003	5-8	26.60	(30.9%)	9.35	(10.9%)	0.73	(0.9%)	15.79	(18.4%)	52.50	(61.0%)	86.0	(100%)
0701-003	10-24	24.27	(51.6%)	8.64	(18.4%)	0.48	(1.0%)	11.35	(24.1%)	44.70	(95.1%)	47.0	(100%)
0701-004	0.5-4	12.86	(24.6%)	2.15	(4.1%)	0.54	(1.0%)	4.59	(8.8%)	20.10	(38.6%)	52.2	(100%)
0701-004	5-8	19.95	(35.6%)	8.11	(14.5%)	0.74	(1.3%)	7.32	(13.1%)	36.10	(64.5%)	56.0	(100%)
0701-004	10-24	13.57	(43.1%)	4.65	(14.8%)	0.21	(0.7%)	5.14	(16.4%)	23.60	(74.9%)	31.4	(100%)
0701-005	0.5-4	10.57	(25.9%)	2.24	(5.5%)	0.57	(1.4%)	8.07	(19.8%)	21.50	(52.5%)	40.9	(100%)
0701-005	5-8	14.34	(20.3%)	6.94	(9.8%)	0.76	(1.1%)	16.16	(22.8%)	38.20	(53.9%)	70.8	(100%)
0701-005	10-24	10.40	(22.8%)	6.95	(15.2%)	0.36	(0.8%)	N/A		17.70	(38.8%)	45.6	(100%)
0701-007	0.5-4	8.66	(27.7%)	2.34	(7.5%)	0.55	(1.8%)	2.01	(6.4%)	13.60	(43.3%)	31.3	(100%)
0701-007	5-8	12.51	(22.4%)	6.54	(11.7%)	0.46	(0.8%)	7.73	(13.9%)	27.20	(48.9%)	55.8	(100%)
0701-007	10-24	15.35	(38.4%)	5.58	(14.0%)	0.34	(0.9%)	8.41	(21.0%)	29.70	(74.2%)	40.0	(100%)
0701-010	0.5-4	12.25	(28.7%)	2.44	(5.7%)	0.30	(0.7%)	5.61	(13.1%)	20.60	(48.3%)	42.7	(100%)
0701-010	5-8	21.03	(33.8%)	12.11	(19.5%)	0.44	(0.7%)	17.26	(27.8%)	50.80	(81.8%)	62.1	(100%)
0701-010	10-24	24.54	(69.8%)	7.36	(20.9%)	0.20	(0.6%)	11.56	(32.9%)	43.70	(124.3%)	35.1	(100%)
0701-011	0.5-4	15.53	(28.0%)	1.69	(3.0%)	0.32	(0.6%)	N/A		17.50	(31.6%)	55.5	(100%)
0701-011	5-8	31.80	(36.8%)	5.94	(6.9%)	0.42	(0.5%)	N/A		38.20	(44.1%)	86.5	(100%)
0701-011	10-24	14.71	(32.5%)	3.40	(7.5%)	0.28	(0.6%)	8.88	(19.6%)	27.30	(60.2%)	45.3	(100%)
Mean	0.5-4	12.75	(28.4%)	2.41	(5.3%)	0.57	(1.3%)	4.82	(10.7%)	20.55	(45.7%)	46.9	(100%)
Mean	5-8	22.62	(33.5%)	7.77	(11.8%)	0.58	(0.9%)	11.85	(17.9%)	42.82	(64.1%)	68.0	(100%)
Mean	10-24	16.58	(41.6%)	5.71	(14.2%)	0.38	(0.9%)	8.65	(21.7%)	31.32	(78.9%)	40.7	(100%)

N/A - not applicable
Source: Table 1 through Table 5 of the Identification and Profile Report (Attachment A)

As it was mentioned earlier, M10 was identified in the plasma of rats and dogs, however, M17 was only found in the plasma of dogs, but was apparently not detected in plasma of rats to date.

The Sponsor's table, below, showing cross-species metabolite comparisons, is inaccurate, since it does not include M17 as present in plasma of humans and dogs [revised table will be submitted by the Sponsor, but it is not available at this time]. It is not clear whether this metabolite is circulating in rats [however, this will also be addressed by the Sponsor]:

	Human		Rat		Dog			
M1								P
M3			U ^a			P		
M4			U ^a	B			B	P
M6			U	B				
Peak A			U					
M9				B				
M10	U	P	U	B ^b	P	U	B ^b	P
M11				B ^b			B ^b	
M13			F	B ^c		F ^d		

	Human	Rat	Dog
M14			B ^c
M15		U	B
M17	U	U	U
M18		F	
M19			P
M21			B P

B - Bile; F – Feces; P – Plasma; U – Urine; ^a M3 and/or M4;

^b M10 and/or M11; ^c M13 and/or M14; ^d in the feces of intact (not bile-duct cannulated) dog

Metabolism by human cytochrome P450 isoforms: The primary cytochrome P450 isozyme responsible for metabolizing vilazodone is CYP3A4, therefore inhibitors of this enzyme can reduce drug metabolism and increase exposure (e.g. ketoconazole increased vilazodone systemic exposure by approximately 50%). CYP2C19 and CYP2D6 were shown to have minor contributions to the metabolism of vilazodone (*GPP-007-CLN-ANR-1997-028*). CYP2C8 may play a role in vilazodone metabolism since this isozyme is also inhibited by vilazodone in microsomal preparations; however, inhibition of CYP2C8 in human hepatocytes was only observed at high vilazodone concentrations (see Clinical Pharmacology Review for further details).

In vitro, vilazodone did not specifically induce the synthesis of mRNA coding for the major cytochrome P450 isozymes in human hepatocytes. Thus, vilazodone appears unlikely to be a source of drug-drug interactions producing enhanced metabolism of other drugs. These studies were supported by *in vivo* drug-drug interaction studies which demonstrated no significant interactions leading to altered PK for probe substrates of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 (see Clinical Pharmacology Review for more details).

Excretion: The mass balance studies have demonstrated that the primary mode of clearance of drug and drug-related material (total radioactivity) is through feces. In mice, rats and dogs, excretion of vilazodone is primarily via hepatic metabolism, with a pronounced first-pass effect. Excretion patterns are similar after oral and IV routes of drug administration in animals.

In rats, excretion of ¹⁴C-vilazodone at oral dose of 1 mg/kg via biliary route was at 7.6%, 30%, and 53% of the total dose eliminated by 1, 4, and 24 h postdose, respectively. Nearly total recovery of radiolabel (97.4-104.1%) was achieved within 72 h (0.02%, 2.3%, 94.8%, and 0.5% in exhaled CO₂, urine, feces, and carcass, respectively after oral dose (*GPP-007-NCD-PKM-1996-016*)).

In dogs (intact and bile-duct cannulated), after a single oral administration of ¹⁴C-vilazodone 10 mg/kg, 96.7% of the dose was eliminated in the feces in intact dogs and < 1% via the urine. Elimination was essentially complete by 48 h. In bile-duct cannulated dogs, 70.7% and 18.0% of the dose was excreted in the feces and bile, respectively within 48 h. Interanimal variability was high. Since the percent of vilazodone excreted in feces from bile cannulated dogs was high (97%), and because vilazodone bioavailability in dogs was low (15.6%), most of the radioactivity found in

feces likely represents unabsorbed drug, although some secretion via the GI epithelium may also contribute (*GPP-007-NCD-PKM-2002-038*)

In humans, similar to nonclinical species tested, recovery of unchanged vilazodone in urine was negligible, accounting for about 1% of administered dose. The excretion of vilazodone was evaluated in 7 healthy male subjects following a single oral dose of ¹⁴C-vilazodone 20 mg (*PGX-08-P1-07*). Recovery of radioactivity in urine was ~20% of the administered dose while just 1.1% of the dose was recovered as unchanged vilazodone. Recovery of radioactivity in feces was ~65% of the administered dose while just 1.8% of the dose was recovered as unchanged vilazodone. Urine and feces combined, during the 14-day period after dosing, accounted for 85% of the dose of radioactivity, but unchanged vilazodone accounted for only 3% of the dose.

As in the nonclinical species tested, fecal excretion accounted for most of the administered dose of vilazodone in humans, but unlike the nonclinical species very little of the dose recovered in the feces of humans was unchanged vilazodone. This difference is likely related to the higher oral bioavailability of vilazodone in humans compared to the nonclinical species at the doses studied. Much of the unchanged vilazodone recovered in the feces of the nonclinical species may represent unabsorbed drug.

The reviews and evaluation of TK studies that were conducted during toxicology studies are included in toxicology studies reviews.

6 General Toxicology

6.1 Single-Dose Toxicity

Mouse: 1) In NMRI (SPF) mice (5/sex/group for the control and MD; 5 males for LD and 5 females for HD), vilazodone was administered at single dose of 0, 1500, 2500 or 3500 mg/kg by oral gavage and mice were observed for 14 days (Study: GPP-007-NCD-TOX-1995-149). Deaths were seen at 2500 (3M) & 3500 mg/kg (2F) at 6 - 23 h postdose. The **oral LD₅₀ was 2307 mg/kg for males and 3797 mg/kg for females**. Clinical signs observed from 3 h to 2 days postdose included reddened tail/ears, locomotor disturbance at ≥ 1500 mg/kg, dyspnea, cyanosis of tail tip, abdominal position at ≥ 2500 mg/kg. Decreased BW gain (50%) and mean BW (9 - 12%) in females at 3500 mg/kg was observed on Day 2 and Day 4 of the study

2) In NMRI (SPF) mice (4-6/sex/group) after i.p. administration of vilazodone at 0, 1000 or 2000 mg/kg. Deaths were seen at 1000 mg/kg (1M, 2F) and 2000 mg/kg (4M, 1F) from 23 h to 3 days postdose; the **i.p. LD₅₀ was 1334 mg/kg for males and > 2000 mg/kg for females**. Locomotor disturbance, cyanosis, feces retention, abdominal position, dyspnea were observed at ≥ 1000 mg/kg until Day 3 postdose. Decreased BW gain at 1000 and 2000 mg/kg was observed on Day 2 of the study. Test material deposition, and focal fibrous organ adhesions was noted in all animals at sacrifice and in 1M (died Day 3). Foreign body granulomas on serosa of stomach and liver, fibroses of spleen/ liver capsules were noted microscopically as response to test material deposition.

Rat: 1) In Hsd/Win:Wu rats (5/sex/group) single dose of 0 or 5000 mg/kg by oral gavage did not cause any deaths (**LD₅₀ for oral dosing was > 5000 mg/kg**). Signs of toxicity included decreased BW gain on Day 2 (47% in males and 66% in females), locomotor disturbance, blood-crusted snout and dyspnea on Day 1 and pale feces on Days 2 to 4.

2) In Hsd/Win:Wu rats (5/sex/group; doses 0 and 2000 mg/kg) single i.p. dose of 2000 mg/kg caused no deaths (**LD₅₀ for i.p. dosing was > 2000 mg/kg**). Signs of toxicity included locomotor disturbance; salivation, dyspnea and blood-crusted snout on Day 1; wet anal region and reduction of BW gain by 59% in males and 66% in females on Day 2. Test article deposition and subsequent inflammation with fibroplastic reaction in the omentum majus (large fold of peritoneum that hangs down from stomach), liver capsule, and perihepatic tissue, secondary liver enlargement and atrophy of the testis and ovary was noted (macroscopically and microscopically).

Dog: In male Beagle (1-3/group) single i.v. doses of 0, 0.4 or 1.2 mg/kg as a bolus and 1.75 mg/kg via a 2 h infusion caused no deaths but strong adverse reactions that included salivation, abnormal respiration, tremors, overactive/agitated behavior, vocalization, abnormal gait and pupil dilation. It was concluded that vilazodone was not tolerated by dogs at i.v. doses of 0.4, 1.2 (bolus) or 1.75 mg/kg (2 h infusion).

6.2 Repeat-Dose Toxicity

Summary of studies shorter than 6 month in duration (2-weeks and 4-weeks) in rats:

EMD 68 843 -2-Week Oral Dose Finding Study in Rats (Study GPP-007-NCD-TOX-1995-172):

The purpose of this non-GLP study was to gain initial experience on the toxicity of vilazodone after oral administration to rats.

HSD:WIN/WU rats, 7-8 weeks old and weighing 148-204 g (5/sex/group), were administered 0, 10, 30, 100, and 300 mg/kg of vilazodone (EMD 68 843; batch 5) in 0.25% aqueous Methocel® (HPMC) by oral gavage at 5 mL/kg once daily for 2 weeks. Morbidity and mortality observations were recorded. Body weight (BW) and food and water consumption were measured. Concentrations of vilazodone and its metabolite M1, later termed M10, were measured in plasma and concentrations of vilazodone were measured in selected tissues. All animals underwent a complete necropsy upon completion of treatment. Organs were weighed and examined microscopically.

Results: No mortality occurred in this study. In females, BWs were increased by 12% and food consumption was increased 13-14% at 30-100 mg/kg/d and both by about 5-6% at 300 mg/kg/day. There was no effect on water consumption. Mild to moderate impairment of the general condition was seen at ≥ 100 mg/kg/d, reddish discoloration of the skin at ≥ 10 mg/kg/day (dose-dependent incidence and severity), salivation at ≥ 100 mg/kg/day, one male with abnormal vocalization at 100 mg/kg/d, and nasal discharge and temporarily open mouths at 300 mg/kg/d.

No gross or organ weight changes were attributed to treatment with vilazodone. Histopathological examination revealed a dose-dependent increase in focal or multifocal accumulation of foam cells in the lung in 6/10 rats at 100 mg/kg/day and in all 10/10 rats at 300 mg/kg/day.

Vilazodone was well-tolerated in rats when administered at doses of 10 and 30 mg/kg/day for 2 weeks.

Plasma concentration data indicated that vilazodone was absorbed and systemic exposure was achieved throughout the study. Plasma concentrations at 1 h post dose increased with repeated dosing from 1.3- to 2.2-fold in males at all doses and 1.3- to 3.4-fold in females at 30-300 mg/kg/day. The increases in plasma concentration were less than dose-proportional in both sexes and at all time points. Plasma and tissue concentrations were generally higher in females than in males. Liver and kidney concentrations were up to 100-fold higher than plasma at 26 h after the final dose, while brain concentrations were comparable to plasma concentrations except at the high dose, where they were lower than plasma in both sexes as shown in the following Sponsor's table:

Mean (N=2) plasma and tissue concentrations of unchanged EMD 68 843							
Values in parentheses are dose-normalized concentrations							
dose [mg/kg]	sex	plasma concentrations [ng/ml]			tissue concentrations [ng/g] at the time of necropsy (approx. 26 h after the last dose)		
		day 1 1 h	day 7 1 h	day 14 approx. 26 h	liver	kidney	brain
10	male	139 (13.9)	180 (18.0)	9.17 (0.917)	222 (22.2)	51.1 (5.11)	-
	female	629 (62.9)	570 (57.0)	4.75 (0.475)	564 (56.4)	88.1 (8.81)	-
30	male	333 (11.0)	742 (17.7)	7.63 (0.254)	594 (19.8)	159 (5.30)	14.5 (0.483)
	female	794 (26.5)	1000 (33.3)	9.84 (0.328)	1010 (33.7)	187 (6.23)	15.6 (0.520)
100	male	594 (5.94)	878 (8.78)	55.3 (0.553)	1750 (17.5)	1500 (15.0)	50.0 (0.500)
	female	1580 (15.8)	2000 (20.0)	60.4 (0.604)	2460 (24.6)	859 (8.59)	53.4 (0.534)
300	male	1100 (3.67)	2030 (6.77)	358 (1.19)	5430 (18.1)	2100 (7.00)	148 (0.493)
	female	1140 (3.81)	3830 (12.8)	1350 (4.50)	18700 (62.3)	8340 (27.8)	470 (1.57)

-: Value below the limit of quantitation (approx. 4 ng/ml for plasma, 50 ng/g for liver and

Study title: EMD 68 843 - 4 week oral toxicity study in rats

Study: GPP-007-NCD-TOX-1995-173 (T 14011; 40/90/95)

This study was reviewed by E.A. Gonzalez Barry, M.S. (IND 54,613; review dated April 4, 1998). The following is the summary of this study.

Vilazodone (EMD 68 843) was administered to (15 M/15 F) rats at 0, 4, 20, and 100 mg/kg/d by oral gavage in 5ml/kg vol. in the vehicle 0.25% methocel. Animals were weighed and clinical observations were conducted regularly during the study. PK data were collected from a satellite group (5 sex/group; 2/sex controls) on days 1 and 28 of study at 4 time intervals. When rats were sacrificed, organs were collected/weighted and selected organs were histologically examined.

Results: No deaths occurred during the study. Briefly, toxic signs reported at ≥ 20 mg/kg included mild clinical, signs (i.e., furless skin). In the HDF, hematology revealed a mild increase in reticulocyte counts and blood urea was also increased at HDM (+14%) and HDF (+10%) at the end of study. Decreased liver weights (absolute and relative in HDM and relative in HDF) and increase in adrenal weights (absolute and relative) in HDM and decrease (relative) in HDF were reported without any morphological correlation to this finding. Histopathology was unremarkable.

One HDF showed a unilateral massive bleeding in one cerebral hemisphere of unknown causes. No other remarkable toxicologic changes were reported in this 4 –week study.

TK parameters reported indicated that peak plasma concentrations were reached after 3 h of drug administration were dose related. At 24 h concentrations of vilazodone were 50 times lower than T_{max} suggesting a short half-life. After 28 days of dosing, the AUC values were on average twice as high as on day 1, suggesting either a lower extent of absorption on day 1 or a decrease in clearance after repeated dosing. In males, AUC increased somewhat in proportion to the dose, and in females AUC values were higher at the HD than for the LD and MD. At all doses (Day 1 and Day 28), concentrations of the drug were higher in females than in male rats. Tissue conc of the drug in liver, kidney, pancreas and adrenals were higher than in brain, but brain conc were lower than in plasma.

Study title: EMD 68 843 - 26 week oral toxicity study in rats with 8 week recovery period

Study no.: GPP-007-NCD-TOX-1995-176 (T 14019)

Study report location: Institute of Toxicology at E. Merck, Darmstadt, Germany

Conducting laboratory and location: Institute of Toxicology at E. Merck, Darmstadt, Germany

Date of study initiation: June 24, 1996

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: EMD 68 843, Batch No. EE77185, 99.4% purity

Key Study Findings:

Male and female rats were dosed orally at 0, 3, 15, and 75 mg/kg/day for 6 months.

Findings were limited to:

Death of one HDM (on study day 105)

Clinical signs at HD: impairment of general condition in 6 HD (3/sex); salivation; slight reddening of hairless skin areas transiently seen in all drug treated rats

Increased leukocytes and neutrophilic segmented granulocytes at HD

Increased urea at HD and MD rats of both sexes

Drug-related histopathological findings were limited to mammary gland and uterus

Methods

Doses: 0, 3, 15, and 75 mg/kg/day

Frequency of dosing: Once a day

Route of administration: Oral gavage

Dose volume: 5 ml/kg

Formulation/Vehicle: Suspension/0.25% aqueous Methocel® K4 Premium (HPMC)

Species/Strain: Rats/HSDCPB:WU

Number/Sex/Group: 20/sex/group

Age: 6-7 weeks old

Weight: Males: 179-208g; females 157-188g

Satellite groups: 3/sex/group (treatment) and 1/sex (controls)

Unique study design: none

Deviation from study protocol: none

Observations and Results:

Mortality - One HDM died on day 105 of the treatment period. Cause of death was not determined, possibly incidental.

Clinical Signs -The behavior and appearance were checked twice a day and daily on weekends.

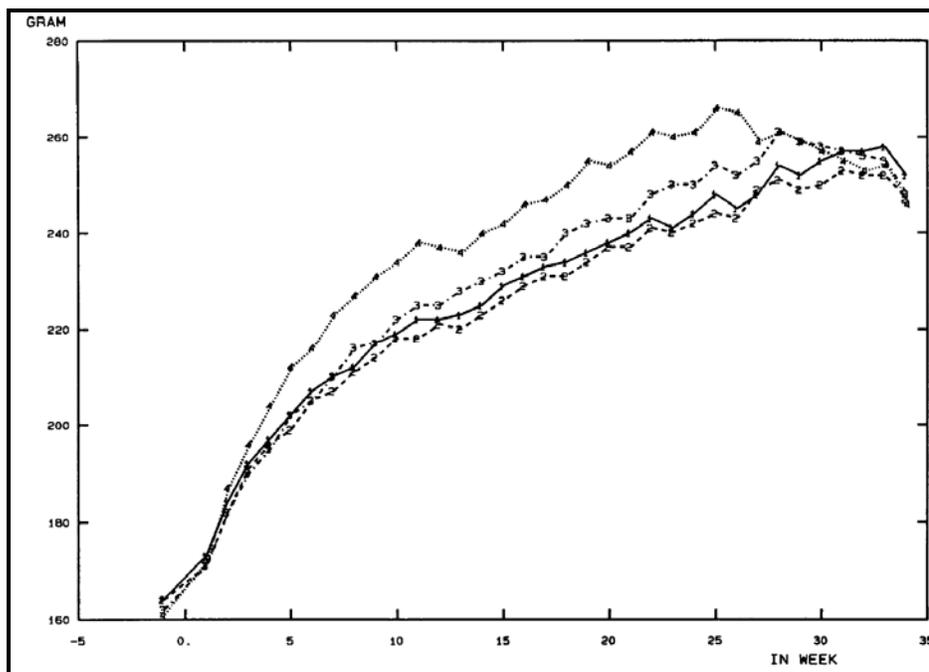
Results: Treatment related clinical findings included slight reddening of hairless skin areas transiently seen in all drug treated rats, salivation, and an **impaired general condition in 6 HD** rats as shown in the following summary table:

Symptoms	Number of rats affected							
	group 1		group 2		group 3		group 4	
	M	F	M	F	M	F	M	F
general condition impaired	1	0	0	0	1	0	3	3
loss of hair	8	6	1	7	4	3	2	5
eye lid closure	0	0	0	0	2	0	1	0
rough coat	0	0	0	0	0	0	1	0
salivation	0	0	0	0	1	0	6	6
discharge of eyes	1	2	2	1	0	0	3	2
skin lesions	0	0	1	2	0	0	1	1
hyperemia	0	0	20	20	20	20	20	20

Body Weights - Rats were weighed once a week.

The results showed an **increase in BW gain in HDF** (triangles) in the study week 3 (+ 9.5%) and week 4 (+ 10%) as shown in the following figure. No changes were observed in males (data not shown).

Sponsor's graph showing increased body weight gain in HD (4, dashed line) compared with control (1, solid line) female rats during 6-month oral dosing with vilazodone



Feed Consumption: The food consumption was determined once a week.

Results: HDF consumed more food (6-7%) than controls which correlated with increased BW gain.

Ophthalmoscopy: The examination was performed before the start of treatment and in weeks 13 and 26 in 10/sex/group.

Results: No findings related to the treatment with vilazodone.

Sperm analysis: The spermatozoa were collected from epididymis of all male rats at scheduled necropsy (due to lack of changes no examination was performed at the end of recovery period)

Results: No differences were seen between treated and untreated rats.

Hematology: The following parameters were evaluated in weeks 4, 13, 26 (10/sex/group) and in week 34 (5/sex/group): WBC (leukocytes), RBC, HGB, HCT, MCV, MCH, MCHC, PLAT, RET, Lymph, NEU-SE (number of segmented neutrophilic granulocytes).

Results: A slight increase in the number of leukocytes and in neutrophilic segmented granulocytes (not statistically significant) was observed in HD rats, which normalized by the end of the 8-week recovery period (week 34), as shown in the following Summary tables provided by the Sponsor.

Leukocytes (LEUK/NL)								
Group	Males				Females			
	week 4	week 13	week 26	week 34	week 4	week 13	week 26	week 34
1	8.52	7.81	6.87	6.60	7.42	5.59	5.18	4.66
2	9.14	8.60	7.83	7.18	6.94	5.20	4.59	4.24
3	8.20	8.22	7.66	7.24	8.04	6.63	6.32	5.46
4	9.11	9.48	10.18++	8.18	7.54	6.88	6.81+	4.26

++ significantly different $p \leq 0.01$; + significantly different $p \leq 0.05$

Neutrophilic segmented granulocytes (SE/NL)								
Group	Males				Females			
	week 4	week 13	week 26	week 34	week 4	week 13	week 26	week 34
1	0.80	0.81	0.89	0.88	0.72	0.61	0.66	0.84
2	0.82	1.15	1.43	0.84	0.59	0.35	0.45	0.62
3	0.83	1.00	1.47	1.28	0.88	0.55	1.03	0.70
4	0.85	1.19	2.18	1.12	0.53	0.76	1.72	0.46

Clinical Chemistry: The following parameters were evaluated: enzymes (ALAT, ASAT & AP), proteins, (TP & ALB), substrates (GLUC, UREA, CREA, TBIL, CHOL, TG) and electrolytes (Na, K, Ca, Cl, and PHOS). Hormone (prolactin and corticosterone) determinations were performed in week 26

Results: Blood urea appeared to be slightly but consistently increased at HD and at MD, but had normalized by the end of the 8-week recovery period (week 34), as shown in the following Sponsor's summary table:

Urea (mmol/l)									
Group	Males				Females				
	week 4	week 13	week 26	week 34	week 4	week 13	week 26	week 34	
1	5.29	5.13	5.54	5.86	5.59	5.57	5.74	6.52	
2	5.48	5.48	5.44	5.20	6.32(+)	6.08	6.24	6.36	
3	5.96(+)	5.81(+)	6.36(+)	6.42	6.71++	6.99++	6.82+	6.04	
4	6.05++	6.25++	6.28(+)	6.54	6.38+	6.11	6.17	6.56	

++ significantly different $p \leq 0.01$; + significantly different $p \leq 0.05$, (+) critical range

Although increased blood urea was also observed in the 4-week rat study at 100 mg/kg it is difficult to interpret these data based on the lack of functional or histopathological correlates.

Other changes observed in the clinical chemistry parameters were only marginally lower or higher than controls (not at abnormal levels), therefore were not considered toxicologically meaningful.

Hormones: Corticosterone was not changed by the treatment with vilazodone but prolactin was slightly but significantly decreased in females of all drug treatment groups (-16%, -56%, and -53% at LD, MD, and HD, respectively).

Urinalysis: The following parameters were evaluated: pH, protein, GLUC, UROB, BIL, blood and sediment.

Results: No toxicologically relevant findings were reported with the exception of increase in number of vilazodone treated rats with proteinuria observed from week 13 onwards until recovery.

Gross Pathology: At the end of study rats were necropsied and examined for gross pathological alterations.

Results: No organ alterations were observed in vilazodone treated rats.

Organ Weights: The specific organs that were weighted are included in the histopathology inventory table (at the end of General Toxicology section) below.

Results: Decreased liver weights (-12% in HDM) and decreased spleen weight (-16% and -14%, in HDM and HDF, respectively) were noted, without histopathological correlates. In HDF the mean uterine weights were lower (31%) than controls possibly due to slightly lower incidence of proestrus/estrus phase.

Histopathology: Tissues examined microscopically in controls and HD rats are listed in the histopathology inventory table.

Adequate Battery: yes

Peer Review: Yes, by two (in-house) pathologists who had the same opinion on reported histopathological findings.

Histological Findings: Drug-related histopathological findings were limited to mammary gland and uterus, and findings are summarized in the following Sponsor's table:

Main kill	Group 1 0 mg/kg		Group 2 3 mg/kg		Group 3 15 mg/kg		Group 4 75 mg/kg	
	15 M	15 F	15 M	15 F	15 M	15 F	15 M	15 F
Mammary gland								
retention of content, mild	0	0	0	0	3	0	4	1
atrophy	0	0	0	0	0	0	1	0
Uterus								
proestrus		3		2		2		1
estrus		4		4		1		3
diestrus		8		9		11		10
irregular cycle		0		0		1		1

Additionally, one MDF (but no other control or drug-treated females) showed mammary hyperplasia and also showed an irregular estrus cycle. No findings were observed after the recovery period.

Special Evaluation: Mild retention of condensed secretion in the excretory ducts of the mammary gland was observed in 3 MDM and 4 HDM (and 1 HDF) and another 1 HDM had atrophy of the gland. These observations are dose-related and could be caused by the endocrine disturbance (no such findings in the recovery males or females).

The HDM that died on study day 105 exhibited adrenal cortex hypertrophy, brown fat atrophy and acute organ changes that include: hyperemia in the liver, dialation of renal tubules, congestion of the right atrium, and interstitial edema of myocardium.

Toxicokinetics:

On days 1, 91, and 182, blood samples were taken 1, 3, 6 and 24 hrs after drug treatment. In addition, 1 hr after the last dose, plasma and tissue (brain, kidneys and liver) samples were obtained at necropsy for drug levels determinations.

Results: The plasma concentrations and AUC values were generally higher in F than in M. The TK parameters are summarized in the following Sponsor's table:

Mean (N = 3) pharmacokinetic parameters of the EMD 68 843 after single and repeated oral administration in rats									
Sex: Male									
parameter	group 2: 3 mg/kg			group 3: 15 mg/kg			group 4: 75 mg/kg		
	day 1	day 91	day 182	day 1	day 91	day 182	day 1	day 91	day 182
t _{max} [h]	3	1 - 3	3	3	3	3	3	3	3 - 6
C _{max} [ng/ml]	12.8	31.4	41.3	196	614	721	552	1720	2060
C _{24 h}	-	-	-	-	-	11.6	74.6	100	339
AUC [ng/ml x h]									
0 - 6 h:	51.4	137	167	738	2230	2910	2560	7080	9510
0 - 24 h:	#	#	#	#	#	4820	6160	15100	25600
AUC/dose [°]									
0 - 6 h:	17.1	45.7	55.7	49.2	149	194	34.1	94.4	127
0 - 24 h:	#	#	#	#	#	321	82.1	201	341
Sex: Female									
t _{max} [h]	1 - 3	1	1	3	3	1 - 3	3 - 6	3	3
C _{max} [ng/ml]	79.5	107	143	520	947	1060	2000	2600	2690
C _{24 h}	-	3.84	-	4.40	10.6	21.4	107	451	745
AUC [ng/ml x h]									
0 - 6 h:	272	459	460	2210	3770	4290	9000	11400	12700
0 - 24 h:	#	720	#	3210	5830	6910	20100	30400	37100
AUC/dose [°]									
0 - 6 h:	90.7	153	153	147	251	286	120	152	169
0 - 24 h:	#	240	#	214	389	461	268	405	495
-: Value below the limit of quantitation (approx. 3 ng/ml)									
#: Value not calculated									
°: AUC [ng/ml x h] / dose [mg/kg]									

Compared to the plasma levels, concentration of vilazodone were on average ~ 30 times higher in liver, 13 times higher in kidneys, and in the brain up to 2 times lower.

Stability and Homogeneity: The concentrations of all doses of vilazodone were determined twice (once after delivery and once on the last day of use) in weeks 2, 14, and 24. Initial concentrations ranged from 93-102% of nominal concentrations (except for the HD solution at week 24, which was 118%). Concentrations at the last day of use ranged from 93-104% of initial concentrations. Dosing was considered acceptable.

Summary: One **HDM died on study day 105** of unknown causes. It is considered by this reviewer that this death was incidental based on the review of other toxicology studies in which much higher doses of vilazodone were used (13-week study at 100, 300, and 1000 mg/kg and 2- year carcinogenicity [HD of vilazodone at 150 mg/kg]) and no life threatening toxicities at doses up to 150 mg/kg were observed. Slight reddening of hairless skin areas was transiently seen in all drug treated rats. **Salivation and impairment of general condition in was observed in 6 HD rats (3/sex).**

In the HDF, the BW was significantly increased from week 2 up to week 27 then normalized at the end of the recovery period. These HDF rats tended to consume somewhat more food than the controls. Clinical pathology assessment revealed increased number of leukocytes and neutrophilic segmented granulocytes in at MD and HD rats. Blood urea appeared to be slightly but consistently higher in the MD and HD groups during the study. In males, the sperm values, although statistically significant different from control, were mostly mildly affected and only little above or below the normal range, and were without an effect on physiological functions, according to drug sponsor. Prolactin was slightly but significantly decreased in LDF and HDF. Retention of condensed secretion in the excretory ducts of the mammary gland was observed in 3 MDM and 4 HDM and 1 HDM had atrophy of the gland. These observations were dose-related and could be caused by the endocrine disturbance (no such findings in the recovery males).

The NOAEL was 75 mg/kg/day and MTD not reached in this study.

Summary of studies shorter than 52 month in duration (2-, 4-, and 26-weeks) in dogs:

Dog subchronic studies of 2, 4, and 26 week duration were reviewed by E.A. Gonzalez Barry, M.S. (IND 54,613; review dated April 4, 1998). The following are the summaries of these studies.

Dose Range Finding Study in Beagle Dogs (Study#: GPP-007-NCD-TOX-1995-174):

The purpose of this non-GLP study was to determine doses for the 4 week toxicity study. Beagle dogs (**1M & 1F**; 36 & 38 months old and weighing 17.1 & 17.7 kg) were administered escalating oral (capsule) doses of **1, 3, 10 and 30 mg/kg/day** (Day 1, Day 2, Day 3 and Days 4-15, respectively) of vilazodone (EMD 68 843; batch 5). Morbidity and mortality observations were recorded. BW, food consumption, hematology and clinical chemistry parameters were measured. All animals underwent a complete necropsy. Organs from all dogs were weighed and examined microscopically. Toxicokinetics (TK) were evaluated in plasma and tissue samples.

Both dogs survived until the scheduled sacrifice. Body weights were reduced by 1.8 kg (male) and 1.0 kg (female). Food consumption was decreased throughout the study (58-66%). Clinical signs were transient and noted almost exclusively on Day 3 (at the 10 mg/kg dose) and included vomiting, salivation, and altered locomotion (increased and decreased). Gross pathology and histopathology revealed no vilazodone-related alterations. In general, doses of up to 30 mg/kg/day were tolerated well by dogs.

TK findings showed good absorption from the gastrointestinal tract. In both sexes, the plasma concentrations of vilazodone and a metabolite (M1) were similar. Maximum plasma concentrations at 4 h after the final dose of 30 mg/kg/day were 1030 and 755 ng/ml for vilazodone and 738 and 715 ng/ml for M1 in the male and the female, respectively. At the time of necropsy (~ 24 h after the last dose) concentrations of the parent (metabolite M1) in the brain were ~ 2 (5) – and in the liver ~ 100 (500) – fold higher than the 24 h concentrations in plasma.

EMD 68 843 - 4-week oral toxicity study in beagle dogs (Study#: GPP-007-NCD-TOX-1995-175)

Vilazodone was given orally (gelatin capsules) to dogs (3/sex/group; BW of 12 to 15 kg; 11 month old) at **0, 3, 10, and 30 mg/kg** once a day, every day for 4 weeks.

Dogs were observed daily, BW and food consumption recorded weekly (before and during the treatment). ECG and blood pressure measurements were collected at pre-dose (week -2) and at week 3 in all HD dogs and controls prior to- and 2 h after dosing. Ophthalmological examination was performed on all dogs prior to treatment and in week 3 of dosing. Clinical pathology examinations were conducted at pre-dose and in week 4 of treatment. Blood samples for PK determinations were collected at 0, 1, 2, 4, 6, and 24 h after dosing on days 1 and 25 of the study. Gross pathology and histopathology examinations were performed on all dogs.

No deaths were observed. Clinical symptoms were seen primarily during the first two weeks of treatment and consisted of fearfulness, salivation, hypokinesia at HD and MD and mydriasis at HD only. Clinical chemistry revealed a slight increase in glucose level in HDM and MDM. There were no other treatment-related findings in the study. The NOAEL was 30 mg/kg.

The PK data showed AUC values proportional to the dose and lack of sex difference in exposure. Mean (M+F) PK parameters are shown in the following Sponsor's table:

parameter	group 2: 3 mg/kg		group 3: 10 mg/kg		group 4: 30 mg/kg	
	Day 3	Day 25	Day 3	Day 25	Day 3	Day 25
t _{max} [h]	2 - 4	1 - 6	2 - 24	2 - 4	2 - 24	2 - 24
C _{max} [ng/ml]	33.1	57.5	223	129	316	498
C _{24h}	-	-	107*	-	202	233
AUC [ng/ml x h]						
0 - 6 h:	128	213	523	495*	900	2000
0 - 24 h:	x	x	2350*	x	4010	6360
AUC/dose ^a						
0 - 6 h:	42.7	71.0	52.3	49.5*	30.0	66.7
0 - 24 h:	x	x	235*	x	134	212

6-month oral toxicity study in beagle dogs (Study-007-NCD-TOX-1997-177)

Vilazodone was given orally (gelatin capsules) over period of 26 weeks to dogs (5/sex/group; 11.4 to 15.2 kg, ~11 month old) at daily doses of 0, **2.5, 10, and 40 mg/kg**. Treatment period was followed by 2 month recovery period (2/sex/group). [The same doses were used in the 1-year study that is reviewed in detail below.]

Dogs were observed daily, BW and food consumption recorded weekly (before and during the treatment). ECG and blood pressure measurements were collected at pre-dose (week -4) and at weeks 2, 11, and 24 in all dogs 2 h after dosing. Ophthalmological examinations were performed on all dogs prior to treatment and in weeks 1, 2, 13 of dosing. Clinical pathology examinations were conducted at pre-dose and in week 1, 2, 13, 23, and 34 of the study. Gross pathology examination and histopathology was performed on all dogs.

Results: One **HDM** died on study Day 155 due to aspiration of vomit and subsequent cardiac failure. The general condition of that dog was comparable to control dogs (occasional vomiting was the only clinical sign observed).

Clinical signs such as fearfulness, salivation, hypokinesia, and mydriasis were seen in HD and MD dogs during the first 1 - 3 weeks of treatment and were probably pharmacological effects of the test article rather than signs of toxicity (similar to

observation in 4-week study). Emesis was occasionally seen at HD throughout the treatment period. A decrease in BW ($\leq 5.6\%$) and food consumption ($\leq 54\%$) was seen at HD during the initial part of the treatment period. No effects on ECG parameters, blood pressure or reflexes were noted.

Corneal opacities were seen in 1 MDF and 3 HDM in Week 2 and 2 HDF in weeks 1 - 2 of treatment but were not seen in the week-3 examination. No corneal opacities were present after the 8 week recovery period. A tendency for reduced tear production was seen in MD and HD dogs.

Slightly lower erythrocyte count ($\leq 13\%$), hemoglobin ($\leq 10\%$), and packed cell volume ($\leq 11\%$) were seen in HDM throughout the treatment period. These effects were reversible (not seen in dogs from the recovery group).

No treatment-related alterations were seen at necropsy. Organ weights and histopathological examinations did not reveal any treatment-related alterations.

Blood samples for PK determinations were collected at 0, 1, 2, 4, 6, and 24 h after dosing on days 3 and 179 of the study. The TK data are summarized in the following Sponsor's table:

Parameter	Plasma levels on day 179 (n=3)					
	2.5 mg/kg		10 mg/kg		40 mg/kg	
	m	f	m	f	m*	f
Cmax (ng/ml)	178	180	319	438	1210	999
AUC 0-24h (ng/mlxh)	613+	582+	3370	3390	14900	13700

m* n=2; + AUC_{0-6h}

The NOAEL was the high dose of 40 mg/kg in this study (the death of one high dose male was due to aspiration of vomit).

Study title: EMD 68 843 – 52 week chronic toxicity study in beagle dogs

Study no:	GPP-007-NCD-TOX-2000-178
Study report location:	Institute of Toxicology, Merck KGaA 64271 Darmstadt, Germany
Conducting laboratory and location:	Institute of Toxicology, Merck KGaA 64271 Darmstadt, Germany
Date of study initiation:	January 16, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	EMD 68 843, lot# EE 774856, 99.4% purity

Key Study Findings

- Beagle dogs were dosed orally (gelatin capsules) at 0, 2.5, 10, and 40 mg/kg/day for 1-year [the same doses used in the 6-month study].
- Three HDM and one HDF died or had to be euthanized prematurely
- Epileptiform convulsions preceded premature deaths
- Transient clinical signs of fearfulness, salivation, hypo/hyperkinesia, and sexual arousal at MD and HD during the first 2-3 weeks of treatment
- Transient, slight decrease in BW and food consumption at MD and HD (first 2-3 weeks)
- Corneal opacities transiently seen at MD and HD at weeks 1-3 of treatment only, which correlated with decreased tear production at that time.
- Increased blood glucose and cholesterol at HD, which resolved fully or partially during recovery
- Increased liver weights in all treated dogs (~8% - 41%), but without histopathological correlates; and resolved at recovery.
- NOAEL was the MD of 10 mg/kg/day based on deaths at the HD of 40 mg/kg/day

Methods

Doses:	0, 2.5, 10, and 40 mg/kg
Frequency of dosing:	Once a day
Route of administration:	oral
Formulation/Vehicle:	Gelatin capsule
Species/Strain:	Dog/Beagle
Number/Sex/Group:	3/sex/group
Age:	9 – 11 month old
Weight:	8.7 – 15.8 kg
Satellite groups:	2/sex/group (recovery)
Unique study design:	Treatment with vilazodone was followed by a 2 month recovery period (2/sex/group)
Deviation from study protocol:	Treatment was staggered in over the first 3 days for mid and high dose animals. All groups consisted of 10 dogs (5 males, 5 females).

Observations and Results

Mortality

Tree HDM and one HDF died or had to be euthanized for humane reasons during the study. The following clinical signs preceded deaths as shown in the Sponsor's table:

Animal no	Found dead/killed	Main symptoms preceding death	Other observations
8311 (F)	killed on day 68 for humane reasons	epileptiform convulsions, lateral position, sleepy – comatose appearance on days 66 and 67, dog was withdrawn from treatment on days 66 and 67	no obvious clinical signs of drug re-action up to day 66
8293 (M)	found dead on day 130	vomiting (vomit contained mucous and blood) on day 129 Note: animal cage was heavily stained with vomit and stool. It is highly likely that the dog went through a convulsive state	no obvious clinical signs of drug reaction up to day 129
8296 (M)	died on day 175	epileptiform convulsions on days 174, 175, withdrawn from treatment on day 174	No obvious clinical signs of drug reaction up to day 74
8276 (M)	Killed on day 355 for humane reasons	severe vomiting on day 68, mucous/bloody nose discharge on day 68; gasping on days 68, 69; withdrawn from treatment on days 69, 70; epileptiform convulsions on day 354	No effect on food consumption up to day 354; slightly decreased BW

Clinical Signs – Dogs behavior and general condition were recorded daily.

Results: Epileptiform convulsions were seen in 2 HDM (#8296 in week 25 [2 incidences] and #8276 in week 51 [this convulsion could have been provoked by the blood sampling procedure on day 355]) and 1 HDF (#8311 in week 10 [2 incidences]); and it appears likely that the other HDM (#8293) also convulsed, based on clinical signs the day before it died. Other clinical signs, fearfulness, salivation, hypokinesia, and mydriasis were observed at MD and HD during the first 2-3 weeks of treatment only. In addition, 2 HDM and 2 MDM showed signs of hyperkinesia, excitement and sexual arousal (pseudo-copulation) during weeks 1-2 of treatment only. Isolated incidences of vomiting were seen in all groups including the controls.

Clinical findings are summarized in the following table:

Clinical sign	Male control	Male LD	Male MD	Male HD	Female control	Female LD	Female MD	Female HD
Fearfulness	0	0	5/5	5/5	0	0	5/5	5/5
Salivation	0	0	3/5	4/5	0	0	4/5	5/5
Hypokinesia	0	0	5/5	5/5	0	0	5/5	4/5
Mydriasis	0	0	4/5	4/5	0	0	5/5	5/5
Excitement	0	0	2/5	2/5	0	0	0	2/5
Vomiting	1/5	4/5	2/5	2/5	2/5	4/5	4/5	3/5

Body Weights – recorded weekly

Results: There was a **transient decrease in BW at MD and HD**. BW was slightly reduced (~ 5% - HDM; ~ 9% HDF) and MD (~4% -MDM; ~7% - MDF) during the first 2-3 weeks of treatment (statistically not significant). Thereafter, dogs regained normal BW and, by week 5 of the treatment period, BW of these dogs was comparable to the controls for the remainder of the study.

Feed Consumption – recorded daily (the dog was offered feed for 2 h a day)

Results: **Food consumption was transiently reduced in HD** (up to ~70%) and MD males and females. This effect was seen during the first week of treatment in MDM, whereas in all other groups, the effect lasted for up to two weeks.

Ophthalmoscopy - Ophthalmological examinations were conducted in all dogs in weeks -5, 12, 24, 49, and 60. Additionally, the Schirmer tear test was performed in all dogs in weeks -7, -2, 2, 11, 28, 52, and 59.

Results: Streak – like **corneal opacities** were seen during the daily clinical observation in weeks 1 to 3 at MD and HD: 4/5 MDM, 1MDF, 4/5 HDM, and 3/5 HDF. These changes were completely resolved in all dogs by week 4 of treatment period and corneal effects were not seen at ophthalmoscopic exams (at weeks 12, 24, 49, or 60).

The Schirmer tear test revealed a reduced tear production in week 2 of treatment in 4/5 HDF and 1/5 MDF (compared to concurrent controls and to the pre-treatment values) but not at later time points (12 weeks or longer).

ECG - ECG and blood pressure measurements were taken in all dogs pre-dose (week - 4) and at weeks 2, 13, 26, 50, both before and 2 h after treatment and in week 59 of the recovery period.

Results: Treatment with vilazodone did **not affect heart rate and ECG parameters** such as PQ-interval, QRS-complex, and QT-interval. There was no evidence for an effect on electrical conductivity and rhythm. There was no treatment-related effect on systolic blood pressure observed.

Hematology – Blood samples for hematological and clinical chemistry were taken by puncture of the jugular vein from dogs that were fasted for about 18 h prior to blood draw at weeks -6, -3, 1, 4, 14, 27, 51, and 60. During treatment period, blood samples were taken before and 2 h after dosing. Additional blood samples were taken from dogs that were prematurely terminated.

For the hematological assessment the following parameters were evaluated: leukocytes (WBC), erythrocytes (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean hemoglobin content (MCH), mean hemoglobin concentration (MCHC), platelets (PLT), reticulocytes (RET), erythrocyte sedimentation rate (ESR1/ESR2), differential blood count (DIFF), absolute number of segmented neutrophilic granulocytes (NEU ABS), lymphocytes (LYMPH ABS), prothrombin time (PT), partial thromboplastin time (PTT) and thrombin time (TT).

Results: Findings were confined to the HD dogs and consisted of elevated RET counts in 2 HDM in week 14 (mean 79% increase versus control; values returned to normal by week 27 in one dog and the second dog died on Day 175). WBC counts were increased by 52%, 70%, and 60% in weeks 27, 51, and 60 (after recovery), respectively; neutrophils were also increased by 27%, 11%, 15% in weeks 4, 27, 51 (but not after recovery), respectively in HDFs.

Clinical Chemistry - the following parameters were evaluated at weeks 4, 14, 27, 51, and after recovery: enzymes (ALAT, ASAT & AP), proteins, (TP & ALB), substrates (GLUC, UREA, CREA, TBIL, CHOL, TG), electrolytes and trace elements (Na, K, Ca, Cl, P, and Fe) and hormones (prolactin).

Results: Treatment-related effects were confined to slight increases in glucose and cholesterol at HD.

Parameter	Week 4		Week 14		Week 27		Week 51	
	M	F	M	F	M	F	M	F
Glucose	↑32%	↑23%	↑11%	NC	↑22%	↑11%	↑18%	↑20%
Cholesterol	↑38%	↑22%	↑61%	↑36%	↑37%	↑36%	↑47%	↑28%

Blood glucose and serum cholesterol levels were slightly but consistently increased in HDM and HDF. Glucose values returned to normal by the end of the recovery period but cholesterol appeared to be higher in treated HDF (↑80%; n=2) when compared to the concurrent control. The examination of an additional blood sample drawn from the HDF (#8311; killed for humane reasons on day 68) had high blood glucose.

There was no treatment-related effect on serum prolactin levels.

Urinalysis – urine samples were collected from all dogs and the following parameters were evaluated: pH, protein, glucose, bilirubin, urobilinogen, blood, and sediment

Results: The pattern of variation did not indicate any treatment-related effect on urinary parameters.

Gross Pathology

At macroscopic examination only findings of spontaneous or sporadic origin were detected in all dogs which were killed as scheduled. Gross pathology observations of dogs that died or were sacrificed in a moribund state (3HDM) revealed only findings that

were described by the study pathologist as agonal changes. No gross pathology findings were noted in 1HDF which was sacrificed in a moribund state.

Organ Weights – organs weighed are marked in the histopathology inventory table.

Results: Increase in liver weights was seen in all vilazodone treated dogs (+42%, +20%, +42% at LDM, MDM, and HDM; +8%, +16%, and +13% at LDF, MDF, and HDF, respectively). There was no dose relationship and no histopathological correlates. The liver weights of the recovery animals were in a normal range. Therefore, the toxicological relevance of the increased liver weights is uncertain. All other observed differences in organ weights were of individual occurrence or BW dependent.

Histopathology – see histopathology inventory table at the end of General Toxicology section

Adequate Battery - yes

Peer Review – yes by another in-house pathologist

Histological Findings – Only findings of spontaneous or sporadic origin were detected in all dogs that survived until scheduled termination at the end of treatment or recovery periods. Evidence of agonal tissue changes were seen in HD dogs that were found dead or had to be euthanized prematurely.

Toxicokinetics – Blood samples were taken from the jugular vein from 3 dogs/sex/group on days 3, 192, and 360 (only 2 HDM) before treatment and 1, 2, 4, 6, and 24 h after dosing and additional samples were collected from 1F and 1M on days 66 and 355, respectively (after epileptiform convulsions occurred).

Results: No sex-dependent differences in PK parameters were noted therefore combined (M+F) mean values are summarized in the following table:

Dose (mg/kg)	Day	AUC _(24h) (ng•hr/ml)	C _{max} (ng/ml)	T _{max} (h)
2.5	3	327*	103	1-4
	192	435*	121	1-2
	360	884	146	2-4
10	3	7950	634	1-4
	192	3340	425	2-4
	360	3310	334	2-4
40	3	10800	877	2-24
	192	19700	1910	2-6
	360	15800	1450	2-4

*: AUC (0-6h) – concentration after 24 h was below the limit of quantification

Dosing Formulation Analysis -

At the end of the treatment period, capsules containing vilazodone kept under the same storage conditions as those used during the treatment period were tested for content and stability.

Results: The analyses show that the capsules contained the amount of vilazodone required in the study protocol and that vilazodone was stable in hard gelatin capsules throughout the treatment period (at treatment initiation it ranged from 97 to 101% and at termination it ranged from 96 to 100% of required content). Dosing was considered acceptable.

Summary: Toxicity of vilazodone at oral doses 0, 2.5, 10, and 40 mg/kg/day (gelatin capsules) was evaluated in dogs during and after 52 weeks of treatment and 8 weeks of drug free recovery.

Four HD dogs (3M/1F) did not survive until scheduled sacrifice. In 3 dogs, death (on Day 68, Day 175, and Day 355) was preceded by epileptiform convulsions. Convulsions were not witnessed in the fourth dog but physical evidence suggested convulsions occurred prior to death (on Day130). There were no clinical chemistry findings (such as hyponatremia) to suggest a metabolic cause for the convulsions. Although the study report stated that these convulsions were epileptiform, an accurate diagnosis could not be made since EEGs were not recorded.

The following comment regarding seizures was offered by the Sponsor: "It is conceivable that EMD 68 843 lowers the threshold for the induction of seizures in beagle dogs and that convulsions can be precipitated in dogs with a certain predisposition. Primary genetic or idiopathic (breed-related inherited) epilepsy has been documented in Beagles (Podell, 1996). This form of epilepsy is mainly seen in dogs aged 1-5 years. This age relationship would also be in line with the type of convulsions seen in this study. Furthermore, induction of seizures has also been reported as an adverse effect for many marketed antidepressants (Potter and Hollister, 1998). This implies that the pro-convulsive effect seen in Beagle dogs is related to an exaggerated pharmacological activity of EMD 68 843 which is known to occur in this class of compounds."

1) Podell, M. *Seizures in Dogs; Veterinary Clinics of North America: Small Animal Practice*, 1996, 26, 779-808.

2) Potter, W.Z., Hollister, L.E.; *Antidepressant Agents In: Katzung, Basic and Clinical Pharmacology*, 7th edition, 1998, 490-494. Appleton & Lange, Stamford, Connecticut

Corneal opacities were transiently seen at MD and HD and correlated with decreased tear production at that time (first 3 weeks of treatment); corneal opacities were not seen late during dosing and no cataracts were found.

NOAEL was at the mid dose of 10 mg/kg/day based on seizures and deaths occurred at the high dose of 40 mg/kg/day.

Histopathology Inventory for NDA 22-567

Study	26-week	52-week	104-week	104-week
Species	Rat	Dog	Rat	Mouse
Abdominal cavity			X	X
Adrenals	X*	X*	X	X
Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)	X		X	X
Brain	X*	X*	X	X
Cecum	X	X	X	X
Cervix		X		
Colon	X	X	X	X
Duodenum	X	X	X	X
Ears			X	X
Epididymis	X*	X	X	X
Esophagus	X	X	X	X
Eyes	X*	X	X	X
Gall bladder	X	X		
Gross lesions	X	X	X	X
Heart	X*	X*	X	X
Harderian Glands			X	X
Ileum	X	X	X	X
Jejunum	X	X	X	X
Kidneys	X*	X*	X	X
Larynx	X		X	X
Liver	X*	X*	X	X
Lung	X	X	X	X
Lymph nodes			X	X
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Mesenteric tissue			X	X
Optic nerves	X		X	X
Ovaries	X*	X*	X	X
Pancreas	X	X	X	X
Parathyroid	X*	X	X	X
Penis			X	X
Pituitary	X*	X*	X	X
Prostate	X*	X*	X	X
Rectum			X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X*	X	X	X
Skeletal muscle		X		
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X	X
Stomach	X	X	X	X

Study	26-week	52-week	104-week	104-week
Species	Rat	Dog	Rat	Mouse
Tail			X	X
Thoracic cavity			X	X
Testis	X	X*	X	X
Thymus	X*	X*	X	X
Thyroid	X*	X*	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X	X
Vagina	X	X	X	X
Zymbal's Glands			X	X

X, histopathology performed

*, organ weight obtained

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: EMD 68 843 - Bacterial mutagenicity assay, Salmonella typhimurium and Escherichia coli

Study no.: GPP-007-NCD-TOX-2004-222 and GPP-007-NCD-TOX-1997-187

Study report location: Central Product Documentation, E. Merck, Darmstadt, Germany

Conducting laboratory and location: Institute of Toxicology Merck KGaA, 64271 Darmstadt, Germany

Date of study initiation: July 18, 1994 [study: GPP-007-NCD-TOX-2004-222] July 23, 1997 [study: GPP-007-NCD-TOX-1997-187]

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: EMD 68 843, Batch # 5, 99.4% purity [study: GPP-007-NCD-TOX-2004-222]; EMD 68 843 (micronized), Batch # EE77485, 99.0% purity [study: GPP-007-NCD-TOX-1997-187]

Key Study Findings:

Vilazodone was negative for mammalian cell mutagenicity (\pm metabolic activation) in bacterial cells in a valid Ames test.

Methods

Strains: Salmonella typhimurium - TA 98, TA 100, TA 102, TA 1535 and TA 1537
Escherichia coli - WP2 uvrA

Concentrations in definitive study: 5, 15.8, 50, 158, 500, 1580, and 5000 $\mu\text{g}/\text{plate}$
For both studies: GPP-007-NCD-TOX-2004 and GPP-007-NCD-TOX-1997-187]

Basis of concentration selection: EMD 68 843 was tested in the range finding experiment using TA 100 and TA 1535 strains in the absence of S-9 at concentrations ranging from 50 up to 5000 $\mu\text{g}/\text{plate}$

Negative control: Solvent, dimethylsulfoxide (DMSO)

Positive control: Without S9: Daunomycin (TA 98), 9-Aminoacridine (TA 1537), N-Ethyl-N'-nitro-N-nitrosoguanidine (TA 100, TA 1535, and

Formulation/Vehicle: E.coli), Cumene hydroperoxide (TA 102);
with S9: 2-Aminoanthracene (all strains,
except TA 102), Benzo(a)pyrene (TA 102)
Solution/ DMSO
Incubation & sampling time: Cells (plate incorporation method) were
exposed to test article for 2 to 3 days

Study Validity: The validity of the assay was assessed by the results obtained for the negative and positive controls. The negative control mutant frequencies were in the regular range and the strain specific positive controls yielded to expected mutant frequencies that were greatly in excess to the negative controls. Thus the genotype of tester strains and activity of exogenous metabolizing system used were confirmed.

Results:

Study: GPP-007-NCD-TOX-2004-222

EMD 68 843 induced toxicity was observed in both range finding and definitive studies which included precipitation of test article in top agar layer at concentrations of ≥ 500 $\mu\text{g}/\text{plate}$ and reductions of bacterial background lawn at concentrations ≥ 1580 $\mu\text{g}/\text{plate}$. In both series of experiments (definitive study), each performed with and without S9, EMD 68 843 showed no increase in the number of reverse mutations of any bacteria strain. The positive controls induced expected increase in cell mutation frequency confirming study validity. Therefore vilazodone was judged to be not mutagenic under these experimental conditions.

Concentration range-finding study: GPP-007-NCD-TOX-1997-187

Cytotoxicity as indicated by a reduction in revertant colonies compared with vehicle control was seen in *Salmonella* strains TA-102, TA-1535 and TA1537 in both assays mostly at ≥ 500 $\mu\text{g}/\text{plate}$. Precipitates were seen in all strains in both assays at ≥ 500 $\mu\text{g}/\text{plate}$. As in the previous Ames assay, vilazodone did not induce an increase in the number of reverse mutations in any strain under any condition in this study. The negative and positive controls met the criteria for a valid assay. In conclusion, vilazodone was considered negative for mutagenicity in the bacterial reverse mutation tests.

***In Vitro* mammalian cell gene mutation test (V79/HPRT) – Study: GPP-007-NCD-TOX-1997-188**

Vilazodone was studied for induction of gene mutations in V79 Chinese hamster cells in vitro. These cells were exposed to the following vilazodone concentrations: 0.158, 0.5, 1.58, and 5.0 $\mu\text{g}/\text{ml}$, without and with S9-mix. The highest concentration tested appeared to be toxic to the V79 cells causing ~ 45% to 22 % reduction in the total growth after the treatment period (higher concentrations were shown to kill all cells in a preceding range finding study).

V79 cells were incubated with vilazodone, positive controls (MNNG, 1 µg/ml and DMBA, 20 µg/ml; without or with S9-mix, respectively) or the solvent (DMSO) for 24 h in the absence and for 2 h in the presence of S9 mix in two separate experiments.

This study was considered valid based on the expected increase in the mutation frequencies caused by the positive controls. The study results were negative showing that vilazodone did not increase mutation frequency of V79 cells as compared to actual solvent controls in the absence and presence of S9-mix.

7.2 In Vitro Chromosomal Aberration Assays in Mammalian Cells

7.2.1 Chromosomal aberration assay in V79 Chinese hamster cells

Study title: EMD 68 843 - In vitro chromosome aberration assay in V79 Chinese hamster cells

Study no.:	T13897, report: GPP-007-NCD-TOX-1995-189
Study report location:	Central Product Documentation (ZPD), Merck, Darmstadt, Germany
Conducting laboratory and location:	Institute of Toxicology at E. Merck, Darmstadt, Germany
Date of study initiation:	February 13, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	EMD 68 843, Batch # 5, 99.4% purity

Key Study Findings:

Vilazodone was clastogenic, without or with metabolic activation, in V79 Chinese hamster lung cells *in vitro*, but only at cytotoxic concentrations.

Methods

Cell line:	V79 Chinese hamster cells
Concentrations in definitive study:	1 st series: 0.158, 0.500, 1.58 µg/ml (-S9); 0.158, 0.500, 1.58, 2.81 µg/ml (+S9); out of 13 concentrations from 0.05 to 50.0 µg/ml. 2nd series: 0.158, 0.158, 2.11, 2.81 µg/ml (-S9); 1.15, 2.11, 2.81 µg/ml (+S9); out of 5 concentrations from 0.158 – 2.81 µg/ml. Concentrations for assessment were based on lack of colony formation at ≥ 0.500 µg/ml -S9 and ≥ 0.889 µg/ml +S9.
Basis of concentration selection:	cytotoxicity in V79 cells at concentrations ≥ 5 µg/ml, in previously conducted studies
Negative control:	DMSO (solvent)
Positive control:	Cyclophosphamide (CPA, 2 µg/ml) – with S9 and

Ethylmethane sulfonate (EMS, 500 µg/ml) – without S9

Formulation/Vehicle: Solution in DMSO

Incubation & sampling time: Incubation 5 hrs, colchicine 11 hr later, and harvest of cultures at 18 hrs

Other: Without and with metabolic activation (±S9 from livers of male Wistar rats induced with Aroclor 1254)

No. of slides per concentration: 4 – 8

No. of metaphases evaluated per slide: 100

Study Validity: The actual negative controls were within the historic negative controls range. Statistically significant and biologically relevant increases in the number of aberrant metaphases for the respective positive controls (in relation to the actual negative controls) were observed.

Results: The cloning efficiencies of V79 cells were not influenced by EMD 68 843 at concentrations ranging from 0.05 to 0.889 µg/ml. Reductions of cloning efficiencies to 43 % of the control values occurred at 1.58 µg/ml without S9 and to 36 % of control values with S9 at 2.81 µg/ml. No colony formation occurred at concentrations ≥ 8.89 µg/ml (without S9) and at 5.0 µg/ml (with S9).

EMD 68 843 decreased (58 - 26% and 52 - 23%) the mitotic activity of V79 calls at concentrations ranging between 0.50 and 1.58 (without S9) and 0.889 and 2.81 µg/ml (with S9), respectively. No evaluable metaphases were present at higher concentrations.

EMD 68 843 caused an increase in the number of aberrant metaphases at higher concentrations of 2.11 and 2.81 µg/ml without S9 and at 2.81 µg/ml with S9 as shown in the following Sponsors' tables.

First experiment:

Without S9

Group	Test Material	Conc. µg/ml	Time h	g	ig	br	ibr	ex	ma	spa	Total Aberr. incl.	Aberr. excl.
1	SOLVENT		5/18	0.3	0.0	2.3	0.0	0.0	0.0	0.3*	2.9	2.6
3	EMS	500	5/18	1.0	0.0	4.5	5.0	8.5	1.0	0.0	20.0	19.0
5	EMD 68 843	0.158	5/18	0.0	0.0	4.0	0.5	0.0	0.0	0.0	4.5	4.5
7	EMD 68 843	0.500	5/18	0.0	0.0	1.5	0.0	0.0	0.0	0.5**	2.0	2.0
9	EMD 68 843	1.58	5/18	0.0	0.0	1.5	0.0	0.5	0.0	0.0	2.0	2.0#

** = dicentric chromosome; # = only 137 mataphases were scored; Time = exposure/preparation; g = gaps; ig = isogaps; br = breaks (chromatid); ibr = isobreaks (chromosomal); ex = exchanges; ma = multiple aberrations; spa = specific aberrations

With S9

Group	Test Material	Conc. $\mu\text{g/ml}$	Time h	g	ig	br	ibr	ex	ma	spa	Total Aberr. incl.	Aberr. excl.
16	SOLVENT		5/18	1.0	0.0	3.5	1.0	0.3	0.0	0.3**	7.1	6.1
17	CPA	2.00	5/18	1.0	0.0	2.0	4.5	3.5	0.0	0.0	11.0	10.0
20	EMD 68 843	0.158	5/18	0.0	0.0	1.5	1.0	0.0	0.0	0.0	2.5	2.5
22	EMD 68 843	0.500	5/18	0.0	0.0	2.5	1.0	1.0	0.0	0.0	4.5	4.5
24	EMD 68 843	1.58	5/18	0.5	0.0	1.0	2.0	0.5	0.0	1.0*/**	5.0	4.5
25	EMD 68 843	2.81	5/18	0.0	0.0	4.5	1.0	5.0	0.5	0.0	11.0	11.0

* = atypical chromosome; ** = dicentric chromosome; Time = exposure/preparation;
g = gaps; ig = isogaps; br = breaks; ibr = isobreaks; ex = exchanges; ma = multiple aberrations; spa = specific aberrations

Second experiment:

Without S9:

Group	Test Material	Conc. $\mu\text{g/ml}$	Time h	g	ig	br	ibr	ex	ma	spa	Total Aberr. incl.	Aberr. excl.
1	SOLVENT		5/18	1.3	0.0	0.8	0.8	0.0	0.0	0.3**	3.2	1.9
2	EMS	500	5/18	2.5	0.0	1.0	3.0	6.5	0.0	0.0	13.0	11.5
5	EMD 68 843	0.158	5/18	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5
9	EMD 68 843	1.58	5/18	0.5	0.0	1.5	3.0	0.5	0.0	0.0	5.5	5.0
31	EMD 68 843	2.11	5/18	1.0	0.5	1.0	6.0	14.0	0.0	0.0	22.5	21.5#
10	EMD 68 843	2.81	5/18	0.5	0.0	3.0	0.5	1.0	0.0	0.0	5.0	4.5##

* = only 136 metaphases were scored; ** = only 34 metaphases were scored;
g = gaps; ig = isogaps; br = breaks; ibr = isobreaks; ex = exchanges; ma = multiple aberrations; spa = specific aberrations

With S9:

Group	Test Material	Conc. $\mu\text{g/ml}$	Time h	g	ig	br	ibr	ex	ma	spa	Total Aberr. incl.	Aberr. excl.
16	SOLVENT		5/18	0.3	0.0	1.3	0.0	0.0	0.0	0.3**	1.9	1.6
17	CPA	2.00	5/18	1.0	0.0	6.5	1.5	4.5	0.0	0.0	13.5	12.5
24	EMD 68 843	1.58	5/18	0.5	0.0	2.0	0.8	0.3	0.0	0.0	3.6	3.1
32	EMD 68 843	2.11	5/18	0.5	0.0	2.3	0.3	3.0	0.0	0.0	6.1	5.6
25	EMD 68 843	2.81	5/18	0.8	0.0	4.8	2.5	4.3	0.0	0.3**	12.7	11.9

** = dicentric chromosome; Time = exposure/preparation;
g = gaps; ig = isogaps; br = breaks; ibr = isobreaks; ex = exchanges; ma = multiple aberrations; spa = specific aberrations

No treatment-related increase of polyploidy cells was observed at any concentration.

In summary, the increases in the number of aberrant metaphases were seen in two independently performed experiments that occurred at the same concentration level and without or with metabolic activation (\pm S9). The percentage of aberrant metaphases for vilazodone-treated cultures exceeded the usual range found in that testing laboratory for the cell line used.

However, the concentration range showing positive effects was narrow (2.11 to 2.81 µg/ml). Cultures treated with higher concentrations of vilazodone were not evaluable therefore a concentration dependency of the increases could not be established. Furthermore, the increases in the number of aberrant metaphases occurred at concentrations that showed cytotoxicity (i.e. a reduction in the mitotic index to 36% of the concurrent negative control).

According to the criteria for the interpretation of obtained results the conducting laboratory concluded that there is an evidence of clastogenic activity in V79 Chinese hamster cells treated with vilazodone at concentrations causing cytotoxicity.

7.2.2 Chromosomal aberration assays in human lymphocytes

Key findings (for studies with and without metabolic activation):

Vilazodone was not clastogenic in human lymphocytes in the absence of metabolic activation.

Vilazodone was clastogenic in human lymphocytes in the presence of metabolic activation, but only at cytotoxic concentrations.

Vilazodone (EMD 68 843) was tested for in vitro clastogenicity in human lymphocytes, in the absence and presence of metabolic activation (\pm S9) in separate studies (reviewed below).

In the presence of metabolic activation:

Study title: EMD 68 843 - In vitro chromosome aberration assay in human lymphocytes [in absence of metabolic activation]

Study no.:	T13966, report: GPP-007-NCD-TOX-1997-190, and GPP-007-NCD-TOX-1997-191 (amendment 1)
Study report location:	Central Product Documentation (ZPD), Merck, Darmstadt, Germany
Conducting laboratory and location:	Institute of Toxicology at E. Merck, Darmstadt, Germany
Date of study initiation:	December 4, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	EMD 68 843, Batch # 6, % purity

Key study findings:

Vilazodone was not clastogenic in human lymphocytes in the absence of metabolic activation.

Vilazodone was investigated in two experimental series for induction of chromosomal aberrations in human lymphocytes *in vitro* without S9 mix.

The following experimental conditions were used in the absence of S9 metabolizing system:

- No. of slides per concentration: 4 (solvent control) and 2 (others)
- No. of metaphases evaluated per slide: 100
- Preparation times: 68 and 77 hrs
- Exposure times: 20 and 29 hrs
- Concentrations evaluated: vilazodone (EMD 68 843): 8.89, 15.8, and 28.1 µg/ml
- Positive control, Mitomycin C (MMC): 0.15 µg/ml
- Negative control: DMSO (also solvent for test article).
- Two independent experiments were conducted (with the same design).

Study validity: The study was valid: the positive control MMC induced the expected clear increase in the proportion of cells with chromosomal aberrations.

Results: The concentrations assessed for chromosomal aberrations were based on a range-finding study of 13 concentrations from 0.281 to 281 µg/ml, where concentrations ≥ 88.9 µg/ml strongly reduced the mitotic index (MI) and markedly affected the structure of the chromosomes (data not provided). For the pivotal experiments, concentrations up to 88.9 µg/ml were used; however, concentrations of 50.0 and 88.9 µg/ml were not evaluated because of excessive toxicity (the relative MIs were $\leq 36\%$).

Vilazodone (EMD 68 843) did not significantly change the proportion of cells with aberrant metaphases in cultures treated with concentrations up to 28.1 µg/ml as shown in the following Sponsors' table. At the highest concentration tested (28.1 µg/ml), the relative mitotic index was significantly decreased, ranging from 44 to 78% of the respective negative controls, see sponsor's table below. [The data of the EMD 68 843-treated cultures in the two experiments were combined for statistical analysis (p-values).]

Sponsor's summary table (from page 17 of report):

4.2.4 Tabulated survey
(cf Table 24)

Test Material	Conc. [µg/ml]	Mean Aberrant Metaphases (excl. gaps) [%]					
		68 h prep. time			77 h prep. time		
		Exp.1	Exp.2	Sign. ^a	Exp.1	Exp.2	Sign. ^a
Solvent		2.8	1.3	"	0.5	0.0	"
MMC	0.15	8.0	9.5	+	10.5	14.5	+
EMD 68 843	8.89	0.5	0.5	ns	0.0	1.0	ns
	15.8	0.5	1.0	ns	0.5	2.8	ns
	28.1	2.0	4.1	ns	2.8	0.8	ns

a: Significance of values:
 ns: not significantly different to the actual negative controls,
 +: significantly different to the actual negative controls,
 ": not examined statistically;
 (cf. Table 23 and 24)

Furthermore, vilazodone did not lead to an increase in the number of polyploid cells.

In conclusion, Vilazodone was not clastogenic in this test system (without S9) under conditions where the positive control exerted potent clastogenic effects.

In the presence of metabolic activation:

Study title: EMD 68 843 - In vitro mammalian chromosome aberration test (human lymphocytes + S9)

Study no.: T14401, report: GPP-007-NCD-TOX-1999-192
 Study report location: Central Product Documentation (ZPD), Merck, Darmstadt, Germany
 Conducting laboratory and location: Institute of Toxicology at E. Merck, Darmstadt, Germany
 Date of study initiation: September 16, 1998
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: EMD 68 843, Batch # EE 77 185, % purity

Key study findings:

Vilazodone was investigated in the presence of exogenous metabolizing system, S9 in three experimental series for induction of chromosomal aberrations in human lymphocytes *in vitro*.

The following methodology was used:

- No. of slides per concentration: 4 (solvent control) and 2 (others)
- No. of metaphases evaluated per slide: 100
- Preparation times: 77 and 96 hrs
- Exposure times: 5 hrs
- Concentrations evaluated: EMD 68 843 at 8.89, 15.8, 28.1, 50.0 and 88.9 µg/ml
- Positive control, cyclophosphamide (CPA): 4.0 µg/ml

Study validity: The positive control CPA induced the expected clear increase in the proportion of cells with chromosomal aberrations.

Results: In the 3 experiments in this study, the mitotic index (MI) was generally markedly (>50%) reduced at concentrations ≥ 28.1 µg/ml; however, the concentration-response relationship was somewhat variable. At concentrations ≥ 158 µg/ml, no metaphases were present. [A change in the pH or the osmotic value of the cell culture medium did not occur in the concentration range tested. Precipitation of the test material was seen at the beginning of the treatment at concentrations ≥ 50.0 µg/ml, but no precipitate was macroscopically visible at the end of the exposure time.]

Treatment of cultures with 15.8 µg/ml EMD 68 843 produced variable results on the proportion of cells with aberrant metaphases in the 3 experiments: #1: no increase (at relative MI of 69%); #2: significant increase (at relative MI of 61%); and #3: no significant increase (at relative MI of 66%).

The sponsors' table of results for clastogenicity in human lymphocytes in the presence of metabolic activation (from page 18 of report):

4.2.5 Tabulated survey of results							
Treatment group	Concentr. [µg/ml]	Prep. time[h]	Rel.mitotic index [%] ^a	Polyploid. metaph.[%]	Aberrant metaph. [%]		
					incl.gaps	excl.gaps	incl.exch.
Experiment 1:							
Solvent control		77	100	0.10	4.0	3.5	0.3
EMD 68 843	8.89	77	74	0.25	3.5	3.0 ^{n.s.}	0
	15.8	77	69	0.10	5.5	3.5 ^{n.s.}	0
	28.1	77	53	0.10	5.5	4.0 ^{n.s.}	0.5
	50.0	77	41	0.11	12.0	9.0**	1.0
Pos. control	4.00	77	65	0.05	10.5	10.5**	3.0
Experiment 2:							
Solvent control		77	100	0.05	2.0	1.5	0
Solvent control		96	100	0.35	2.3	1.5	0
EMD 68 843	15.8	77	61	0.15	7.0	6.5**	0.5
	28.1	77	78	0.60	5.5	4.5*	0
	50.0	77	44	1.05	3.5	2.0 ^{n.s.}	0
	50.0	96	45	0.40	6.0	4.5*	0
Pos. control	4.00	77	73	0.20	17.0	15.0**	3.5
Experiment 3:							
Solvent control		77	100	0.25	2.5	2.3	0.3
Solvent control		96	100	0.40	4.8	4.5	0.3
EMD 68 843	15.8	77	66	0.30	6.5	5.0 ^{n.s.}	0
	28.1	77	53	0.10	7.5	7.0**	0
	50.0	77	45	0.75	6.0	5.5*	0
	50.0	96	68	0.45	7.5	6.5 ^{n.s.}	0
	88.9	77	21	1.45	2.0	1.5 ^{n.s.}	0.5
	88.9	96	25	2.50	7.0	6.0 ^{n.s.}	0
Pos. control	4.00	77	65	0.15	11.5	11.0**	2.5
a: % of solvent control sig. = **: p ≤ 0.01 *: 0.01 < p ≤ 0.05 n.s.: p > 0.05 (not significant)							

In summary: The conclusion in the report was that "...EMD 68 843 was clastogenic in human lymphocytes in the presence of S9 mix at cytotoxic concentrations. However, the toxicological relevance and the possibility of an indirect induction of genotoxicity by cytotoxicity needs to be investigated." Although there was no clear, consistent effect on the proportion of cells with aberrant metaphases at concentrations that did not markedly reduce the mitotic index, this Reviewer considers vilazodone to be positive for clastogenicity (in the presence of metabolic activation) in this study.

7.3.1 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)**Study title: EMD 68 843 - Micronucleus test in rats after oral administration**

Study no.: T13932; Report: GPP-007-NCD-TOX-1997-193
 Study report location: Central Product Documentation (ZPD), Merck, Darmstadt, Germany
 Conducting laboratory and location: Institute of Toxicology at E. Merck, Darmstadt, Germany
 Date of study initiation: May 29, 1995
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: EMD 68 843, lot # 6, % purity

Key Study Findings:

Vilazodone was not clastogenic in the micronucleus test in rats *in vivo*.

Methods

Doses in definitive study: 200, 632 and 2000 mg/kg
 Frequency of dosing: Once
 Route of administration: Oral gavage
 Dose volume: 10 ml/kg
 Formulation/Vehicle: Solution in 0.25% Methocel K4M Premium
 Species/Strain: Rats, Hsd/Win:WU
 Number/Sex/Group: 5/sex/group
 Satellite groups: 3/sex/1 h/24 h/48 h
 Basis of dose selection: The highest dose was selected to produce toxicity but no mortality (preliminary dose-finding study)
 Negative control: 0.25% aqueous solution of Methocel K4M Premium
 Positive control: Cyclophosphamide at 16.5 mg/kg

Study Validity: PK assessment demonstrated systemic exposure and dosing appeared to be adequate (dose-ranging study). The negative control (vehicle) values were all in the range of historical controls of the laboratory. The positive control group (cyclophosphamide) showed a statistically significant increase in the number of polychromatic erythrocytes with micronuclei.

Results: No statistically significant or biologically relevant increase in the number of polychromatic erythrocytes with micronuclei per 1000 PCE was observed in any of the Vilazodone-treated groups.

Therefore, Vilazodone was not mutagenic in the micronucleus test in rats *in vivo* under condition of this study where positive control exerted potent mutagenic effect.

7.3.2 Chromosomal Aberrations in the Bone Marrow of Treated Rats**Study title: EMD 68 843 – Induction of Chromosomal Aberrations in the Bone Marrow of Treated Rats**

Study no.:	GPP-007-NCD-TOX-1999-195
Study report location:	Central Product Documentation (ZPD), Merck, Darmstadt, Germany
Conducting laboratory and location:	(b) (4)
Date of study initiation:	
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	EMD 68 843, lot # EE 79485; 100.1% purity

Key Study Findings:

Vilazodone was negative for chromosome aberrations in rat bone marrow cells at doses up to 2000 mg/kg.

Methods

Doses in definitive study:	200, 633 and 2000 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Solution in 0.25% hydroxypropylmethylcellulose (HPMC)
Species/Strain:	Rats, CrI:CD®BR (CD); (b) (4)
Number/Sex/Group:	6/sex/group (24h); 6/control&HD (48h)
Basis of dose selection:	Dose range finding in 3/sex (1500, 1750, 2000mg/kg)
Negative control:	0.25% HPMC
Positive control:	Cyclophosphamide (CPA) at 40mg/kg

Slides from animals treated with HPMC, vilazodone or positive control (CPA) were examined, uncoded, for mitotic index (MI) or percentage of cells in mitosis, based on 1000 cells scored per animal. Only cells with 40-42 chromosomes were considered acceptable for analysis of structural aberrations. Any cell with more than 42 chromosomes (polyploid, endoreduplicated and hyperdiploid cells) observed during this search was noted and recorded separately.

Study Validity: CPA-treated animals exhibited increases in chromosomal aberrations such that the frequency of aberrant cells in the positive control group was significantly greater than that observed in the concurrent vehicle control group.

Results: Animals treated with vilazodone at all doses exhibited frequencies of cells with structural chromosome aberrations which were similar to and not significantly different from those observed in concurrent vehicle control groups at both sample times.

Historical vehicle control ranges were not exceeded in any group of animals treated with vilazodone at either sample time.

It is concluded that vilazodone was unable to induce chromosome aberrations in the bone marrow cells of rats treated with vilazodone up to 2000 mg/kg; an acceptable maximum dose for this valid and adequately conducted assay.

Other Genetic Toxicity Studies

Study title: EMD 68 843 - In vitro mammalian cell gene mutation test (V79/HPRT)
(Study No: T13929; Report: GPP-007-NCD-TOX-1997-188)

Vilazodone at concentrations 0.158, 0.500, 1.58, and 5.00 µg/ml (with and without S9 mix) was investigated for induction of gene mutations in V79 Chinese hamster cells *in vitro* in two series of experiments. A weak toxicity was observed at the highest concentration tested i.e. a 45% to 22% reduction in total growth after the treatment period (higher concentrations killed all cells in the preceding range finding study).

V79 cells were exposed to vilazodone, respective positive (N-Methyl-N'-nitro-N-nitrosoguanidine – without S9 or 7,12-dimethyl- benz[a]anthracene– with S9) or negative control (vehicle DMSO) for 24 h in the absence and for 2 h in the presence of S9 mix.

Results: The positive controls induced the expected increase in the mutation frequency. Vilazodone in the absence and presence of S9 mix, did not relevantly increase the mutation frequency of the V79 cells as compared to the actual solvent controls. According to the predetermined criteria for the evaluation of results, EMD 68 843 was negative in this test system.

In conclusion, Vilazodone was not mutagenic in V79 mammalian cell gene mutation test under conditions where the positive controls exerted potent mutagenic effects.

Study title: EMD 68 843: Measurement of unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure (GPP-007-NCD-TOX-1995-194)

Vilazodone was tested for its ability to induce unscheduled DNA synthesis (UDS) in the livers of orally dosed male rats using an *in vivo/in vitro* procedure.

Male Wistar rats (5/group) were dosed once with vilazodone at 632.5 or 2000 mg/kg by oral gavage. Positive controls (5M) for 12-14 h experiment were dosed orally at 75 mg/kg with 2-acetamidofluorene (2-AAF) and 5M were dosed with Dimethylnitrosamine (DMN) for 2-4 h experiment, and negative control rats (5M) received vehicle (0.25% hydroxypropylmethylcellulose).

Animals of experiment 1 (12-14h) and experiment 2 (2-4h) after dosing were killed and their livers perfused to provide a primary culture of hepatocytes. Cultures were made from three animals in each dose group and were treated with [³H] thymidine. Six slides

from each animal were prepared with fixed hepatocytes, processed and examined microscopically for the net grain count (NNG), the number of grains present in the nucleus minus the mean number of grains in three equivalent areas of cytoplasm, was determined for each of two of the three slides, each animal and dose group.

Results: None of the cells from the negative control animals was considered to be in repair (mean NNG value of less than zero). Group mean NNG values were increased by 2-AAF and DMN treatment to more than 5, with more than 50% cells considered to be in repair. In this study the vehicle control NNG value was consistent with both published and historical control data, and the system was shown to be sensitive to two known DNA damaging agents requiring metabolism for their action. The assay was therefore accepted as valid.

Vilazodone at 632.5 or 2000 mg/kg did not produce a group mean NNG value greater than - 2.6 nor were any of the cells scored considered to be in repair at either dose,

In conclusion, vilazodone was negative in this study (failed to induce UDS detectable under the conditions of this study).

8 Carcinogenicity

Background: Carcinogenicity protocols for mouse and rat were presented to the Executive CAC on July 14, 1998. The Sponsor had proposed to use doses of 0, 6, 20 and 70 mg/kg/d po in B6C3F1 mice and 0, 6, 20 and 70 mg/kg/d po in HSDCPB: WU Wistar rats. The Committee agreed with the Reviewer (Dr. Norman Huang) that MTDs could not be determined in the 13-week mouse studies [doses of 0, 5, 15, 45, and 135 mg/kg/d po to B6C3F1 mice] and the 26-week rat studies [doses of 0, 3, 15 and 75 mg/kg/d po to HSDCPB:WU Wistar rats] that were used as basis for dose selections presented at that time. The Committee also concurred with the Reviewer that pharmacokinetic data could not be used for dose selection because of the positive results from the chromosome aberration test. Thus, no concurrence on dose selection could be made at that time. The Committee recommended that the Sponsor perform dose range-finding studies in mouse and rat at higher doses.

Subsequently, the Sponsor had submitted 13-week studies in mice and in rats, using much higher doses, i.e., 0, 135, 270, and 540 mg/kg/d to B6C3F1 mice and 0, 100, 300 and 1000 mg/kg/d to HsdCpb:WU Wistar rats (reviewed by Dr. Linda Fossom; IND 54,613, N-017)

Based upon these studies the Sponsor had revised the proposed dosing to 0, 15, 45 and 135 mg/kg/d for mice and 0, 7.5, 25, and 75 mg/kg/d (started in July, 1998, before they received the recommendations from the initial Executive CAC meeting) plus a higher dose of 150 mg/kg/d (added with a concurrent control group in April, 1999) for rats. The Executive CAC (4/11/00) agreed that these doses were reasonable, based on the results of 13-week studies in rats and mice.

8.1 Rat carcinogenicity study

Study title: EMD 68 843 - Carcinogenicity study in rats with oral administration

Study no.:	T14059, Report: GPP-007-NCD-TOX-2004-154
Study report location:	Merck KGaA, Pharma Ethicals, Global Preclinical R&D; 64271 Darmstadt, Germany
Conducting laboratory and location:	Merck KGaA's Institute of Toxicology, Bldg. U9 Darmstadt, Germany
Date of study initiation:	July 7, 1998
GLP compliance:	Yes (approved electronically)
QA statement:	Yes (approved electronically)
Drug, lot #, and % purity:	EMD 68 843, lot # EE 77485, 99.4 – 99.51% purity (up to October 19, 1999) and lot # EE 79485, 99.62 – 99.71% purity (from October 20, 1999)
CAC concurrence:	Yes

Key Study Findings

- Male and female Wistar (HsdCpb:WU) rats were dosed orally at 0, 7.5, 25, 75 and 150 mg/kg/d for 104 weeks (from 8 weeks of age).
- Adequacy of dosing was not demonstrated in the current study, but the doses had been approved by the E-CAC, based on findings at 300 mg/kg in a 13-week study.
- There were no biologically relevant, drug-related increases in incidences of neoplasms.
- Non-neoplastic findings were limited to increases in incidence/severity of fibrohistiocytic granulomas in mesenteric and mediastinal lymph nodes in both males and females at the HD.

Adequacy of Carcinogenicity Study and Appropriateness of Test Models

The study was conducted according to standard procedures based on recommendations of applicable guidelines to assess the carcinogenic potential of vilazodone. Wistar rats, used in this study, were commonly used as second rodent species in addition to mice. Moreover, *in vivo* and *in vitro* studies have demonstrated that the metabolism of vilazodone was similar in man and in rats. Treatment required concentrations of all test article samples were within the predefined acceptance (+/- 10%). The HD was selected based on results of the 13-week toxicity study in the same species and strain (doses: 0, 100, 300, and 1000 mg/kg/d) which was reviewed by Dr. Fossom (IND 54,613; N017 – April 18, 2000). As reproduced from Dr Fossom's review and in agreement with current reviewer assessment, "overload phenomena" evidenced by; mild to massive deposition of crystalline material (drug/metabolites) in the intestinal mucosa, associated with reactive histiocytosis, granulocyte infiltrates and enlarged villi, and an immune response in mesenteric lymph nodes in ~1/3 of animals after 13 weeks

of dosing were observed at ≥ 300 mg/kg/d dose. Although there was no effect on mortality or body weight loss at any dose in this 13 week study, it seemed likely that with longer (2-year) treatment duration the “overload phenomenon” would result in overt toxicities.

Based on results of these 13-week studies, the Executive CAC had agreed with the higher doses proposed (and already being administered) for carcinogenicity studies in rats (0, 7.5, 25, and 75 mg/kg/day and a higher dose of 150 mg/kg/day added 9 months later with a concurrent control group) as reflected in Meeting Minutes of April 11, 2000 (included in the Attachments)

Main toxicological findings related to treatment with vilazodone included non-neoplastic lesions of minimal to severe fibrohistiocytic granulomas in the mesenteric lymph nodes (up to 82% incidence) and in the mediastinal lymph nodes (up to 100% incidence) in male and female rats at the high dose. In addition to the “overload phenomena” identified in the 13-week study (presumably deposits of the test material) deposition of crystalline material was also observed in these granulomatous lesions of lymph nodes and within macrophages in the lung at 150 mg/kg/d.

The exposure to vilazodone during the study period was verified in TK groups of rats; the exposure (AUC_{0-24}) at the high dose was 15x (males) and 40x (females) of the human exposure at therapeutic dose of 40 mg/day. The organs and tissues from all rats of all study groups were histologically examined. Animal survival was sufficient for an adequate assessment of tumorigenic potential. Therefore, it is concluded that this is a valid carcinogenicity study.

Evaluation of Tumor Findings

The Sponsor’s analyses showed no statistically significant positive dose response relationship or pairwise difference between control and any of vilazodone treated groups in any of the tested tumor types.

Statistical review and evaluation of the results of this study was independently conducted by the statistical reviewer, Mohammad Nagem, Ph.D. (see Statistical Review for statistical methods and references). The only statistically significant finding was a dose response relationship ($p < 0.05$) for histiocytic sarcoma (hemolymphoreticular system) in female rats (2/150 in controls and 3/60 in HD). However, these tumors were present in much higher rate in male controls (7/150) versus none at HDM. In general, all tumors seen in vilazodone-treated and control rats were part of the range of tumors to be expected in this strain of rats at their age. Therefore it can be concluded that vilazodone administered to rats daily for 2 years did not affect the overall incidence of tumors in either sex.

The study results were presented to the Executive CAC on October 5, 2010. The Committee concurred that the study was acceptable, noting that selection of the high

doses had received prior concurrence. The Committee also concurred that there were no drug-related neoplasms found.

Methods

Doses:	0, 7.5, 25, 75 and 150 mg/kg/day
Frequency of dosing:	Once a day
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension/0. 25% aqueous hydroxypropyl methylcellulose
Basis of dose selection:	Rat 26-week toxicology studies with dosing of 0, 6, 20, 70 mg/kg (no MTD) and rat 13-week study (0, 100, 300 and 1000 mg/kg) in which 300 mg/kg dose exceeded the MTD.
Species/Strain:	Rat, Wistar HsdCpb:WU
Number/Sex/Group:	100/sex – controls; 50/sex/group
Age:	About 8 weeks old; males and females
Animal housing:	Individually in Type III Makrolon® cages
Paradigm for dietary restriction:	Food and water ad libitum
Dual control employed:	no
Interim sacrifice:	no
Satellite groups:	5/sex/group for TK
Deviation from study protocol:	150 mg/kg/day group with a concurrent control group was added ~ 9 months after initiation of the main study*. Executive CAC did not comment on the split study design.

* See the details in the Background section above.

Observations and Results

Mortality and general condition of the rats were checked twice daily on working days and once daily on off-days.

Results showed that there was no effect of treatment with vilazodone on rat survival during the study; 223 of 700 main group rats did not survive until the end of the treatment period. The overall mortality rate was 31.7 %.

The distribution of the mortality rate among groups and genders is listed in the following Sponsor's tables:

Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
0 mg/kg		7.5 mg/kg		25 mg/kg		75 mg/kg		0 mg/kg		150 mg/kg	
100m	100f	50m	50f	50m	50f	50m	50f	50m	50f	50m	50f
35	29	9	10	13	15	26	17	11	20	14	24
35%	29%	18%	20%	26%	30%	52%	34%	22%	40%	24%	48%

Groups 1 + 5		Group 2		Group 3		Group 4		Group 6	
0 mg/kg		7.5 mg/kg		25 mg/kg		75 mg/kg		150 mg/kg	
150m	150f	50m	50f	50m	50f	50m	50f	50m	50f
46	49	9	10	13	15	26	17	14	24
31%	33%	18%	20%	26%	30%	52%	34%	24%	48%

Clinical Signs

Behavior and general conditions of rats were checked twice daily on working days and once daily on off-days.

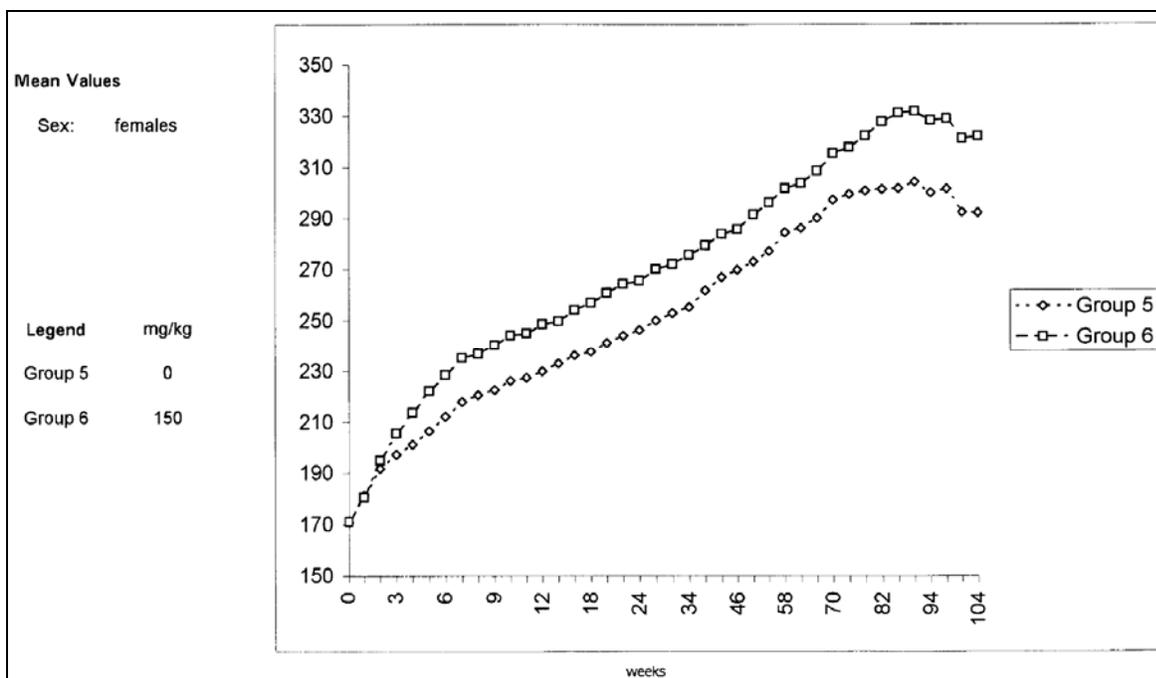
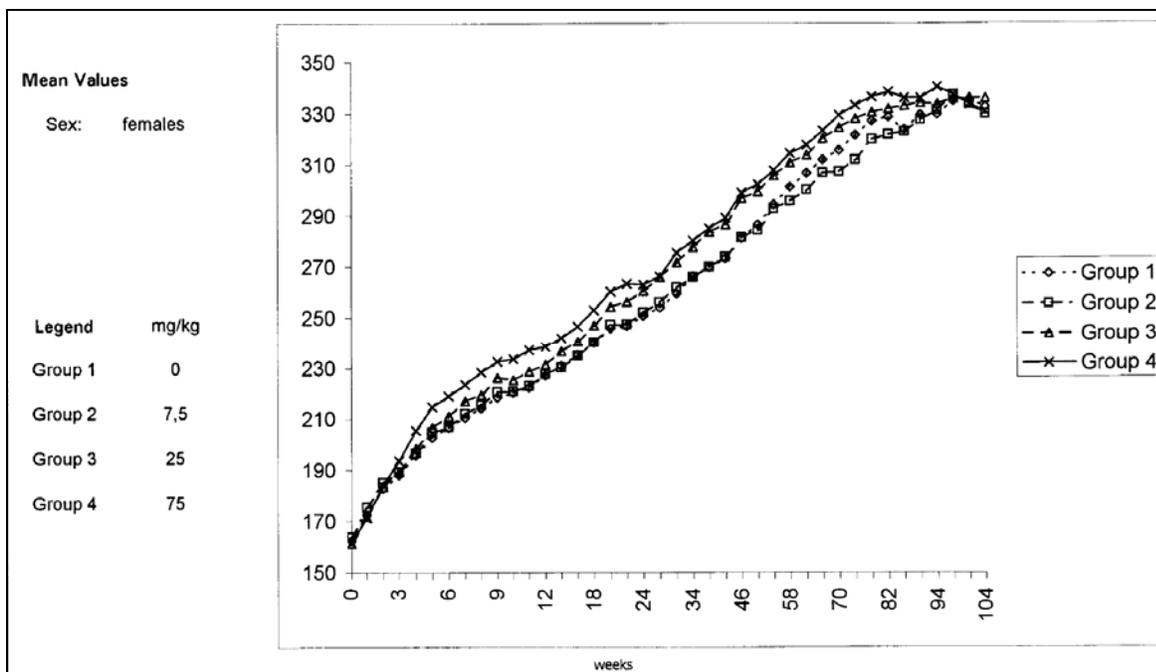
Results: Reddening of ears and feet (hyperemia) were noted in all treated rats with a dose-related increase in severity during the first 8 weeks of treatment and increased salivation during the last half of the study at 75 and 150 mg/kg/day. The following incidence of palpable masses/nodules was assessed: 16, 8, 12, 6 and 14% in males and 43, 42, 22, 32, and 34% in females at 0 (combined controls), 7.5, 25, 75, and 150 mg/kg/day, respectively.

Body Weights

Each rat was weighed once a week in weeks 1 – 12, once every 2 weeks in weeks 14 – 26, once every 4 weeks in week 30 up to the end of the treatment, and in week 104.

Results: Generally, there was no effect of treatment with vilazodone on BW in male rats at all dose levels except slight increase at 7.5 mg/kg/day during the first 11-12 weeks of treatment. The treatment with vilazodone caused increase BW in female rats at 75 mg/kg/day (significantly higher between week 4 and 50) and caused a slight increase in BW at 25 mg/kg/day during that treatment period. At 150 mg/kg the BW and BW gain of the female rats were significantly increased during the whole treatment period except for the first 2 weeks.

These BW data from female rats are presented in the following sponsor's graphs:



Feed Consumption

Food consumption was determined for each rat once a week in weeks 1 – 12, once every 2 weeks in weeks 14 – 26, once every 4 weeks in week 30 up to the end of treatment, and in week 104 by weighing the food which had not been consumed.

Results: Generally, food consumption was not impaired the treatment with vilazodone in both male and female rats.

Ophthalmology: Eye examinations (pupillary reflex, anterior parts, fundus) and examinations by slit lamp were performed before start of treatment in 50/sex/control and 25/sex/treatment group and in the same animals in weeks 31/32 (only groups 1 to 4), 52, 72, and in weeks 99/100 (groups 1, 4, 5 and 6).

Results: No treatment related alterations were observed.

Clinical Pathology: Blood samples for hematological tests (WBC, RBC, HGB, HCT, MCV, MCHC, PLT, RET, Lymph, NEU, EOS, BASO, MONO) were taken at the end of the treatment period starting in week 102 from all surviving rats. Food was withdrawn for approximately 18 h during which time rats had been kept in metabolism cages.

Results: At the end of the treatment period, hematology revealed mild increases in red blood count (≤ 1.1 -fold) but within the historical control range in all treated groups. Statistically significant increases in reticulocytes (≤ 1.7 -fold), platelets (≤ 1.4 -fold), and neutrophilic granulocytes (≤ 1.5 -fold) and decreased lymphocytes ($\leq 27\%$) occurred at ≥ 75 mg/kg/day. An increase in white blood count ($\leq 62\%$) was also noted in both sexes at 150 mg/kg/day.

Prolactin levels varied over time and no clear test article effect could be detected.

Gross Pathology: All rats of the main groups surviving until the end of their scheduled treatment period were sacrificed and all were subjected to a detailed necropsy.

Results: Treatment with vilazodone did not result in any increase in either overall tumor incidence or in any tumor type. There was no statistical relationship to the incidence of fatal tumors in treated animals. All tumors seen in treated and control rats were part of the range of tumors to be expected in this strain of rat (Wistar HsdCpb:WU).

Histopathology

Organs from all rats were examined microscopically. The list of organs and tissues evaluated histopathologically is included in the inventory table on page 58.

Peer Review; Yes, all tumors.

Neoplastic

According to the Sponsor, there were no statistically significant tumor findings in treated rats as compared to their controls. Statistical analyses performed by Dr. Nagem for dose response relationship or pairwise comparisons revealed significant increase in histiocytic sarcoma in the hemolymphoreticular system of females (3/50 – HDF versus 2/150 – controls). However, these tumors were present in much higher rate in male controls (7/150) versus none at HDM. In general, all tumors seen in vilazodone-treated and control rats were part of the range of tumors to be expected in this strain of rats at their age. A negative dose relationship was seen in females in the incidence of malignant tumors due to a decreased incidence of malignant uterine tumors at HD (14 – controls; 2 – HD). Additionally, a negative trend in the incidences of pituitary tumors in treated males (14 - controls vs 4 - HD) and females (12 – controls vs 11 – HD) was observed.

These and other statistically significant changes were considered to be of little or no toxicological significance within the context of this study.

Non Neoplastic

Statistically significant increases in non-neoplastic lesions in the heart, lung and lymph nodes (mesenteric and mediastinal) were observed in vilazodone treated rats, as shown in the following Sponsor's table.

Dose (mg/kg/day)	MALES					FEMALES				
	0	7.5	25	75	150	0	7.5	25	75	150
No. Animals Examined	150	50	50	50	50	150	50	50	50	50
Heart										
Myocardial Fibrosis	79 (53%)	25 (50%)	32 (64%)	41 (82%)	44 (88%)	45 (30%)	20 (40%)	26 (52%)	40 (80%)	40 (80%)
Auricular Dilatation	9 (6%)	3 (6%)	9 (18%)	10 (20%)	5 (10%)	1 (1%)	0 (0%)	0 (0%)	4 (8%)	7 (14%)
Early Atrial Thrombi	9 (6%)	2 (4%)	3 (6%)	6 (12%)	6 (12%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (10%)
Lung										
Foamy Macrophages with Eosinophilic Material	2 (1%)	2 (4%)	3 (6%)	1 (2%)	9 (18%)	1 (1%)	0 (0%)	0 (0%)	3 (6%)	12 (24%)
Fibrohistiocytic Granulomas	0 (0%)	3 (6%)								
Lymph Nodes – Mesenteric										
No. Animals Examined	144	47	50	48	50	132	46	45	48	49
Fibrohistiocytic Granulomas ^a	0 (0%)	1 (2%)	9 (18%)	35 (73%)	41 (82%)	0 (0%)	0 (0%)	1 (2%)	27 (56%)	35 (71%)
Sinus Histiocytosis	50 (35%)	21 (45%)	21 (42%)	24 (50%)	32 (64%)	58 (44%)	21 (46%)	22 (49%)	41 (85%)	15 (31%)
Lymph Nodes – Mediastinal										
No. Animals Examined	3	0	0	1	4	2	0	0	0	33
Fibrohistiocytic Granulomas	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	29 (88%)

^a - Extended from lymph nodes into adjacent tissues such as jejunum.

In the heart, there was increase in the incidence of myocardial fibrosis in all groups of rats. This change is common in old rats and can be exacerbated by alterations in cardiac perfusion. Histologically, an increase in the incidence of minimal to severe atrial auricular dilatation in rats at ≥ 75 mg/kg/day was seen (14% vs. 1-6% in controls). This change was often associated with early atrial thrombi (6, 4, 6, 12 and 12% in males at 0, 7.5, 25, 75 and 150 mg/kg/day, respectively, and 10% in high-dose females vs. 0% in controls). In the lungs, there was an increase in the incidence ($\leq 24\%$ vs. 2% in

controls) and severity of foamy macrophages with eosinophilic material in HD-M and HD-F (this finding was also present in previous rat studies). In females this finding was associated with fibrohistiocytic granulomas ($\leq 6\%$ vs. none in controls). In several rats outlines of crystals was seen within these macrophages.

An increase in fibrohistiocytic granulomas in both sexes, in the mesenteric lymph nodes and in mediastinal lymph nodes (HD rats), was observed in vilazodone treated rats but not in the controls. The granulomatous lesions corresponded to the enlargement of mesenteric lymph nodes (2, 15 and 78% at 0, 75 and 150 mg/kg/day, respectively) and white nodules/discoloration (1, 2, 6, 16 and 29% at 0, 7.5, 25, 75 and 150 mg/kg/day, respectively, for both sexes combined) were seen in the abdominal and thoracic cavities at necropsy. In the granulomatous lesions, outlines of crystals were often seen, presumably deposits of the test article. This correlated with the high quantities of vilazodone present in mesenteric lymph nodes. Additionally, there was an associated increase in sinus histiocytosis in treated males ($\leq 64\%$ vs. 35% in control males) and in females at 75 mg/kg/d (85% vs. 44% in controls).

Toxicokinetics

Blood samples from the satellite groups on day 1 and in weeks 26, 52, and 98, at 1, 3, 6, and 24 hrs after dosing were used for the TK evaluation.

Results: The TK data indicated that exposure to vilazodone was higher in female than in male rats throughout the duration of the study. At 150 mg/kg, peak plasma concentrations in male rats were on average 0.69, 2.30, 1.74, and 1.64 $\mu\text{g/ml}$ and in females 1.60, 3.47, 3.19, and 5.19 $\mu\text{g/ml}$ on day 1 and in weeks 26, 52, and 98, respectively. Peak plasma concentrations at the lower doses (7.5 and 25 mg/kg) were generally reached between 1 and 3 h and at the higher doses between 3 and 6 h suggesting a somewhat slower rate of absorption at the higher doses.

At all doses and on all study days, C_{max} and AUC values in female rats were higher (on average 2-fold) than those in male rats as shown in the following Sponsor's table.

Dose mg/kg	Parameter	Day 1		Day 176/177		Day 358		Day 685	
		male	female	male	female	male	female	male	female
7.5	T max (h)	1-3	3	3	1-3	3	1-3	3-6	1-3
	Cmax (ng/mL)	48.9	206	196	405	227	365	330	475
	AUC (0-6 h)	221	736	715	1610	856	1450	1350	1960
	AUC (0-24 h)	#	#	#	#	#	#	#	3400
25	T max (h)	3	3	3	3	3	3	3-6	3
	Cmax (ng/mL)	243	567	1000	2170	944	2240	1030	1540
	AUC (0-6 h)	956	2130	3710	8630	3630	9080	4210	6400
	AUC (0-24 h)	#	#	6460	18000	6890	19600	9980	15800
75	T max (h)	3-6	3-6	3-6	3	3-6	3-6	3-6	3
	Cmax (ng/mL)	711	1120	1730	3610	1400	1790	1840	2120
	AUC (0-6 h)	2950	4490	7120	14600	5930	8430	7870	9210
	AUC (0-24 h)	5390	10700	15600	34100	15300	23600	22000	26200

Dose mg/kg	Parameter	Day 1		Day 176/177		Day 358		Day 685	
		male	female	male	female	male	female	male	female
150	T max (h)	3	1-6	3-6	3-6	3-6	3-6	3-24	3-6
	Cmax (ng/mL)	691	1600	2300	3470	1740	3190	1640	5190
	AUC (0-6 h)	2810	5990	9970	16700	7800	15400	7750	26000
	AUC (0-24 h)	7690	19600	26600	53900	22000	55000	26200	92900

Vilazodone concentrations (Week 98) were also measured in small intestine, mesenteric lymph nodes and liver as shown in the following Sponsor's table:

Tissue Concentrations (µg/g)	Males			Females		
Dose (mg/kg/day)	7.5	75	150	7.5	75	150
Number of animals	1	2	5	0	2	5
Small intestine	-	4810	6190	-	6335	4324
Mesenteric lymph nodes	1390	1110	6748	-	8230	11408
Liver	-	-	26	-	-	947

- No samples available

The liver concentrations were approximately 40 times higher in male and 350 times higher in females than those of plasma 24h after drug administration in Week 98. In view of these high tissue concentrations and the poor solubility of vilazodone in aqueous medium, it is likely that the crystalline deposits noted at necropsy and histopathology represented deposits of test article.

Dosing Formulation Analysis

The analyses of the test material concentrations revealed that the required concentrations of all samples analyzed were within the predefined acceptance limits (+/- 10%) throughout the dosing period.

Summary: There was no effect of treatment with vilazodone (doses: 7.5, 25, 75 and 150 mg/kg/d; 1.8x, 6x, 18x, and 36x the HED, respectively on mg/m² basis and human therapeutic dose of 40 mg/d) on the incidence of neoplastic or hyperplastic tumors and survival rate as compared to controls. All tumors seen in treated and control rats were within the range of tumors to be expected in this strain of rats. The only statistically significant observations included increases in non-neoplastic lesions; minimal to severe fibrohistiocytic granulomas in the mesenteric lymph nodes (up to 82% incidence), often with extension into adjacent tissue, observed in treated rats of both genders and in mediastinal lymph nodes (up to 100% incidence) of HD rats, but not in controls.

8.2 Mouse carcinogenicity study.

Study title: Carcinogenicity study in mice

Study no.: 990411; Report: Gpp-007-NCD-Tox-2003-199
 Study report location: Merck KGaA, Frankfurter Strasse 250, Darmstadt, Germany

Conducting laboratory and location:



Date of study initiation: May 17, 1999
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: EMD 68843, lot # EE 77485 of 99.4% purity
 CAC concurrence: Yes

Key Study Findings:

- Male and female B6C3F1 mice were dosed orally at 0, 15, 45, and 135 mg/kg/d, for at least 105 weeks.
- Adequacy of dosing was demonstrated by increased premature mortality at HD in males and a trend for increased premature mortality in females; and the doses had been approved by the E-CAC, based on findings 13-week studies.
- Biologically relevant, drug-related increases in incidences of neoplasms were limited to hepatocellular carcinomas in males at HD and mammary gland adenocarcinomas in females at MD and HD.

Adequacy of Carcinogenicity Study and Appropriateness of Test Models:

The study was designed to assess the carcinogenic potential of vilazodone following oral administration to male and female B6 C3 F1 mice (widely accepted rodent species for evaluating the carcinogenic potential of a wide range of toxic substances) for 104 weeks. The doses for this bioassay (0, 15, 45, and 135 mg/kg/d) were selected in accordance with the Executive CAC recommendations (Meeting Minutes of April 11, 2000). The selection of doses was based on two 3-month studies in the same strain of mice; 1) at doses of 0, 5, 15, 45, and 135 mg/kg/d and 2) at doses 0, 135, 270, and 540 mg/kg/d. Mice exposed to vilazodone at ≥ 270 mg/kg/d exhibited histiocytosis in multiple organs caused by crystal deposition (possibly test article) and associated degenerative and inflammatory reactions along with bone marrow myeloid hyperplasia and splenic erythropoiesis (Study: GPP-007-NCD-TOX-1999-198 reviewed by Dr. Fossom, IND 54,613, N-017).

In this 2-year study, a trend towards decreased survival (statistically not significant according to the Sponsor) was seen in vilazodone treated mice; at the HDM (78% versus 87% in controls) and HDF (70% versus 79% in controls). The dose response relationship or differences between control and any of the treatment group survivals was assessed to be statistically significant (see statistical review by Dr. Nagem) in male but not in female mice. Treatment with HD was associated with findings of histiocytosis in multiple organs caused by crystal deposition and associated degenerative and inflammatory reactions (similar to those observed in the 13-week study with higher doses). Based on these findings a 135 mg/kg/d dose was considered to be the MTD in this carcinogenicity study. All animals of treatment groups were exposed to vilazodone during the study period. The organs and tissues from all animals of all study groups were histologically examined. Animal survival was sufficient for an adequate assessment of tumorigenic potential. Therefore, it is concluded that this is a valid carcinogenicity study.

Evaluation of Tumor Findings

Hepatocellular adenomas and carcinomas were present in males and females in all groups including control. Although statistical significance was noted at some doses of vilazodone in both sexes, there was no clear dose response relationship. It should be noted that the incidence of hepatic adenomas and carcinomas in the treated and control groups in this study were within the National Toxicology Program (NTP) historical control database rates for B6 C3 F1 mice.

There was an increase in acinar hyperplasia and a statistically significant increase in adenocarcinomas in the mammary glands in MDF and HDF. An increased incidence of mammary adenocarcinomas in rodents has been previously associated with administration of drugs known to elevate serum prolactin including drugs acting directly or indirectly on activation of 5-HT receptors. The 2-week mechanistic study (PGX-08-PC-01) with B6C3F1 female mice administered vilazodone at 45 and 270 mg/kg/d demonstrated increases in serum prolactin (on Day 7 by 5- and 5.5-fold and Day 14 by 3- and 7- fold, respectively) suggesting hormonally mediated mechanism for the mammary gland findings.

There were statistically significant increases in thyroid gland follicular adenomas in MDM; and in thyroid gland follicular hyperplasia in males at all doses and females at \geq 45 mg/kg/d. There was a trend toward a dose response for both the hyperplasia and adenomas; however, in controls there was an unusually high background level of both. Moreover, the incidence of thyroid follicular adenoma in the vilazodone-treated groups was within the historical data range for males, and slightly above the NTP historical data for the adenomas observed in the females. Further analysis of selected animals (study: GPP-007-NCD-TOX-2004-250) indicated a correlation between thyroid stimulating hormone (TSH)-positive pituitary lesions and thyroid neoplasms in both control and vilazodone treated mice. In a 3-month study (PGX-09-PC-01), treatment of B6 C3 F1 male mice with vilazodone at 45 and 135 mg/kg/d resulted in small, but statistically

significant increases in TSH. Taken together, these results suggest that overstimulation of the thyroid may have contributed to the hyperplasia and adenomas observed in this 2-year carcinogenicity study in mice. The increased incidence of tumor findings in thyroid glands of mice (statistically significant in MDM according to the Sponsor but not significant according to Dr. Nagem statistical review), was not considered biologically relevant by the Executive CAC or this Reviewer.

In summary, the Sponsor's analysis demonstrated a statistically significant positive dose response relationship in the incidence of the thyroid, mammary gland and liver tumors in both male and female mice. Pairwise comparisons showed statistically significant increase in the incidence of hepatocellular adenomas in HDM, MDF and HDF as compared to their respective controls.

Statistical review and evaluation of tumor findings in this study was independently conducted by the statistical reviewer, Mohammad Nagem, Ph.D. According to his analysis, increased incidences of mammary gland adenocarcinomas and adenoacanthomas combined in HDF and increased incidences of hepatocellular adenomas, carcinomas, (separately and combined) in HDM were statistically significant by trend and pairwise tests in mice treated with vilazodone as indicated in the following table:

Organ name	Tumor name	Control n = 120	15 mg/kg N = 60	45 mg/kg N = 60	135 mg/kg N = 60	Trend test P-value	Pairwise test (C vs H) P-value
Mammary gland – (females)	Adenocarcinoma + adenoacanthoma	3	1	6	8*	< 0.0017*	< 0.0069*
	Adenocarcinoma	3	1	6	7	< 0.0050*	(< 0.0166)
Liver – (males)	Adenoma + carcinoma	36	32	20	40*	< 0.001*	< 0.001*
	Adenoma	22	23	14	27*	< 0.001*	< 0.001*
	Carcinoma	15	11	10	19*	< 0.0012*	< 0.0016*

No statistically significant dose response relationship or differences between the control and any of the treated groups in survivals across treatment groups in female mice, that was similar to the Sponsor's analysis. However, dissimilar to the Sponsor's analysis, the dose response in mortality in male mice was statistically significant (p=0.0440; see Statistical Review and Evaluation for further details).

Methods

Doses: 0, 15, 45 and 135 mg/kg/day
 Frequency of dosing: Once daily, 7 days/week for 24 months
 Dose volume: 10 ml/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: Solution/0.25% hydroxypropyl-methylcellulose

Basis of dose selection: Two 13-week toxicity study: GPP-007-NCD-TOX-1998-197 (doses: 0, 5, 15, 45, and 135 mg/kg/d; MTD was not reached) and GPP-007-NCD-TOX-1999-198 (doses: 0, 135, 270, and 540 mg/kg/d; increased liver and spleen (up to 395%) weights; histiocytosis of multiple organs caused by crystal deposition (possibly the test article) and associated degenerative and inflammatory reactions; also bone marrow myeloid hyperplasia and splenic erythropoiesis at ≥ 270 mg/kg/d. MTD was at 135 mg/kg/d.

Species/Strain: Mice/B6 C3 F1
 Number/Sex/Group: 60/sex/group; 120/sex/vehicle
 Age: About 4 weeks old and weighed ~ 16-18 g at the beginning of the study
 Animal housing: Wire cages 28.5x10.5x13h cm
 Paradigm for dietary restriction: Ad libitum
 Dual control employed: No
 Interim sacrifice: No
 Satellite groups: TK – 48/sex/group; serum prolactin and blood chemistry – 6/sex/group (control and high dose)

Observations and Results

Mortality

Observations were made twice a day (morning and late afternoon). Animals found dead before the end of the study or sacrificed "in extremis" were subjected to a complete necropsy and the organs selected for the gross pathology examination were removed and histologically evaluated.

Results showed a trend toward decreased survival observed in vilazodone-treated animals (mainly at the HD compared to the control) as shown in the following Sponsors' table:

Dose (mg/kg/day)	Mortality (%)							
	Week 60	Males			Females			
		Week 87	Week 102	Week 106	Week 60	Week 88	Week 102	Week 106
0	0.00	5.00	12.50	13.33	2.50	10.00	20.00	22.50
15	0.00	3.33	6.67	10.00	1.67	8.33	20.00	30.00
45	1.67	5.00	16.67	20.00	0.00	1.67	13.33	18.33
135	5.00	13.33	21.67	23.33	0.00	8.33	23.33	35.00

The statistical analyses performed by Dr. Nagem revealed that the dose-response in mortality rate was statistically significant in male ($p < 0.0440$) but not in female mice.

Clinical Signs

Animals were observed daily for clinical signs. In addition the examination for physical appearance and palpable masses were performed and recorded once every 4 weeks during the study.

Results demonstrated an increase in palpable masses was observed near the end of dosing period at 135 mg/kg/d as shown in the following Sponsors' table at week 104:

Dose (mg/kg/day)	Males			Females		
	% ^a	No. ^b	Cumulative ^c	% ^a	No. ^b	Cumulative ^c
0	2.88	3/104	5/120	1.06	1/94	4/120
15	0.00	0/54	0/60	0.00	0/45	0/60
45	2.00	1/50	3/60	3.92	2/51	3/60
135	4.26	2/47	6/60	13.64**	6/44**	9/60

^a percent of surviving mice with palpable masses

^b number of surviving mice with masses/number of surviving mice

^c total number of mice with masses/total number of mice over length of study

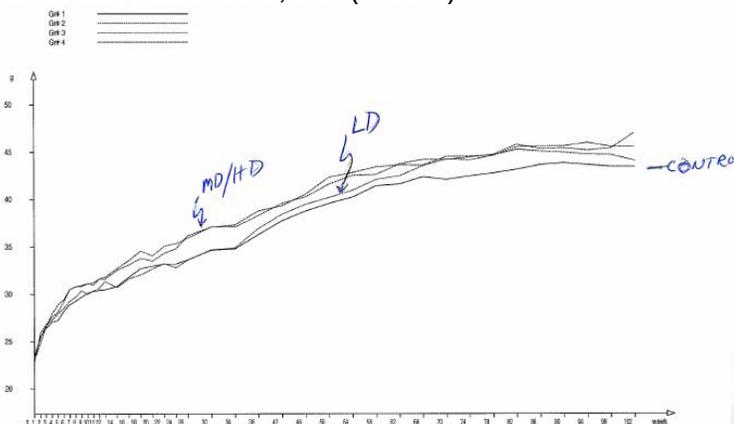
** p<0.01, vs. control

Body Weights

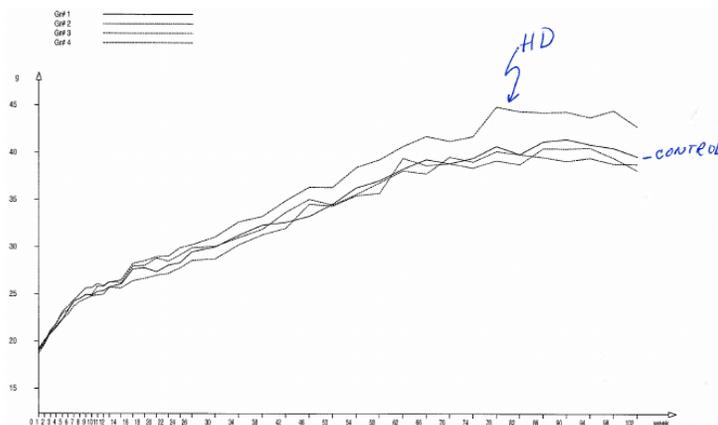
Each animal was weighed prior to the beginning of the treatment period (on Day 0, the day before the start of treatment), at weekly intervals for the first 12 weeks of the study, once every two weeks from week 14 to week 26 and once every 4 weeks thereafter.

Results: In general vilazodone had no adverse effects on body weight throughout most of the 24 months study in mice as indicated in the following sponsors' graphs excerpted from Dr. Fossoms' memo to the IND 54,613 (N-057)

Male mice



Female mice



There was a gradual increase in mean body weight in HDF showing 4 – 7% increase vs. controls in weeks 12 – 74 and 8 - 11% increase vs. controls in weeks 78 - 102. Mean body weights of males at all dose levels were also slightly higher (3 – 7%) than those of controls but did not show any dose-correlation.

Feed Consumption

Amount of food consumed was recorded, for each animal, weekly for the first 12 weeks, for one week every 2 weeks from week 14 to week 26 and for one week every 4 week thereafter.

Results: There were no relevant changes in food consumption in either sex at any dose.

Clinical Pathology

The following parameters were measured in all the surviving mice at the final sacrifice; hematological: erythrocytes, hemoglobin, leukocytes, hematocrit, mean corpuscular volume (MCV), MCHC, mean corpuscular hemoglobin (MCH), and platelets (a differential WBC was performed on the blood smears from HD and control groups).

Results: Hematological changes at Week 108 examination included the following parameters:

- increased leukocytes (2.6-fold - HDM and 46% - HDF)
- increased platelets (1.4-fold - HDM and 64% - HDF)
- decreased hemoglobin (9% - HDM and 8% - HDF)
- decreased hematocrit (8.5 % both HDM and HDF)
- decreased erythrocytes (4.6% - HDM only)
- decreased MCV (4.3%- HDM; 7%-HDF) and MCH (4.3%-HDM; 7%-HDF)

Gross Pathology

At the end of treatment period each not fasted surviving animal as well as all mice sacrificed before the end of treatment were bled to death and subjected to a complete necropsy. Blood was collected for clinical pathology and plasma level determination from all animals killed at the end of the treatment period.

The list of organs and tissues evaluated histopathologically is included in the inventory table on page 58.

Results: Treatment-related changes were observed in the following organs of males and females: liver, kidneys, mesenteric, inguinal and mediastinal lymph nodes, spleen and, in males, urinary bladder.

Kidneys - Treatment-related changes were noted in both HDM and HDF, consisting of increased incidences of paleness (HDM - 35% vs 11 % in control group) and presence of whitish area at the cortico-medullary junction (HDF - 27% vs 0% in control group).

Liver – An increased incidence of masses or nodules, compared to controls, were noted in vilazodone-treated mice as summarized in the following table:

	0 mg/kg/day	15 mg/kg/day	45 mg/kg/day	135 mg/kg/day
Males	35%	48%	43%	67%
Females	19%	30%	37%	33%

Mesenteric lymph nodes - Increased size was noted in both sexes treated with the high dose (HDM - 33% vs 8% control; HDF - 45% vs 6% control). Increased size was also seen in other lymph nodes (i.e. iliac lymph nodes in HDM, and mediastinal lymph nodes in HDF). These changes were sometimes correlated with histopathological treatment-related changes (presence of vacuolated histiocytes and inflammation).

Spleen – Increased incidences, as compared to controls, of paleness and increased size were noted in animals of the high dose groups (paleness: HDM - 83% vs 5% - control; HDF - 92% vs 1% - control; increased size: HDM - 28% vs 16% - control; HDF - 73% vs 37% - control).

Urinary bladder - Increased incidences of dilation and repletion were noted in males high and mid doses (dilation: 55% and 32% vs 4%; repletion 50% and 25% vs 3%, respectively at HDM, MDM and controls). The increased incidence of macroscopic observation of dilated organ was correlated with distended wall seen microscopically, and very rarely associated with mucosal ulcer and inflammation.

Subcutaneous tissue – An increased incidence of masses or nodules (confirmed by histology as mammary tumors), was noted in HDF – 15% and MDF – 10% versus 5% in controls.

Histopathology

The organs and tissues from all animals of all study groups to be histologically examined were embedded in paraffin blocks, sectioned and stained with hematoxylin and eosin.

Peer Review: Yes; the consensus was obtained on all cases and this report submitted to the NDA is the final result of that assessment.

Neoplastic

The thyroids, liver and mammary gland were the main target organs of neoplastic and/or non-neoplastic changes in both sexes. In addition, findings of hyperplastic/tumoral or non-tumoral changes were observed in kidneys, urinary bladder,

jejunum, ileum, lymph nodes (mesenteric, inguinal and mediastinal), spleen, bone marrow and mesentery in both sexes.

Thyroids - An increase in the incidences of proliferative (neoplastic and/or non-neoplastic) changes of follicular cells occurred in a dose-related manner in males of all treated groups and in HDF and MDF as shown in the following sponsors' table.

<i>HYPERPLASTIC AND NEOPLASTIC MODIFICATIONS OF THYROID GLANDS IN MALES</i>				
Group No.	1	2	3	4
Number of Animals examined	120	60	60	60
Dose (mg/kg)	0	15	45	135
Follicle (s) Hyperplasia, Slight	26 (22%)	22 (37%)	26 (43%)	29 (48%)
Follicle (s) Hyperplasia, Moderate	4 (3%)	9 (15%)	17 (28%)	23 (38%)
Total incidence of follicle hyperplasia	30 (25%)	31 (52%)	43 (72%)	52 (86%)
Follicle (s) Adenoma (single)	3 (3%)	1 (2%)	7 (12%)	4 (7%)
Total incidence of follicle adenoma	3 (3%)	1 (2%)	7 (12%)	4 (7%)
<i>HYPERPLASTIC AND NEOPLASTIC MODIFICATIONS OF THYROID GLANDS IN FEMALES</i>				
Group No.	1	2	3	4
Number of Animals examined	120	60	60	60
Dose (mg/kg)	0	15	45	135
Follicle (s) Hyperplasia, Slight	39 (33%)	19 (32%)	27 (45%)	22 (37%)
Follicle (s) Hyperplasia, Moderate	19 (16%)	12 (20%)	19 (32%)	16 (27%)
Follicle (s) Hyperplasia, Severe	1 (1%)	2 (3%)	1 (2%)	2 (3%)
Total incidence of follicle hyperplasia	59 (49%)	33 (55%)	47 (78%)	40 (67%)
Follicle (s) Adenoma (single)	8 (7%)	2 (3%)	4 (7%)	8 (13%)
Follicle (s) Adenoma (multiple)	2 (2%)	1 (2%)	3 (5%)	1 (2%)
Total incidence of follicle adenoma	10 (8%)	3 (5%)	7 (12%)	9 (15%)

Statistical analysis of the data provided by the sponsor showed trends toward increased incidence of adenoma significant ($P=0.015$) in males and proximal to significance ($P=0.058$) in females, and incidence significantly increased, compared to controls, in MDM. The increased incidence of thyroid follicle hyperplasia showed statistical significance in male and female mice with $p \leq 0.001$ in males and $p \leq 0.009$ in females. Individual groups also appeared to be increased in incidence compared to controls, with $p < 0.001$ for all treated male groups and MDF, and $p < 0.05$ for HDF.

The historical incidence for thyroid follicular cell adenomas and carcinomas in B6 C3 F1 control mice (2-year NTP methylcellulose gavage studies) is summarized in the following sponsors' table:

Thyroid follicular neoplasm		Males	Females
Adenoma	Mean	13/199 (6.5%)	8/198 (4.1%)
	Range	1/49 - 6/50 (2 - 12%)	1/50 - 3/49 (2 - 6%)

Comparison with these data indicates that the incidence of thyroid follicular adenoma in the vilazodone-treated groups was within the historical data range for males, and slightly above the NTP historical data for the adenomas observed in the females.

Given the high incidence of proliferative thyroid lesions occurring in mice of all groups including controls, but with higher incidence in vilazodone treated mice, the possible role of TSH in these processes was examined in the study entitled:

“EMD 68 843 – TSH Immunohistochemistry study on pituitaries of mice (GPP-007-NCD-TOX-2004-250, T15768)

Note: This study was submitted to the IND 54,613 (N-057) on 12-27-2004 and reviewed by Linda Fossom, Ph.D. The present reviewer agrees with Dr. Fossom’s assessments, therefore the following is directly reproduced from Dr. Fossom’s document:

“.....the Sponsor conducted a retrospective study, where immunohistochemical analysis (including morphometry) for TSH was conducted on paraffin sections of pituitaries that were available from dosed female mice that had severe multifocal thyroid hyperplasia and control female mice that had moderate (none had severe) multifocal thyroid hyperplasia (plus all male mice with pituitary lesions: 5 male mice with pituitary adenomas and 2 males with pituitary hyperplasia) from the carcinogenicity study; pituitaries from 52 mice were examined for histology, but only 27 were “considered to be suitable for a morphometrical investigation.” These 27 mice included “19 of the 80 females with pituitary adenomas (4 without thyroid lesion, 15 with thyroid lesions)” and all 5 males with pituitary adenomas or hyperplasia. [The study report also says that “about 2/3 of the thyroid adenomas in each group [of female mice] were associated with pituitary hyperplasias and/or adenomas.”]

The results of the immunohistochemical analysis are presented in the table, below.

Table: Findings of TSH-containing cells in hyperplastic/neoplastic vs normal tissue in anterior pituitary of female mice, arranged by whether the mice also showed hyperplasia or neoplasia in thyroid tissue.

Thyroid	Females examined	Pituitary	Pituitary	Pituitary
		no lesion	hyperplasia and/or adenoma	remaining normal tissue
			TSH pos. / neg.	TSH pos. / neg.
No lesion	7 (4-0-0-3)	7		7 / 0
No lesion	4 (4-0-0-0)		1 / 3	4/0
Hyperplasia (slight)	1 (0-0-0-1)		-/1	1 /-
Hyperplasia (moderate)	6 (6-0-0-0)	1	3 / 2	3/ 2 *
Hyperplasia (severe)	4 (0-2-1-1)	0	4 / 0	0/ 3 *
Adenoma (single)	9 (1-1-2-5)	4	3 / 2	8/ 1
Adenoma (multiple)	7 (3-1-3-0)	2	4 / 1	3/ 4

(group 1-group 2-group 3-group 4) * no normal tissue available in one mouse

It was noted that “Most female mice with thyroid hyperplasias (8 of 10 mice) and half of the mice with thyroid adenomas (8 of 16 mice) exhibited concurrent pituitary hyperplasias and/or adenomas with few to abundant TSH immunoreactive cells...and the remaining normal pituitary tissue often showed no or only a few TSH immunoreactive cells (compare numbers printed in bold in the table above.” The 5 pituitary adenomas observed in males were negative for TSH, but 1 of the 2 hyperplasias seen in HDM had TSH-immunoreactive cells with a concurrent low number of TSH cells in the remaining normal tissue (as seen in females).

Based on the morphometric analysis, there was no difference in the TSH-positive cell density in normal pituitary tissues in controls (mean of 170 cells/mm² with range of 0-374 for 17 control female mice) compared with high-dose (mean of 164 cells/mm² with range of 0-331 for 10 HDF) female mice.

The study report concluded that “most thyroid hyperplasias and half of the thyroid adenomas were associated with pituitary hyperplasias or adenomas that contain few to abundant TSH positive cells.” It was also noted a “concurrent reduction or complete lack of TSH secreting cells in the remaining unaffected pituitary tissue in all of these mice” consistent with a feedback mechanism acting locally in the pituitary. Additionally, “TSH expressing hyperplastic and neoplastic lesions appear to have interfered with mechanisms that maintain an endocrine homeostasis in the pituitary-thyroid axis in a lot of female mice,” which resulted in over stimulation of thyroid growth. Finally, “The higher incidence of thyroid proliferative lesions in dose mice suggests a drug-related effect, but this enhancement of proliferation was demonstrated to be mostly indirectly, e.g. mostly TSH driven.”

However, regarding a dose-related effect, it should be noted that, of the 27 mice that could be analyzed: 1) There was no difference in the density of TSH-positive cells in the 3 controls and 3 HDF that had normal pituitaries and thyroids: the mean TSH-positive cell density was 189 (148-250) cells/mm² for controls and 272 (207-331) cells/mm² for HDF. 2) There was no apparent difference in the density of TSH-positive cells in the female mice that had thyroid adenomas (see table, below). 3) The number of mice that could be analyzed was small and the results were very variable.

Table: Density of TSH-positive cells in normal tissue, hyperplasia, and adenomas in the pituitaries of female mice that had thyroid adenomas (from Dr. Fossom review).

DOSE	MOUSE #	TSH-POSITIVE CELLS PER MM ² IN PITUITARY		
		normal	hyperplasia	adenoma
Controls	2528	58		8
	2554	245		
	2570	0		48
	2589	337		
LD	2698	303	0	0
	2702	0		1

DOSE	MOUSE #	TSH-POSITIVE CELLS PER MM ² IN PITUITARY		
		normal	hyperplasia	adenoma
MD	2830	0	75	
	2855	420		
	2813	0	177	4
	2852	99		0
	2859	0	0	7
HD	2936	41	0	
	2937	282		
	2940	52		
	2976	192		
	2977	18		51

In conclusion, this supportive immunohistological and morphometric study of pituitaries from selected control and vilazodone treated mice with thyroid adenomas or hyperplasia suggested that most thyroid hyperplasias and half of the adenomas were associated with pituitary hyperplasias or adenomas. The higher incidence of thyroid proliferative lesions in dosed mice suggests a drug-related effect (mostly indirect, TSH driven). These pituitary lesions contained variable numbers of TSH-positive cells, whereas the unaffected pituitary tissue showed a near or complete absence of TSH-positive cells.

Liver - Hepatocellular adenomas and carcinomas were present in male and female mice of all groups including controls. The incidence of adenomas was significantly increased in LDM and HDM but not in MDM group (lack of dose relationship) but increased dose-dependently in MDF and HDF. Carcinomas increased significantly in HDM (32% vs. 13% in controls) as well as in LDF and HDF (15% vs. 7% in controls) as shown in the following Sponsor's table:

MALE MICE : TOTAL NUMBER OF ANIMALS WITH LIVER TUMORS				
Group No.	1	2	3	4
Number of animals	120	60	60	60
Dose (mg/kg)	0	15	45	135
Hepatocellular Adenoma, single	18 (15%)	20 (33%)	9 (15%)	11 (18%)
Hepatocellular Adenoma, multiple	4 (3%)	1 (2%)	1 (2%)	10 (17%)
Hepatocellular Carcinoma, single	13 (11%)	5 (8%)	4 (7%)	11 (18%)
Hepatocellular Carcinoma, multiple	2 (2%)	4 (7%)	2 (3%)	2 (3%)
Hepatocellular Adenoma(s) and Carcinoma(s) (A)	-	2 (3%)	4 (7%)	6 (10%)
Total animals with liver hepatocellular tumors	37 (31%)	32 (53%)	20 (33%)	40 (67%)
Total incidence of adenoma	22 (18%)	23 (38%)	14 (23%)	27 (45%)
Total incidence of carcinoma	15 (13%)	11 (18%)	10 (17%)	19 (32%)

FEMALE MICE : TOTAL NUMBER OF ANIMALS WITH LIVER TUMORS				
Group No.	1	2	3	4
Number of animals	120	60	60	60
Dose (mg/kg)	0	15	45	135
Hepatocellular Adenoma, single	12 (10%)	4 (7%)	11 (18%)	8 (13%)
Hepatocellular Adenoma, multiple	1 (1%)	-	-	3 (5%)
Hepatocellular Carcinoma, single	7 (6%)	8 (13%)	4 (7%)	6 (10%)
Hepatocellular Carcinoma, multiple	1 (1%)	1 (2%)	2 (3%)	1 (2%)
Hepatocellular Adenoma(s) and Carcinoma(s) (A)	-	-	1 (2%)	2 (3%)
Total animals with liver hepatocellular tumors	21 (18%)	13 (22%)	18 (30%)	20 (33%)
Total incidence of adenoma	13 (11%)	4 (7%)	12 (20%)	13 (22%)
Total incidence of carcinoma	8 (7%)	9 (15%)	7 (12%)	9 (15%)

(A) Animals with combined hepatocellular adenoma and carcinoma were not included in the counts of the previous categories (animals with adenoma or carcinoma).

In addition, the number of mice having metastatic hepatocellular carcinomas (seen in the lung) was: 4/120 (control-M); 5/60 (LDM); 3/60 (MDM); 1/60 (HDM); and 1/120 (control-F); 2/60 (LDF); 3/60 (MDF); 7/60 (HDF).

According to the Sponsor, the statistical analysis of the data showed significant trends towards increase for adenoma in both sexes and carcinoma in males, mainly related with significantly increased frequencies at the high dose. Similarly, significant trends emerged at the statistical analysis of combined adenoma plus carcinoma.

The historical incidence of hepatocellular adenomas and carcinomas in 2-year NTP methylcellulose gavage studies in B6 C3 F1 control mice is as follows:

Hepatocellular neoplasm		Males	Females
Adenoma	Mean	86/199 (43.2%)	70/200 (35%)
	Range	16/50 - 26/49 (32-53%)	11/50 - 20/50 (22-40%)
Carcinoma	Mean	63/199 (31.7%)	46/200 (23%)
	Range	10/49 - 21/50 (20-42%)	7/50 - 14/50 (14-28%)
Combined	Mean	126/199 (63.3%)	96/200 (48%)
	Range	24/50 - 36/50 (48-72%)	20/50 - 27/50 (40-54%)

Comparison with the above data indicates that the incidence of hepatocellular adenomas and carcinomas observed in the vilazodone-treated groups was within the NTP historical range.

Mammary gland - Statistical analysis performed by the Sponsor and Dr Nagem, showed a significant trend toward increase of the neoplastic pathology due to treatment, and group incidences significantly higher than controls at MDF and HDF as shown in the following table:

Finding	Controls	15 mg/kg/day	45 mg/kg/day	135 mg/kg/day
Adenocarcinoma	3% (3/118)	2% (1/57)	11% (6/57)*	14% (8/56)**
Acinar hyperplasia	7.6%	7%	14%	28.6%

* includes one that had metastasized to the lung

** includes one adenoacanthoma

The historical incidence for mammary carcinoma in 2-year NTP gavage methylcellulose studies in B6 C3 F1 female control mice is 1/200, i.e. 0.5% (range 0-2%).

The incidence of mammary adenocarcinoma at MDF and HDF were therefore above the NTP historical data for this strain.

Non Neoplastic

Non neoplastic findings were noted in multiple organs. In the high-dose group, the kidneys showed paleness in males (35% vs. 11% of controls) and white areas at the cortico-medullary junction in females (27% vs. 0% of controls). Also in the high dose group, enlargement of the mesenteric lymph nodes (33% vs. 8% in males; 45% vs. 6% in females) as well as other lymph nodes, e.g., iliac and mediastinal, was noted. Spleens were also pale (83% vs. 5% in males; 92% females) and enlarged (28% vs. 16% in males; 73% vs. 37% in females). These changes often correlated with histological findings of histiocytosis (vacuolated histiocytes often containing crystal cleft-shaped foreign material, presumed to be test article) and pyrogranulomatous inflammation noted in these and other organs (ileum, jejunum, bone marrow, mesenterium, mammary gland interstitium, kidneys, and liver) in response to vilazodone crystals. The organs most extensively involved in this process were the ileum, jejunum, mesenteric lymph nodes and spleen, suggesting that the test article was deposited in the small intestine, accumulated within macrophages and was distributed either by these cells or via the lymphatics and blood to different organs. The extent of organ distribution and the severity of inflammation varied among animals of the same dose groups. Increased incidence of hematopoiesis compared to controls was noted in the spleens of males (90% vs. 27%) and females (92% vs. 73%) and the livers of females (15% vs. 3%) at 135 mg/kg/day. Dose-related findings of urinary bladder distention were seen macroscopically (32-55% vs. 4% of controls) and microscopically (30-57% vs. 4% of controls) in the males at ≥ 45 mg/kg/day. This was not usually associated with obstructive or inflammatory processes.

Toxicokinetics

Blood samples were collected from all vilazodone treated animals (3/sex/group) on Day 1 and in Weeks 25, 52 and 105 at 1, 3, 6, and 24 hrs after dosing.

Results of this study show that Vilazodone was rapidly absorbed with t_{max} at approximately 1 h in both sexes on all sampling days. $AUC_{0-24 h}$ was roughly dose-proportional at Day 1 and Week 25, but not at Week 52 and at Week 105-sampling days, where inconsistent alterations were noted in both sexes. Exposure ($AUC_{0-24 h}$) increased with repeated dosing from Day 0 to Week 52 in males and females. Similar trends were observed in C_{max} values. No meaningful gender differences were noted at any time in the study.

Vilazodone exposure in mice carcinogenicity study is summarized in the following Sponspr's table:

Dose (mg/kg/day)	C_{max} (ng/ml)								
	Day 1	Males			Females				
		Week 25	Week 52	Week 105	Day 1	Week 25	Week 52	Week 105	
15	718	854	3151	455	414	833	1126	1078	
45	2220	2241	2796	2505	1627	2526	4006	3122	
135	1787	4673	5334	3116	2742	4534	7940	4076	
Dose (mg/kg/day)	AUC_{0-24h} (ng•h/ml)								
	15	3511	3682	17582	2564	3600	3770	3809	5443
	45	9464	11526	23859	15183	10454	15427	19110	21987
	135	25674	39503	64372	44206	31783	34726	57211	48362

Dosing Formulation Analysis:

Test article formulations were checked for concentration five times for each treatment group during the administration period.

All preparations were within the predefined acceptance limits for this type of formulation (suspension: 90-110% of the nominal concentration). Tests of stability of vilazodone in frozen plasma were performed at 2 concentrations (low and high in the range of the calibration curve: 0.5 and 100 ng/ml). The percent concentration ratios (% Recovery) of the frozen versus the fresh samples were 97.8% and 99.6% at 0.5 and 100 ng/ml respectively.

Other

Prolactin and blood chemistry determinations were performed in additional satellite groups of mice (controls – 6/sex and HD – 6/sex). Blood samples were collected from the abdominal aorta of these animals after 13 weeks of dosing.

The following blood chemistry parameters were analyzed in the serum samples: creatinine, urea, ALP, SGOT/AST and SGPT/ALT.

Results of this study demonstrated a 1.4-fold increase in prolactin in HDM and HDF as compared to their controls. No meaningful changes were seen in clinical chemistry parameters.

Summary: A trend towards increased mortality was observed at the HDM (78% versus 87% in controls) and HDF (70% versus 79% in controls). The dose response in mortality rate was statistically significant in male but not in female mice.

The thyroids, liver and mammary gland were the main target organs of neoplastic and/or non-neoplastic changes in both sexes of B6 C3 F1 mice treated with vilazodone

at 15 (LD), 45 (MD) and 135 mg/kg/d (HD); doses being 1.8x, 5.5x , and 16.5x the HED on mg/m² basis and human therapeutic dose of 40 mg/d.

A statistically significant, dose-dependent increase in thyroid follicular cell hyperplasia in males at all doses and in MDF and HDF was identified. Total follicular adenomas increased, not dose-dependently, in MDM (statistically significant) and HDM. Elevated TSH levels were observed in rats treated with the same doses of vilazodone in another 3 month study suggesting an overstimulation of the thyroid as a contributing factor to observed findings.

Mammary gland adenocarcinomas were increased in MDF and HDF and these findings correlated with increased plasma prolactin level assessed in another study in mice treated with the same doses of vilazodone.

A statistically significant increase in hepatocellular neoplasms was observed at LDM and HDM but not at MDM and these observed rates of increase were within the National Toxicology Program (NTP) historical control database rates for B6 C3 F1 mice.

9 Reproductive and Developmental Toxicology

The reproductive and developmental studies performed with vilazodone included:

Study Type and Duration	Species	Dose by oral gavage (mg/kg/day)	Study No. (Ref.)
8 Week Male fertility	Rat / HSD/CPB:WU	0, 5, 25, 125	GPP-007-NCD-TOX-1997-025
5 Week Female fertility	Rat / HSD/CPB:WU	0, 5, 25, 125	GPP-007-NCD-TOX-1997-179
10 Day Embryo-fetal development (range finding)	Rat / HSD/CPB:WU	0, 50, 100, 200	GPP-007-NCD-TOX-1997-180 and amendment GPP-007-NCD-TOX-2004-251
12 Day Embryo-fetal development	Rat /HSD/CPB: WU	0, 8, 40, 200	GPP-007-NCD-TOX-1997-183
13 Day Embryo-fetal development (range finding)	Rabbit / New Zealand White	0, 5, 10, 50	GPP-007-NCD-TOX-1997-181 and amendment GPP-007-NCD-TOX-1997-182
13 Day Embryo-fetal development	Rabbit / New Zealand White	0, 2, 10, 50	GPP-007-NCD-TOX-1998-184
25 Day Pre- and postnatal development (range finding)	Rat / Sprague Dawley	0, 160	GPP-007-NCD-TOX-2001-185
39 Day Pre- and postnatal development	Rat / Sprague Dawley	0, 5, 25, 125	GPP-007-NCD-TOX-2002-186

9.1 Fertility and Early Embryonic Development

Separate studies were conducted with males or females treated; however, they are reviewed together here.

Study title: EMD 68 843 – Female fertility study with oral administration to rats Male fertility study with oral administration to rats

Study no.:	GPP-007-NCD-TOX-1997-179 (F) and GPP-007-NCD-TOX-1997-025 (M)
Study report location:	Institute of Toxicology of Merck KGaA, Darmstadt, Germany
Conducting laboratory and location:	Institute of Toxicology of Merck KGaA, Darmstadt, Germany
Date of study initiation:	February 6, 1997 (F); February 28, 1997 (M)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	EMD 68 843, lot # EE77185, 99.4% purity

Key Study Findings:

- Male or female rats were dosed orally at 0, 5, 25, or 125 mg/kg/day, starting 4 weeks (males) or 2 weeks (females) before mating;
- Males treated at 125 mg/kg/d showed a reduced fertility index (76% versus 96% in controls) without any changes in weight or microscopic findings in male sexual organs that would correlate with reduced fertility.
- Treatment with vilazodone had no effect on female rat fertility or reproductive function.

Methods

Doses:	0, 5, 25, and 125 mg/kg/d
Frequency of dosing:	Once a day
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension/hydroxypropylmethylcellulose (HPMC)
Species/Strain:	Rats/Wistar, HSDCPB: WU
Number/Sex/Group:	100 F and 100 M
Satellite groups:	none
Study design:	Female study: treatment started two weeks before mating and was continued until GD7. Treated females were mated with untreated males; males were changed after 1 week. GD0 was defined as the day sperm was detected in the female. Females were sacrificed on GD20 and underwent a complete necropsy. Male study: after 4 weeks of treatment males were mated

with untreated females for 2 weeks. Mating was considered successful if implantation sites could be determined in the uterus. After 7 weeks of treatment males were necropsied. Females of this study were killed on GD 15 and content of uteri was examined.

Deviation from study protocol: Not reported

Observations and Results:

Mortality, clinical observations and body weights were recorded. Liver and adrenals were weighed. Uterine contents were examined and mating and reproductive indices were assessed. Mating was considered successful if implantation sites could be determined in the uterus. Male fertility was considered successful if live embryos were produced.

Fetuses were examined macroscopically for external and internal malformations and were weighed and sexed.

Mortality

No deaths occurred in the study.

Clinical Signs

Mild hyperemia of the furless skin was observed in females at ≥ 25 mg/kg/d.

Body Weight

Pre-mating BW gains (F) were slightly increased (38% from Day 0 to Day 14) at ≥ 25 mg/kg/day. BW gains in all groups were similar to controls during gestation. Decreased BW gain in HDM was noted in week 1 of treatment only.

Feed Consumption: no meaningful changes

Dosing Formulation Analysis: performed at the beginning and the end of dosing; assay results showed 96 to 103% (F) and 98 to 101% (M) of the nominal concentrations.

Necropsy: There was no gross pathological findings and no changes in organ weights at any dose therefore histopathological examination was not performed.

In male fertility study the reproductive performance was shown as the number of females with sperms in the vaginal smear (in parenthesis the number of rats that did not become pregnant) as summarized in the following Sponsor's table:

Parameter	Group	1	2	3	4
	(mg/kg/day)	0	5	25	125
Number of days paired	1	2	7	7	5 (1)
	2	12 (1)	11	9	9 (1)
	3	7	6	8 (1)	7 (1)
	4	4	1	1	3 (1)
	1 - 4	25 (1)	25	25 (1)	24 (4)
	5 - 14	1	0	6	6
Median until pregnancy		2	2	2	2
Rats without sperm		0	0	0	0
Males paired		25	25	25	25
Females paired		25	25	25	25
Females pregnant		24	25	24	21
Females with live embryos		24	25	24	19
Mating index		100	100	100	100
Male fertility index		96	100	96	76

Fertility Parameters: When females were treated (and paired with un-treated males), estrous, mating and fertility parameters were unaffected. Mating index was 100% and the fertility index (number of pregnant females) was similar to controls across all groups. Time to successful mating and number of females with live fetuses were unaffected. The numbers of corpora lutea, implantations and pre-implantation loss were similar to controls in all treated groups. No complete resorptions occurred. Early resorptions were slightly increased at ≥ 25 mg/kg/d but were not considered to be drug related.

When males were treated (and paired with untreated females), mating index was 100% in all dose groups, but at the HD only 19/25 males produced live fetuses showing a reduced fertility index (76% versus 96% in MD, and 100% in LD). Although reduced fertility index was observed, there were no microscopic effects on testes, epididymis, prostate and seminal vesicles or any weight changes of these organs to correlate with reduced fertility.

In summary, the NOAEL for female fertility was 125 mg/kg/d (30-times human therapeutic dose based on body surface area conversion). The NOAEL for male fertility was 25 mg/kg/d (6-times human dose of 40 mg).

9.2 Embryonic-Fetal Development

9.2.1 Embryo-fetal development in rabbits.

Preliminary embryotoxicity and teratogenicity study (GPP-007- NCD-TOX-1997-181) with oral administration of vilazodone at doses of 5, 10, and 50 mg/kg/d to pregnant New Zealand White rabbits (5/group) resulted in maternal toxicities that included clinical signs of reduced defecation, diarrhea and reduced water consumption at ≥ 10 mg/kg and decreases in BW ($\leq 7.5\%$) and food consumption during the treatment period (significant at 50 mg/kg). No developmental toxicities were observed.

Based on the results of this study, doses of vilazodone at 2, 10, and 50 mg/kg/d were selected for the main study which is reviewed in the following section.

Study title: Embryotoxicity and teratogenicity study with EMD 68 843 administered by oral gavage in albino rabbits

Study no: GPP-007-NCD-TOX-1998-184
 Study report location: Merck KGaA, Frankfurter Strasse 250, D-64293 Darmstadt, Germany

Conducting laboratory and location:

(b) (4)

Date of study initiation: April 14, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: EMD 68 843, lot# EE 77185, 99.4% purity

Key Study Findings

- Pregnant New Zealand White rabbits (22/dose) were dosed orally at 2, 10, and 50 mg/kg/d (actual doses were 1.6, 7.8, and 35.8 mg/kg, respectively) on GD 6-18.
- HD exceeded an MTD for maternal toxicity; 3 dams were found dead during dosing. Two additional HD does were euthanized (on GD21 and GD27) due to spontaneous abortions (1 MD dam was also euthanized [GD25] due to abortion). There was dose-related reduction in BW gain and food consumption, increase in post implantation loss at HD.
- Developmental toxicities: decrease of fetal weight at MD and HD (- 6% versus control), particularly of male fetuses (- 8.5% versus control), reduced fetal weight at birth, no malformations at any dose level.
- The NOAEL for maternal toxicity was 2 mg/kg/d based on deaths at two higher doses (although the death at the MD was due to abortion).
- Similarly, NOAEL for fetal developmental toxicity was also at 2 mg/kg/d based on reduced fetal BW and delayed ossification at higher doses.

- Vilazodone was not teratogenic at any dose up to 24x the MRHD of 40 mg (based on body surface area conversion) in rabbits.

Methods

Doses:	0, 2 (LD), 10 (MD), and 50 (HD) mg/kg/d
Frequency of dosing:	Once a day
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	1% aqueous carboxymethyl cellulose
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	22 females/group, but only 16-19/group became pregnant.
Satellite groups (TK)	3/group (Study: GPP-007-NCD-PKM-1999-006)
Study design:	Female rabbits were artificially inseminated with semen samples from donor bucks. Receptal (hormone) was injected twice; 3 weeks prior to insemination and immediately after insemination. Does were treated with vilazodone or vehicle from Day 6 to Day 18 of gestation. Necropsy was conducted on GD28.
Deviation from study protocol:	Actual doses administered were 1.6, 7.8 and 35.8 mg/kg/d

Observations and Results**Mortality:**

Three HDF were found dead during the dosing period (between GD 8 and GD 17). Two HDF (on GD 21 and GD 27) and one MDF (on GD 25) were euthanized because premature abortion was detected. Of the 3 HDF which died prematurely, 2 appeared to be pregnant and 1 was not pregnant.

SUMMARY OF PERFORMANCE OF INSEMINATED FEMALES				
Group	1	2	3	4
Dose (mg/kg bw/day)	0	2	10	50
Number of inseminated females	21*	22	22	22
Number of pregnant females	19	19	20	16
Pregnancy rate (%)	90	86	91	73
Number of females used for calculations	19	19	19	10
* = One female died immediately following insemination.				
Reason for exclusion	INCIDENCES#			
Death during insemination:	1(4)	-	-	-
Death prior to necropsy:	-	-	-	3(68,77,88)
Abortion:	-	-	1(57)	2(84,86)
Implantation sites only:	-	-	-	2(82,87)
Not pregnant at termination:	2(7,14)	3(26,33,37)	2(46,64)	5(67,69,70,81,83)

- in brackets – animal # that was excluded from calculations

It should be noted that of the 22 dams/ dose, 2 or 3 were not pregnant (at termination) in the control, LD, and MD groups; however, 5 were not pregnant in the HD group. The fewer pregnancies at HD would not be drug-related and were presumed to be a random effect due to failure of the hormonal treatment during insemination (see discussion under necropsy findings below).

Additional findings in the HD group included 2 with implantation sites only and 2 with abortions. The total number of pregnant HDF was 16 (including those with implantation sites only and those with abortions). However, it was noted that the 5 HDF which were not pregnant at termination on Day 28 showed ovaries with hemorrhagic follicles. According to the Sponsor; "This may indicate an overreaction to the second hormone injection, subsequently resulting in non-test substance related unfertilized females. Therefore the ultimate pregnancy rate in the high dose group may be between 73 and 95%. One non-pregnant control female (animal 14) also showed at necropsy comparable follicular changes in the ovaries. The pregnancy rate in the control group, therefore, was considered to be between 90-95%." [It was noted that in the preliminary study (the same study conditions) with the same HD, no such findings were observed.]

Clinical Signs

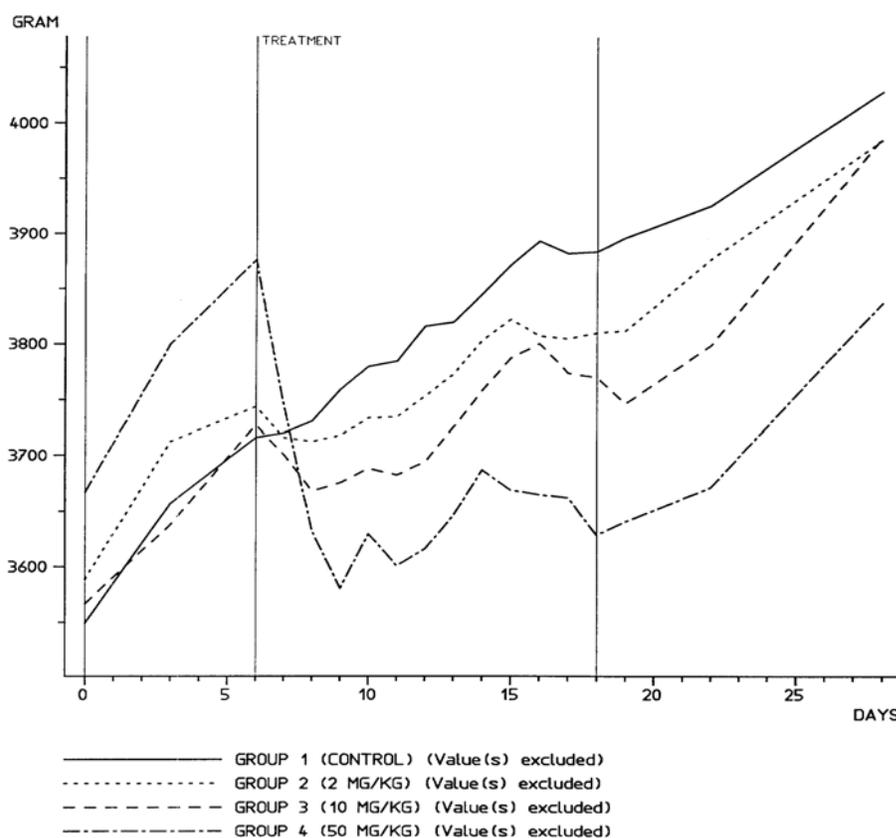
Clinical signs were recorded daily from GD0 until termination (GD28).

Results of clinical observations included: reduced feces production (in 2 at LD, 3 at MD, but all at HD) that could have reflected decreased food consumption; and red appearance of the urine in 4 HDF on a few occasions during the study period without further pathological correlates.

Body Weight

BW was determined at periodic intervals during pregnancy.

The results showed treatment related decrease in BW gain observed in all groups treated with vilazodone as shown in the following graph. After the first 3 days of dosing (GD9), dams (that survived and were pregnant at termination) at LD (n=19), MD (n=19), and HD (n=10) lost 0.7%, 1.4%, and 7.6% of their GD6 BW, respectively, while controls gained 1.2% (based on mean values in sponsor's summary table on report pages 31-32 of 178). During dosing, HD dams failed to gain significant weight, but after dosing was discontinued (after GD18) BW gain increased in all dose-groups. On GD28, LD and MD, dams had gained 6.5% and 7.0% of their GD6 BW, respectively, while controls had gained 8.4%; however, HD dams still weighed slightly (1%) less than their GD6 weights (based on mean values in sponsor's summary table on report pages 31-32 of 178).



Feed Consumption

Food consumption was determined at periodic intervals during pregnancy.

A dose-dependent decrease in food consumption was observed in all treatment groups and paralleled decreased BW gains.

Toxicokinetics:

Plasma samples (from 3/group) were collected on GD6 and GD18, 1, 3, 6, and 24 h after dosing.

Results showed that peak plasma concentrations were reached generally 1-3 h post dose. C_{max} increased with dose in a greater than dose-proportional manner from 2-10 mg/kg/d and less than dose-proportionally from 10-50 mg/kg/d on both sampling days (G6 and G18). A similar trend was noted for AUC which increased with repeated dosing, but this increase was more than dose proportional (GD18) suggesting that the clearance was reduced at HD; TK study results are shown in the following summary table:

Daily Dose (mg/kg)	2	10	50
AUC _{0-6h} (ng•h/ml) - GD 18	297	3160	16300
AUC ₀₋₂₄ (ng•h/ml) - GD 6	NC	3310	12800
AUC ₀₋₂₄ (ng•h/ml) - GD 18	NC	5680	59800
C_{max} (ng/ml) - GD 6	45.7	402	804
C_{max} (ng/ml) - GD 18	67.3	829	2990
T_{max} (ng/ml) - GD 6	1-3	3	1-6
T_{max} (ng/ml) - GD 18	1-3	1-3	3

Dosing Formulation Analysis:

Dosing formulation analysis revealed lower than nominal concentrations of vilazodone by approximately 80%, 78% and 71.5% of nominal (average of three formulation analysis; actual doses were 1.6, 7.8, and 35.8 mg/kg for nominal doses of 2, 10, and 50 mg/kg, respectively).

Necropsy:

On Day 28 of gestation, all does were sacrificed and subjected to post-mortem examination and external, thoracic and abdominal macroscopic findings were recorded.

Macroscopic examination at necropsy revealed follicular hemorrhages in HDF (these females were not pregnant). This finding was attributed to an overreaction to the second hormone injection rather than an effect of treatment with vilazodone.

Cesarean Section Data:

[Note that only 10 dams out of 22 in the HD group were pregnant at termination, due to premature deaths, abortions, complete post-implantation losses, and lack of pregnancy, as presented above.]

The post implantation loss was increased at HDF as shown in the table below. This increase was associated with a higher incidence of implantation scars due to early embryonic death.

Data compiled from sponsor's summary table (on report page 77 of 178)

	control	LD – 2 mg/kg	MD–10mg/kg	HD–50 mg/kg
No of dams	19	19	19	10
Corpora lutea	192	192	200	105
Pre-implantation loss	35	19	11	18
% of corpora lutea	18.2	9.9*	5.5**	17.1
Implantation sites	157	173	189	87
% of corpora lutea	81.8	90.1*	94.5**	82.9
Post-implantation loss	6	10	12	8
% of implantation sites	3.8	5.8	6.3	9.2
Embryonic/fetal death-total	5	8	12	7
% of implantation sites	3.2	4.6	6.3	8
Embryonic resorptions	2	3	7	3
% of implantation sites	1.3	1.7	3.7	3.4
Fetal resorptions	3	5	5	4
% of implantation sites	1.9	2.9	2.6	4.6

*/**: Fisher's exact test; alpha levels 5%/1%.

Offspring (fetuses):

There was no treatment related mortality detected among fetuses from dams treated with vilazodone. Sex ratios were comparable in treated and control groups.

Signs of developmental toxicity included an increase of the post-implantation loss, due to early embryonic/fetal resorptions in litters of HDF. Furthermore, based on total fetuses per group, mean fetal weights were decreased at MD and HD (↓~ 6% versus control, at both doses [Dunnet-test]). This effect was attributable largely to an 8.5% decrease in males at both doses, but only 3.5% and 4.4% decreases in females at MD and HD, respectively. Additionally, based on litters, the decreases in fetal weights (11.6% at both MD and HD) were not statistically significant...Reduced fetal weight was associated with a slight delay in fetal ossification (skeletal variation) at MD and HD, in the anterior fontanelle, sternebrae, caudal vertebral arches, pubic bones and bones of the fore and hind feet (apparently mostly in fetuses of lower BW). No treatment related fetal malformations were seen at external, visceral or skeletal examination.

In summary, the oral dosing of pregnant rabbits with vilazodone at 2, 10, and 50 mg/kg/d from GD6 through GD18 revealed maternal toxicity and developmental toxicity but not malformations. The NOAEL for maternal toxicity was at 10 mg/kg/d based on deaths at HD (the death of one MD doe was due to abortion). NOAEL for fetal developmental toxicity was at 2 mg/kg/d, based on reduced fetal BW and delayed ossification at higher doses (≥ 10 mg/kg).

9.2.2 Embryo-fetal development in rats.

In the dose-range finding study oral administration of vilazodone at doses of 50, 100, and 200 mg/kg/d to pregnant Wistar rats (from GD 6 to GD 15) caused a decrease in BW gain in all females during treatment. With the exception of the slightly lighter fetuses at HD, there was no impairment of reproductive parameters. No malformations were reported in any fetuses.

Based on the results of this study, doses of vilazodone at 8, 40, and 200 mg/kg were selected for the main study reviewed in the following section:

Study title: Developmental toxicity study with oral administration of EMD 68 843 to rats

Study no.:	GPP-007-NCD-TOX-1997-183
Study report location:	Institute of Toxicology, Merck KGaA, 64271 Darmstadt, Germany
Conducting laboratory and location:	Institute of Toxicology, Merck KGaA, 64271 Darmstadt, Germany
Date of study initiation:	February 6, 1997
GLP compliance:	Yes (signed and dated)
QA statement:	Yes (signed and dated)
Drug, lot #, and % purity:	EMD 68 843, lot# EE 77 185, 99.4% purity

Key Study Findings

- Pregnant Wistar rats (25/dose) were dosed orally at 8, 40, and 200 mg/kg/d during the period of organogenesis (GD6 to GD17).
- Maternal toxicity at HD only: slight, transient hyperemia of the furless skin was noted throughout the treatment period; BW gain was reduced (BW were ~3-4% less than controls throughout dosing); food and water consumption were also reduced.
- Fetal toxicity at HD only: fetal BW was decreased (~12%) compared to controls; ossification (a skeletal variation) was delayed and this finding correlated with fetal BW reduction observed in this group.
- NOEL for developmental toxicity was at the MD of 40 mg/kg/d. The LOEL for developmental toxicity was the HD of 200 mg/kg/day, based on lighter fetuses and weaker skeletal ossification. No teratogenic effect was found.

Methods

Doses:	0, 8, 40, 200 mg/kg/d
Frequency of dosing:	Once a day
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension in 0.25% aqueous hydroxypropylmethylcellulose
Species/Strain:	Rats/Wistar, HSD CPB:Wu
Number/Sex/Group:	25 females/group

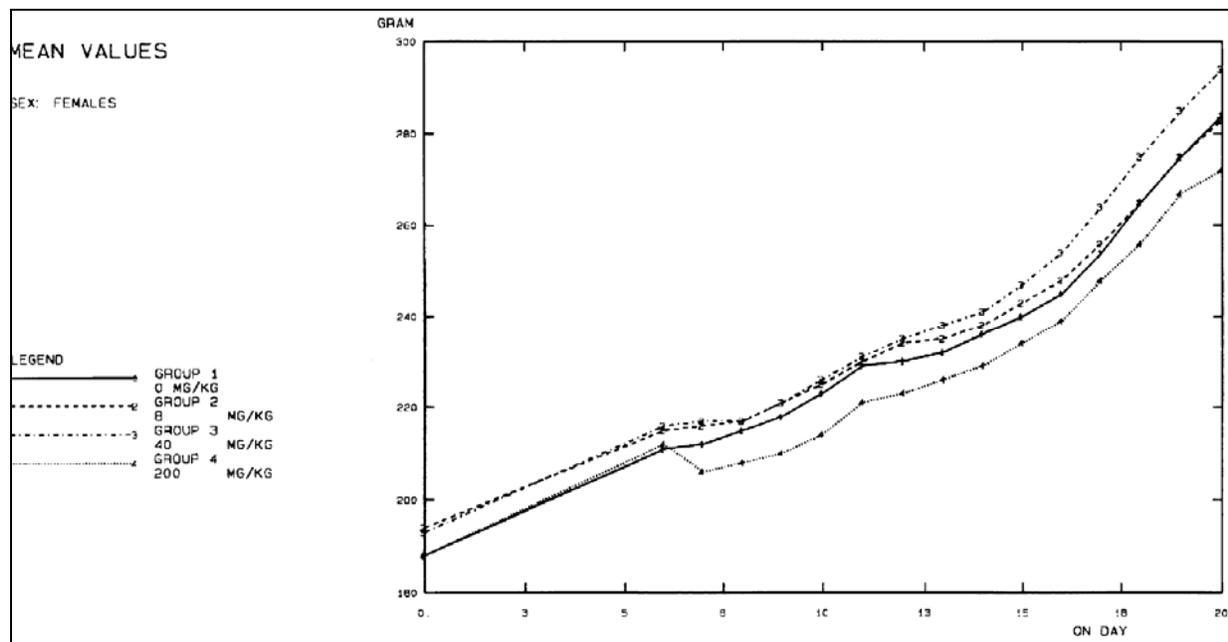
Satellite groups: N/A
 Study design: Vilazodone was administered to pregnant rats from GD6 to GD17. The fetuses were delivered by c-section on GD20 and examined

Observations and Results

Mortality: None of the animals died and none were euthanized.

Clinical Signs: Slight hyperemia was observed in MDF and HDF after drug administration.

Body Weight: HDFs lost weight at the beginning of dosing (they weighed ~3% less than their GD6 weight on GD7-8 and ~3-4% less than controls from GD7-GD20), but subsequently gained weight at a rate similar to the other groups, as shown on the following Sponsor's figure below.



Feed Consumption: Food consumption was not different than control in any vilazodone treated group.

Toxicokinetics: Not performed in this study. [However, TK was conducted on GD6 and GD15 in the dose-range finding study at 0, 50, 100, 200 mg/kg/d,]

Dosing Formulation Analysis: The assays revealed dosing formulations being within 96 to 104% of the nominal concentrations.

Necropsy: The number of corpora lutea determined in both ovaries revealed similar frequencies in rats treated with vilazodone and those of control.

Cesarean Section Data: There was no effect of treatment on implantations or resorptions. No differences in the frequency of live fetuses or fetal sex ratio were noted. Fetal weights were reduced ~12% for both males and females at HD (based on mean fetus weight per dam), similarly to the effect observed in dose range finding study.

Offspring:

No external, skeletal or visceral changes were observed in fetuses of any study group. However, sternum, coccygeal vertebrae and distal phalanges (a skeletal variation) were less ossified in the fetuses of HDF as summarized in the following table:

Table compiled from sponsor's summary table (table 45, on report pages 69-72/72).

Findings	C-M	LDM	MDM	HDM	C-F	LDF	MDF	HDF
# of litters examined	15	14	12	12	14	15	13	13
# of fetuses examined	59	58	69	66	83	77	67	58
Ossified sternum 1	3	0	0	0	0	0	0	0
2	0	0	3	0	0	0	0	0
3	3	1	0	10	0	0	2	5
4	0	11	7	15	10	14	6	29
5	5	12	25	35	8	15	36	42
6	89	76	66	40	82	72	55	24
Coccygeal vertebrae 0	7	4	8	6	1	3	3	6
1	0	16	10	21	5	12	16	22
2	14	2	16	15	15	8	22	24
3	31	36	36	43	32	28	30	31
4	38	28	28	14	42	40	30	17
5	5	11	1	0	5	9	0	0
6	2	0	0	0	0	0	0	0
7	2	4	0	0	0	0	0	0
Distal phalanges (r/l) 0	28/39	39/39	37/50	53/59	29/25	45/45	36/56	66/70
1	19/18	8/17	16/15	10/8	20/20	3/12	11/7	7/7
2	8/8	8/4	9/5	8/3	8/17	5/2	4/6	9/7
3	9/8	6/13	12/18	10/10	10/14	15/12	22/13	4/5
4	14/6	14/16	11/3	3/11	18/10	14/14	15/12	7/8
5	22/21	24/12	15/9	16/8	15/13	17/15	13/7	6/3

Weaker skeletal ossification correlated with reduced fetal BW observed at HD. NOEL for developmental toxicity was at MD (40 mg/kg/d). No teratogenic effect was observed in this rat study at doses up to 200 mg/kg/d.

9.3 Prenatal and Postnatal Development

Pilot pre- and postnatal toxicity study (GPP-007-NCD-TOX-2001-185) with vilazodone at 160 mg/kg/d administered by oral gavage to Wistar female rats from the gestation day (GD) 6 until lactation day (LD) 7 was conducted to determine whether this dose is suitable as the HD for the main study.

Decreased BW gain and lower food consumption was observed at this dose. A postnatal survival was lower and BW of pups was significantly reduced. Based on reduced pup weights and survival index at 160 mg/kg/d, the HD for the main study was selected at 125 mg/kg/d (main study reviewed in the following section).

Study title: EMD 68 843 Pre-and postnatal development study including maternal function by oral route in rats

Study no.: GPP-007-NCD-TOX-2002-186
 Study report location: Merck KGaA, Pharma Ethical Preclinical R&D; Frankfurter Strasse 250; 64293 Darmstadt, Germany

Conducting laboratory and location:



Date of study initiation: January 31, 2001
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: EMD 68843, lot#: EE 77485, 99.4% purity

Key Study Findings

- Pregnant Wistar rats (25/dose) were dosed orally at doses of 0, 5, 25, and 125 mg/kg/day during the period of organogenesis, and through parturition and lactation (GD6-LD21).
- Maternal generation (F0) toxicity:
 - reduction in maternal BW gain (GD6-GD8) and food consumption (throughout dosing) during gestation (at MD and especially HD) and decreased food consumption during lactation (especially HD);
 - increased implantation loss and increased number of still-born pups (HD);
 - reduced number of live-born pups and fewer females with live pups at weaning (HD);
 - decreased probability of survival due to decreased birth index, and decreased viability at LD4 and at weaning (LD21) at MD and HD; increased number of cannibalized pups (dose-dependent, especially at HD).
- F1 generation as pups:
 - decreased BW from birth to weaning (MD and HD);

- delayed development, based on a longer time of surface righting reflex and pinna detachment; delayed vaginal opening, but not preputial separation (all dose groups); longer time in fur appearance at HD only.
- F1 generation as adults:
 - No effect on tests of locomotor activity or learning and memory.
 - Reproductive effects: Decreased fertility index (HD); decreased BW gain from weaning to sexual maturity (MD and HD). Caesarean-section data showed lower number of corpora lutea, implantations, and live fetuses along with reduced weight of pregnant uteri at HD as compared to controls.

Methods

Doses:	0, 5, 25 and 125 mg/kg/d
Frequency of dosing:	Once a day from GD 6 to LD 21
Dose volume:	10 ml/kg/d
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension/aqueous 0.25% hydroxypropyl-methylcellulose
Species/Strain:	Rats/Crl:CD (SD) BR
Number/Sex/Group:	25/females/group
Satellite groups:	none
Study design:	Animals were observed for survival, clinical signs, behavior and parturition. BW was recorded during pregnancy and lactation. Females were allowed to give birth and rear their young (F1 generation) up to LD 21 of. Pups were observed for survival and abnormalities, and weighed during lactation. On day 21 post-partum 1M and 1F per litter (F1) were selected to be reared until sexual maturity while F0 dams with their remaining pups were necropsied. After reaching sexual maturity selected F1 animals were mated. Pregnant F1 females were sacrificed on day 20 of pregnancy when the uterus content was examined.
Deviation from study protocol:	Not reported

Observations and Results

Clinical signs were recorded daily (physical appearance, behavior, possible reaction to treatment) and BW was recorded during pregnancy on GD 0, 6, 8, 10, 12, 14, 16, 18, and 20 and during lactation on LD 0 (parturition day), 7, 14, and 21.

Food consumption was recorded on GD 6, 8, 10, 12, 14, 16, 18, and 20 and on LD 7, and 14.

F1 generation observations included: external abnormalities at birth, live and stillbirths, mortality, sex (Day 0), pup weight (Days 0, 4, 8, 12, 21), appearance of fur (daily from Day 4), eruption of incisors (daily from Day 7), eye opening (daily from Day 12), pinna detachment (daily from Day 4), surface righting reflex (daily from Day 4), pinna reflex

(daily from Day 11), corneal reflex (daily from Day 12), papillary reflex (daily from Day 12), traction reflex (daily from Day 4), grip reflex (daily from Day 4), chimney test (Day 14).

Effects on F0 dams:

F₀ Dams

Survival:

All survived until the end of the study. The length of gestation was similar in all experimental groups and parturition was not affected.

Clinical signs:

No findings

Body weight (% difference versus control)

Gestation BW: decreased slightly between GD6-GD8 (the first days of dosing) at HD (↓2.3%), with no gain/loss at MD, slight gain at LD (↑1.6%), and larger gain in controls (↑2.6%); however, BWG was similar across groups from GD8-GD20, see fig. 1.

Lactation BW: BWGs during lactation (for dams with live pups at weaning) were similar across all dose groups, see fig. 2.

Feed consumption (% difference versus control)

Gestation: food consumption was decreased (vs controls) at MD (↓~15%) and HD (↓~30% at GD8, then ~12% for remaining days) throughout gestation/dosing.

Lactation: food consumption during lactation (for dams with live pups at weaning, measured at LD7 and LD14) was decreased (vs controls) at MD (↓~17% at LD7, ~12% at LD14) and HD (↓~27% at LD7, ~19% at LD14).

Uterine content:

Decreased number of live-born pups (82% vs. 91%); increased post-implantation loss (18% vs. 9%) and stillbirths (43% vs. 10%) at HD versus controls; sex ratio was unaffected as shown in Sponsor's tables (below)

Necropsy observation:

No abnormalities observed.

Toxicokinetics:

Not performed

Stability and homogeneity:

All preparations were within predefined acceptance limits for this suspension (90 – 110% of the nominal concentration)

Other:

Dose-dependent increase in number of cannibalized pups and decreased number females with live pups at weaning at HD as shown in the following Sponsor's tables.

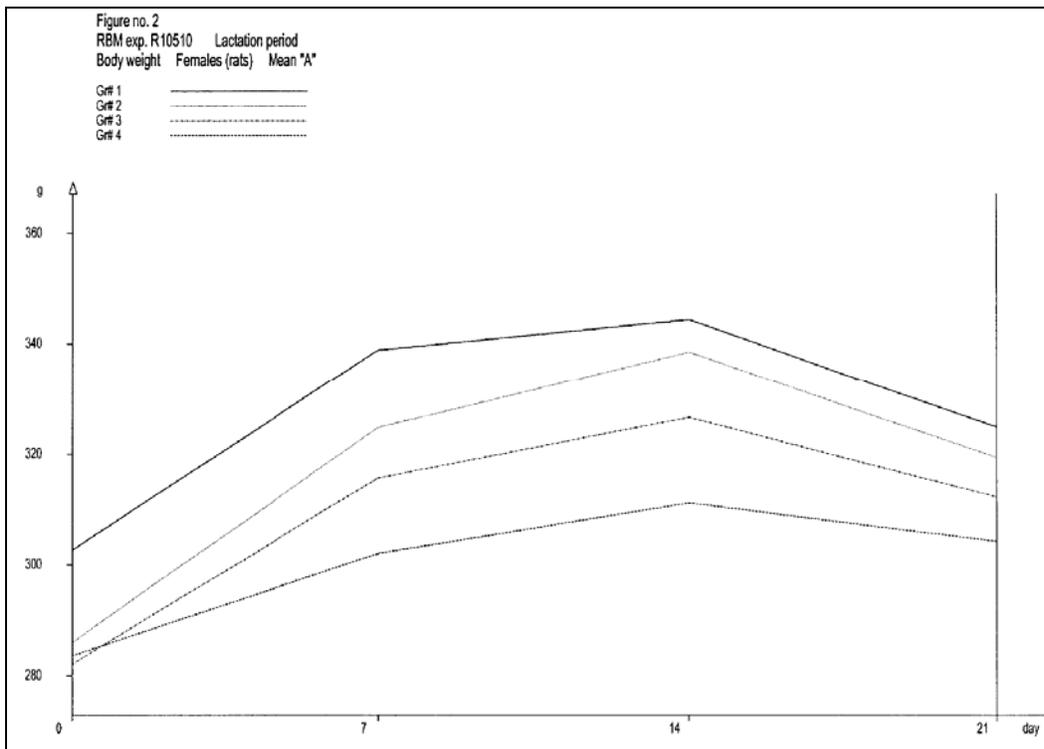
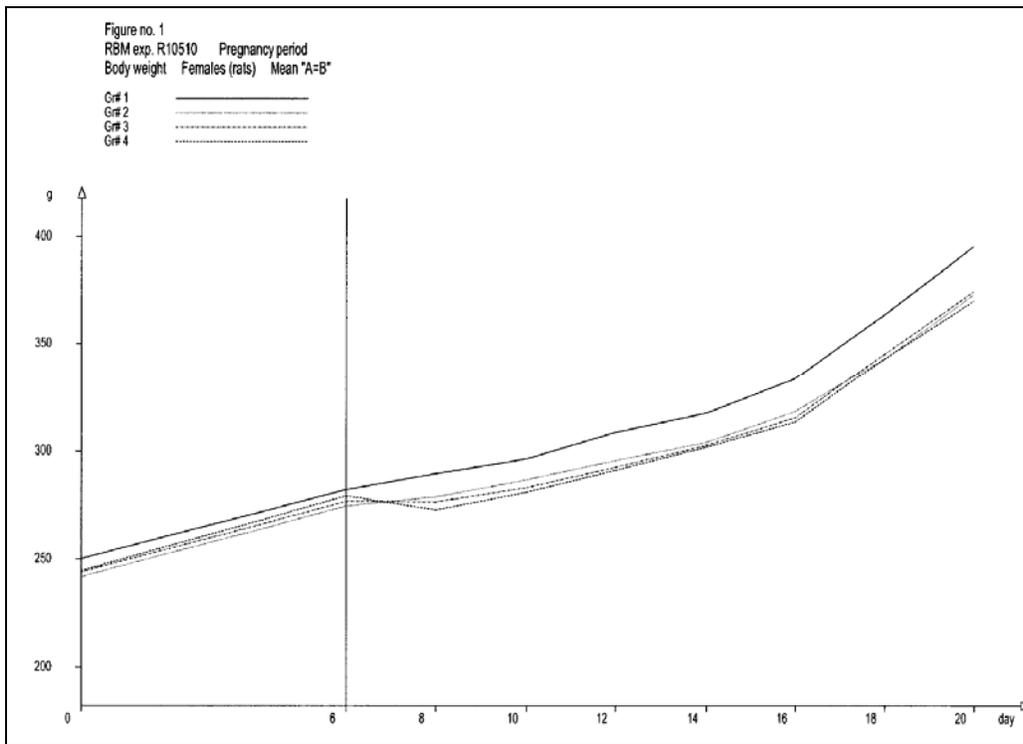
When all dams were considered for calculating parameters:

Dose (mg/kg/day)	0	5	25	125	(P)
Fertility	21	23	21	23	
Parturitions	21	23	21	23	
With live pups at birth	21	23	21	23	
Pregnancy index	21/21 100.00%	23/23 100.00%	21/21 100.00%	23/23 100.00%	>0.05
With stillborn pups	2/21 9.52%	1/23 4.35%	2/21 9.52%	* 10/23 43.48%	.0001
With all stillborn pups	0/21 0.00%	0/23 0.00%	0/21 0.00%	0/23 0.00%	>0.05
With live pups at weaning	21/21 100.00%	23/23 100.00%	21/21 100.00%	* 17/23 73.91%	.0001

When females with live pups at weaning were used for calculations:

Dose (mg/kg/day)	0	5	25	125	(P)
No. of females	21	23	21	23	
Implantations	340	344	337	355	
Stillborn	2/340 0.59%	1/344 0.29%	2/337 0.59%	*** 17/355 4.79%	.0001
Live born	309/340 90.88%	313/344 90.99%	303/337 89.91%	*** 291/355 81.97%	.0001
Total born	311/340 91.47%	314/344 91.28%	305/337 90.50%	* 308/355 86.76%	.0087
Live born (males)	163/309 52.75%	150/313 47.92%	148/303 48.84%	158/291 54.30%	>0.05
Live born (females)	146/309 47.25%	163/313 52.08%	155/303 51.16%	133/291 45.70%	>0.05
Live born (undetectable)	0	0	0	0	
Cannibalized	1	9	29	100	
Chosen at weaning	42	46	42	34	

The following figures illustrate lower body weight gain in pregnant rats treated with vilazodone (Fig. 1) and distribution of BW gain of the same female rats during lactation period (Fig. 2) in comparison with controls (solid line).



Effects on F1 pups:

F₁ Generation

Dose (mg/kg/day)	0	5	25	125	
Live pups at day 21(no.)	14.67 1.906 (21)	13.00 3.344 (23)	12.67 2.477 (21)	7.35 4.987 (23)	P ***
Birth index(%)	91.10 9.202 (21)	90.40 10.208 (23)	89.08 8.711 (20)	82.22 13.727 (23)	P *
Viability index on day 4(%)	100.00 0.000 (21)	95.70 12.624 (23)	92.07 13.211 (21)	63.23 41.296 (23)	N ***
Weaning index(%)	99.66 1.559 (21)	99.41 1.974 (23)	95.96 5.601 (21)	95.50 7.209 (17)	N **

Survival: Pup survival to LD4 was significantly lower, especially at HD, as shown in the Sponsor's table (above). At HD 6/23 females that delivered lost their whole litter before weaning. Survival to weaning (LD21) was also slightly decreased at MD and HD.

Clinical signs: No clinical signs observed

Body weight: Decreased (9 – 17% at birth and 11 – 15% at weaning) at MD and HD

Pup weight (g)	Control		5 mg/kg/day		25 mg/kg/day		125 mg/kg/day	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Male	6.8	47.3	6.4	46.5	6.2*	41.0*	5.7*	42.0*
Female	6.4	45.2	6.1	45.4	5.8*	39.7*	5.3*	38.4*
Male + female	6.6	46.2	6.3	45.9	6.0*	40.2*	5.5*	40.4*

* statistically significant difference

Feed consumption: Decreased at MD and HD during gestation (GD8–GD20) by 16 – 30% and during lactation period by 17 – 37% (LD7) and 12 – 33% (LD14)

Physical development: Longer time to show surface righting reflex and pinna detachment at ≥LD; increased time to show traction (7-19%) and to vaginal opening (6.1%); decreased % success in chimney test (76-58% vs.87%) at ≥25 mg/kg/d; increased time to fur appearance at HD.

Neurological assessment: No statistically significant effects in Water maze and Open-field tests were observed among experimental groups

Reproduction No difference in the mating index and the mean pre-coital time.

F₂ Generation Affected parameters at HD included: decreased fertility index (no. of animals mating/no. of pregnant females) by 20% (76% vs. 95% in controls); decreased no. of corpora lutea (22%), implantations (17%), live fetuses (males 29%; females 8%); lower uterus weight (19%), testes (2.4%) and ovary (14%). No effects on resorptions and no externally malformed fetuses were found.

Other: Reduced BW from weaning (Day 21) to sexual maturity (Day 84) by 5 - 8% at MD and HD and during pregnancy (8.5%) at HD.

In summary: Vilazodone was administered to pregnant female rats from implantation (GD 6) through pregnancy, parturition and lactation to weaning of progeny (postnatal Day 21) at oral doses of 5, 25, and 125 mg/kg/day. Toxicity in the maternal generation (F0) consisted of reduction in BW gain and food consumption (MD and HD); reduced number of live born pups and females with live pups at weaning (HD); increased implantation loss and number of stillborn (HD); increased number of cannibalized pups (dose-dependent, at MD and especially HD); decreased probability of survival, based on decreased birth index, decreased viability index on Day 4 and decreased weaning index (at LD21) at MD and HD.

The NOAEL for maternal toxicity was the LD of 5 mg/kg/d based on reduced BW and reduced food consumption at higher doses.

F₁ generation pups showed decreased BW from birth to weaning (MD and HD); and developmental delays, namely a longer time to show surface righting reflex and pinna detachment (all doses); delayed vaginal opening (but not preputial separation) and delayed traction reflex (MD and HD); longer time to fur appearance and deficit in chimney test (HD only).

Regarding reproductive performance, F1 rats showed decreased fertility index (HD), but no effect on mating. BW gain was decreased from weaning to sexual maturity (MD and HD); at sexual maturity (PND84), mean body weights at HD were ~5% lower than controls. Caesarean section data showed lower number of corpora lutea, implantations, and live fetuses (F₂ generation), along with reduced weight of pregnant uteri at HD compared to controls. The NOAEL for offspring toxicities was the LD of 5 mg/kg/d based mainly on reduced BW from birth to weaning and from weaning to sexual maturity.

10 Special Toxicology Studies

Impurities

The limits for vilazodone HCl impurities are at (b) (4), except for those impurities with structural alerts for genotoxicity which were not evaluated in non-clinical studies. The limits for these impurities were at the level of (b) (4) ppm (except (b) (4)) to allow a human exposure of (b) (4) µg/day. An exposure of (b) (4) /day equates to (b) (4) ppm for vilazodone HCl at human dose of 40 mg/day. Impurities with structural alerts for genotoxicity are summarized in the following Sponsors' table

(b) (4)

(b) (4) impurity ((b) (4)) and (b) (4) impurity ((b) (4)) were evaluated in acute general toxicology and genotoxicity assays *in vitro* and *in vivo* which are reviewed in the following section.

Toxicity of (b) (4) Impurity:

(b) (4) - **Acute Toxicity Study in Rats after Oral Administration;** Study GPP-007-NCD-TOX-2001-221:

(b) (4) impurity (b) (4) at 5000 mg/kg was administered rats (3/sex/group) in aqueous Methocel® K4M premium solution. Rats were sacrificed after a 2 week post dose observation period and gross examination conducted. The results showed the only findings of locomotor disturbance and dyspnea observed from 1h to 6h post dose. Therefore (b) (4) was considered to be without acute toxicity at 5000 mg/kg in rats.

(b) (4) - **Bacterial Mutagenicity Assay, *Salmonella typhimurium* and *Escherichia*** (Ames test; Study: GPP-007-NCD-TOX-2000-171).

(b) (4) (batch 99/MT002-16) was tested for its ability to induce reverse mutations in *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537, and *Escherichia coli* strain WP2uvrA pkM101 in a GLP study using the plate incorporation test with and without addition of liver S9 mix in two series of experiments. In this assay, (b) (4) was dissolved in dimethyl sulfoxide (DMSO) and tested at concentrations ranging from 5.00 to 5000 µg/plate. Precipitation of the test material on the agar plates occurred at concentrations > 500 µg/plate. Toxicity to the bacteria was not observed.

The validity of the study was confirmed by the expected mutant frequencies assessed for the positive controls (daunomycin, N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine and cumene hydroperoxide served as strain specific in the absence of S9 and 2-aminoanthracene and benzo[a]pyrene were used for testing the bacteria in the presence of S9 mix) versus negative control (solvent).

Results: In the first series of experiments, (b) (4) showed clear increases in the number of revertants in TA 1535 strain with and without S9 activation and weaker increases in TA 100 strain as shown in the following Sponsor's summary tables:

Test Material	Concentration [µg/plate]	+/- S9- Mix	Mean revertant colonies / plate		
			TA 98	TA 100	TA 102
Solvent control		-	12	97	276
(b) (4)	5	-	14	88	293
	15.8	-	11	80	285
	50	-	12	97	269
	158	-	12	101	225
	500	-	7	113	222
	1580	-	11	125	273
	5000	-	10	116	189
Solvent control		+	22	116	345
(b) (4)	5	+	24	112	354
	15.8	+	23	109	330
	50	+	19	116	367
	158	+	26	148	324
	500	+	25	210	348
	1580	+	28	206	313
	5000	+	19	223	300
Positive controls	Name	-	DAUN	ENNG	CUM
	Conc. [µg/plate]		2	5	200
	Revert./plate		215	629	427
	Name	+	2-AA	2-AA	B(a)p
	Conc. [µg/plate]		1	1	10
	Revert./plate		248	404	960
Test Material	Concentration [µg/plate]	+/- S9- Mix	Mean revertant colonies / plate		
			TA 1535	TA 1537	WP2
Solvent control		-	9	4	93
(b) (4)	5	-	12	4	101
	15.8	-	16	3	108
	50	-	20	4	99
	158	-	29	5	95
	500	-	60	2	107
	1580	-	64	6	103
	5000	-	33	2	99
Solvent control		+	13	5	95
(b) (4)	5	+	11	7	128
	15.8	+	11	6	116
	50	+	14	6	123
	158	+	45	9	117
	500	+	124	8	145
	1580	+	149	7	135
	5000	+	178	7	116
Positive controls	Name	-	ENNG	9-AA	ENNG
	Conc. [µg/plate]		10	50	5
	Revert./plate		388	311	979
	Name	+	2-AA	2-AA	2-AA
	Conc. [µg/plate]		1	2	10
	Revert./plate		85	51	1065

In the second series of experiments only TA 1535 was tested and the following data were obtained:

Test Material	Concentration [µg/plate]	+/- S9- Mix	Mean revertant colonies / plate		
			TA 1535		
Solvent control (b) (4)		-	9		
	5	-	8		
	15.8	-	14		
	50	-	16		
	158	-	25		
	500	-	61		
	1580	-	53		
	5000	-	46		
Solvent control (b) (4)		+	11		
	5	+	11		
	15.8	+	15		
	50	+	14		
	158	+	37		
	500	+	116		
	1580	+	151		
	5000	+	144		
Positive controls	Name Conc. [µg/plate] Revert./plate	-	ENNG 10 310		
	Name Conc. [µg/plate] Revert./plate	+	2-AA 1 63		

In conclusion, (b) (4) was mutagenic in the Salmonella typhimurium tester strain TA 1535 with and without addition of S9 mix.

(b) (4) - **In Vitro Chromosome Aberration Assay in V79 Chinese Hamster Cells** - Study GPP-007-NCD-TOX-2002-165

(b) (4); batch 98/TY/027) was evaluated in two experimental series for the induction of chromosomal aberrations in V79 Chinese hamster cells in a GLP *in vitro* assay in the presence and absence of S9 metabolic activation system. The impurity was evaluated at concentrations 28.1, 50, 88.9, and 158 µg/ml (first series) and at 50, 88.9, 118, and 158 µg/ml (second series).

In the range-finding assay, precipitation occurred at concentrations ≥ 88.9 µg/ml and cytotoxicity was observed at ≥ 88.9 µg/ml (-S9) and ≥ 2810 µg/mL (+ S9).

Results: The study was considered valid based on a clear increase of aberrant metaphases caused by the positive controls in both series of experiments. This study results are summarized in the following Sponsor's table:

Treatment Group	Concentr. [µg/ml]	Prep. time[h]	Rel.mitotic index [%] ^a	Polyploid. metaph.[%]	Aberrant metaph. [%]		
					incl.gaps	excl.gaps	exchanges
Without S9 mix:							
Exp. 1 (EZ 1343)							
Solvent control		25	100	6.90	1.2	1.2 ^d	0.30
(b) (4)	28.1	25	193	5.75	1.0	1.0 ^{n.s.}	0.5
	50.0	25	150	5.95	5.0	5.0 ^{**}	3.5
	88.9 ^{PE}	25	141	7.30	5.0	4.0 [*]	2.5
	158 ^{PE}	25	30	6.40 ^b	14.0	12.0 ^{**c}	4.0
Pos. Control	500	25	204	6.80	12.5	12.0 ^{**}	9.0
Exp. 2 (EZ 1344)							
Solvent control		25	100	4.08	1.0	0.75	0
(b) (4)	50.0	25	77	4.90	5.5	5.0 ^{**}	3.0
	88.9 ^{PE}	25	66	4.80	2.5	2.5 ^{n.s.}	1.0
	118 ^{PE}	25	82	6.70	3.0	2.5 ^{n.s.}	1.0
	158 ^{PE}	25	86	5.60	4.0	4.0 ^{**}	1.5
Pos. Control	500	25	92	4.50	7.5	5.5 ^{**}	3.5
With S9 mix:							
Exp. 1 (EZ 1356)							
Solvent control		25	100	5.75	1.5	0.75	0.25
(b) (4)	28.1	25	126	6.60	2.0	1.0 ^{n.s.}	0
	50.0	25	120	7.15	4.5	3.5 [*]	1.5
	88.9 ^{PE}	25	136	6.50	9.0	8.5 ^{**}	6.0
	158 ^{PE}	25	138	4.65	12.0	11.5 ^{**}	9.0
Pos. Control	4.00	25	126	7.10	12.0	11.0 ^{**}	8.0
Exp. 2 (EZ 1357)							
Solvent control		25	100	4.63	2.0	1.75	0.25
(b) (4)	50.0	25	106	4.15	6.5	5.0 [*]	4.5
	88.9 ^{PE}	25	78	4.45	7.5	7.0 ^{**}	7.0
	118 ^{PE}	25	93	4.30	8.5	8.0 ^{**}	3.5
	158 ^{PE}	25	111	3.95	1.0	0.5 ^{n.s.}	0.5
Pos. Control	4.00	25	108	4.35	21.0	20.0 ^{**}	18.5

PE = Precipitate of test material until end of the exposure time; a = % of solvent control; b = 906 evaluated metaphases; c = 90 evaluated metaphases; d = 329 evaluated metaphases; ** p ≤ 0.01; * 0.01 < p ≤ 0.05; ns = not significant (EZ = internal study number)

(b) (4) at cytotoxic and non-toxic concentrations ≥ 50 µg/ml (without and with S9 mix) increased the chromosome aberration rate up to maximum 12% versus 1 to 2%

observed in negative controls. No treatment related increase of polyploid cells was observed. In conclusion, (b) (4) impurity was considered clastogenic in this *in vitro* system. The drug substance, vilazodone was also clastogenic while tested in the same V79 cell system therefore it seems likely that clastogenic effect observed by treatment on cells with vilazodone is caused by its (b) (4) impurity.

(b) (4) - **Measurement of Unscheduled DNA Synthesis in Rat Liver using an In Vivo/In Vitro Procedure** (Study GPP-007-NCD-TOX-2002-166):

Using an *in vitro/in vivo* procedure, (b) (4); batch 98/TY/027) was tested for its ability to induce unscheduled DNA synthesis (UDS) in livers of rats orally administered (b) (4) in this GLP study.

Han Wistar rats (4/sex/group) were administered 0, 800 or 2000 mg/kg (b) (4) or the positive control (10 mg/kg dimethylnitrosamine or 75 mg/kg 2-acetamidofluorene). Following a 2-4 h or 12-14 h post dose period, rats were euthanized, primary hepatocyte cultures prepared and induction of UDS evaluated.

Results: Clinical signs in the 2000 mg/kg group included vocalization in one female in the 2-4 h experiment and lethargy in one male at 12-14 h experiment. Plasma concentrations in satellite TK rats averaged 2.5 µg/ml over a 48 h post dose period. Concentrations in the liver were 3.2 to 11.2 times greater than plasma concentrations but in the bone marrow, concentrations were similar to plasma concentrations.

Negative (vehicle) control animals gave group mean NNG (net grain count) values of 0% to 0.7% [males] or 0.5% to 3.0% [females] cells in repair. Group mean NNG values were increased by the positive controls (DMN and 2-AAF) treatment to more than 14.8 [males] and 13.2 [females] and more than 50% cells found to be in repair [male and females]. In this study the vehicle control NNG value was consistent with both published and historical control data, and the system was shown to be sensitive to two known DNA damaging agents requiring metabolism for their action. Therefore the assay was considered valid.

Treatment with 800 or 2000 mg/kg (b) (4) did not produce a group mean NNG value greater than 0.9 [males and females] nor were any more than 3.0% [males] or 5.7% [females] cells found in repair at either dose.

In conclusion, rats administered (b) (4) impurity up to 2000 mg/kg showed no induction of UDS in hepatocytes, therefore, (b) (4) was considered to have no genotoxic activity in this test system.

(b) (4) - **Micronucleus Test in Rats after Oral Administration** (Study GPP-007-NCD-TOX-2001-167):

In this GLP study, Wistar rats (5/sex/group/timepoint, 6-8 weeks old and weighing 155-241 g) were administered (b) (4); batch 98/TY/027) once by oral gavage at

0, 200, 356, 633 or 2000 mg/kg at 10 mL/kg in a Methocel® K4M (HPMC) vehicle). Bone marrow was harvested 24 and 48 h post dose in the 2000 mg/kg dose group and at 24 h post dose in the lower doses and 16.5 mg/kg cyclophosphamide positive control groups. Bone marrow was examined for micronucleated polychromatic erythrocytes (PCEs) and for any change in the number of PCEs relative to normochromatic erythrocytes (NCEs).

Results: Clinical signs of diarrhea were observed at ≥ 200 mg/kg as well as piloerection and locomotive disturbance in one rat at 356 mg/kg. No biologically relevant increases in the number of PCEs or variation in PCE:NCE ratio were observed. The positive and negative controls demonstrated the validity of this assay. It was concluded that (b) (4) impurity was negative for clastogenicity in this mammalian micronucleus test system *in vivo*.

Toxicity of (b) (4) Impurity:

(b) (4) - **Acute Toxicity Study in Rats after Oral Administration**; Study: GPP-007-NCD-TOX-2001-170

A GLP single-dose toxicity study was conducted to evaluate the acute oral toxicity of (b) (4) batch 96/TR/010/01) impurity of vilazodone. HsdCpb rats (3/sex/group) were orally administered doses of 100, 500 or 5000 mg/kg in aqueous Methocel® K4M premium (HPMC) solution.

Mortality occurred within 60 min at 5000 mg/kg (6/6 rats) and within 23 h at 500 mg/kg (5/6 rats). Clinical signs at 500 mg/kg and 5000 mg/kg included piloerection, incomplete eyelid closure, blood-crusted snout, salivation, sunken flanks, retention of feces, abdominal position, fibrillar tremor, locomotor disturbance, dyspnea, and cleaning. Signs were observed within 1-15 min after administration and in some cases, persisted up to 5 days. Decreased body weight was observed in one surviving rat at 500 mg/kg.

Surviving rats (all at 100 mg/kg and one at 500 mg/kg) were sacrificed after a 2 week post dose observation period and gross examinations detected no abnormalities.

Conclusion: (b) (4) was lethal at doses > 500 mg/kg following a single oral dose in the rat.

(b) (4) - **Bacterial mutagenicity assay, *Salmonella typhimurium* and *Escherichia coli***; Study: GPP-007-NCD-Tox-1997-161

(b) (4) impurity was assessed for mutagenic potential using *Salmonella typhimurium* tester strains TA 98, TA 100, TA 102, TA 1535, and TA 1537, and *Escherichia coli* WP2 *uvrA* pkM101. The plate incorporation test with and without addition of liver S9-Mix from Aroclor 1254-pretreated rats was used. Two independent experimental series were performed. (b) (4) was dissolved in dimethylsulfoxide (DMSO) and tested at concentrations ranging from 5.00 to 5000 $\mu\text{g}/\text{plate}$.

Daunomycin, N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine and cumene hydroperoxide served as strain specific positive control compounds in the absence of S9 Mix. 2-Aminoanthracene and benzo[a]pyrene were used for testing the bacteria and the activity of the S9 Mix.

Each treatment with the substances used as positive controls led to a clear increase in revertant colonies, thus showing the expected reversion properties of all strains and good metabolic activity of the S9 Mix used. Therefore, the study was considered to be valid.

In summary: (b) (4) impurity ((b) (4)) showed no increase in the number of revertants of any bacteria strain, with and without S9 Mix, therefore it was concluded that (b) (4) impurity was not mutagenic under the experimental conditions described.

(b) (4) - **In Vitro Chromosome Aberration Assay in V79 Chinese Hamster Cells**; Study: GPP-007-NCD-TOX-2003-169

(b) (4) batch 96/TR/010/01) was evaluated for the induction of chromosomal aberrations in V79 Chinese hamster cells in a GLP *in vitro* assay in the presence and absence of an Aroclor-induced rat liver S9 metabolic activation system.

Method: The impurity was evaluated at concentrations ranging from 15.8 to 889 µg/ml (-S9) and 88.9 to 889 µg/ml (+S9). In the range-finding assay the concentrations from 2.81 to 2810 µg/ml were used.

In the definitive studies, the following (b) (4) impurity concentrations were evaluated: 15.8, 50, 88.9, 158, 281, and 889 µg/ml (-S9 mix) and 88.9, 281, and 889 µg/ml (+S9 mix); positive controls: 250/500 µg/ml ethymethansulfonat (- S9) and 4 µg/ml cyclophosphamide (+ S9). DMSO was used as solvent/negative control. Exposure time of 5, 25, and 35 h (- S9) and 5 h (+ S9) was employed and 100 metaphases/slide were evaluated (three experiments were conducted).

Results: The positive controls induced the expected clear increase in number of cells with chromosomal aberrations. Precipitation occurred at concentrations ≥ 889 µg/ml. Cytotoxicity was observed at ≥ 88.9 µg/ml (-S9) at the longer exposures of 25 and 35 h.

(b) (4) impurity caused increase in aberration frequency up to 18% (dose-dependent in Experiment 2) and up to 16.5% (Experiment 3) at longer exposure time (25 – 35 h) without S-9 mix. This effect of increased aberrant metaphases was mainly observed at cytotoxic concentration (158 µg/ml) and was associated with increased poliploid cells. No such effects were seen at non-toxic concentrations or in the presence of S9 mix. This study results are summarized in the following Sponsor's summary table:

Treatment Group	Concentr. [µg/ml]	Prep. time[h]	Rel.mitotic index [%] ^a	Polyploid. metaph.[%]	Aberrant metaph. [%]		
					incl.gaps	excl.gaps	exchange
Without S9 mix:							
Exp. 1 (EZ 1389), Exp.time 5hrs:							
Solvent control		25	100	3.9	2.5	2.3	0.0
(b) (4)	88.9 ^S	25	120	4.6	4.0	3.0 ^{ns}	0.5
	281 ^S	25	102	5.5	2.5	1.0 ^{ns}	0.0
	889 ^{S,PE}	25	74	6.0	3.5	3.5 ^{ns}	1.0
Pos. Control	500	25	98	4.6	13.0	13.0 ^{**}	5.0
Exp. 2 (EZ 1405), Exp. times 25 and 35hrs:							
Solvent control		25	100	6.1	1.8	1.5	0.3
Solvent control		35	100	4.8	2.3	1.8	0.0
(b) (4)	15.8	25	87	5.5	3.0	3.0 ^{ns}	0.5
	50.0	25	90	9.5	5.5	4.5 [*]	0.0
	158 ^S	25	27	23.1	15.0	14.0 ^{**}	3.0
	158 ^S	35	51	27.4	20.0	18.0 ^{**}	6.0
Pos. Control	250	25	90	6.4	15.5	15.0 ^{**}	9.0
Exp. 3 (EZ1419), Exp. times 25 and 35hrs:							
Solvent control		25	100	5.3	1.3	0.8	0.0
Solvent control		35	100	5.0	2.0	1.3	0.5
(b) (4)	15.8	25	86	5.1	3.5	3.0 [*]	1.5
	50.0	25	93	5.9	1.0	1.0 ^{ns}	0.0
	88.9 ^S	25	68	7.1	6.0	5.5 ^{**}	1.5
	158 ^S	25	29	11.4	17.0	16.5 ^{**}	5.5
	158 ^S	35	78	20.0	8.5	7.0 ^{**}	1.5
Pos. Control	250	25	66	4.8	13.0	12.5 ^{**}	8.0

a: % of solvent control; S: plated as a suspension; PE: Precipitation of test material until the end of exposure time; EZ: Internal study number

Statistical significance: **: $p \leq 0.01$; *: $0.01 < p \leq 0.05$; ns: not significant

Treatment Group	Concentr. [µg/ml]	Prep. time[h]	Rel.mitotic index [%] ^a	Polyploid. metaph.[%]	Aberrant metaph. [%]		
					incl.gaps	excl.gaps	exchanges
With S9 mix:							
Exp. 1 (EZ 1390), Exp. time 5hrs:							
Solvent control		25	100	4.4	3.5	3.0	0.0
(b) (4)	88.9 ^S	25	111	3.3	2.5	1.5 ^{ns}	0.0
	281 ^S	25	101	5.0	2.0	2.0 ^{ns}	0.0
	889 ^{S,PE}	25	82	5.2	2.5	1.5 ^{ns}	0.5
Pos. Control	4.00	25	146	3.3	28.5	28.5 ^{**}	22.0
Exp. 2 (EZ 1392), Exp. time 5hrs:							
Solvent control		25	100	5.7	2.0	2.0	0.3
Solvent control		35	100	5.9	3.8	2.8	0.3
(b) (4)	88.9 ^S	25	82	4.7	3.5	3.5 ^{ns}	0.0
	281 ^S	25	79	4.9	5.0	4.5 ^{ns}	0.5
	889 ^S	25	82	5.8	3.5	3.5 ^{ns}	0.5
	889 ^S	35	123	5.6	3.0	3.0 ^{ns}	1.0
Pos. Control	4.00	25	116	4.3	28.5	27.5 ^{**}	9.0

a: % of solvent control; S: plated as a suspension; PE: Precipitation of test material until the end of exposure time; EZ: Internal study number

Statistical significance: **: $p \leq 0.01$; *: $0.01 < p \leq 0.05$; ns: not significant

In summary: Treatment of V79 cell cultures with (b) (4) impurity increased the proportion of cells with aberrant chromosomes at increased exposure times (25 and 35 h) and at cytotoxic concentration in the absence of S9 mix. In addition, a treatment related increase of polyploid cells was observed under the same experimental condition. Therefore, it was concluded that (b) (4) impurity is clastogenic at cytotoxic concentration under the condition of this *in vitro* study.

Conclusions:

The Sponsor considers (b) (4) impurity as having negligible potential for the risk of genotoxicity based on negative findings in two *in vivo* assays (bone marrow micronucleus assay and induction of hepatic unscheduled DNA synthesis in rats). We do not agree with this assessment and consider (b) (4) impurity as genotoxic based on two positive responses in Ames test and in chromosome aberration assay with V79 cells *in vitro*. Moreover, the Sponsor's proposed limit for (b) (4) impurity, of not more than (b) (4) (as an unspecified impurity) is considered not acceptable. It has also been noted that 7 impurities with structural alerts for genotoxicity are (b) (4) controlled at the level of (b) (4) ppm each which increases the risk of the patient's exposure to this class of potentially genotoxic impurities.

Based on this evaluation the following recommendation was communicated to the Sponsor on 10/15/10: "In addition to 6 potential genotoxic (b) (4) impurities (b) (4)

(b) (4), we consider (b) (4) (another (b) (4) impurity) to be genotoxic, based on the positive in vitro findings for mutagenicity (Ames test) and clastogenicity (chromosomal aberration test in V79 Chinese hamster cells). Consequently, your proposed limit for (b) (4) of not more than (b) (4), as an unspecified impurity, is not acceptable. Therefore, update your proposed in-process and/or drug substance specifications to control this impurity at a level of not more than (b) (4) ug/day clinical exposure, as you have done for each of the other 6 impurities. You need to provide an updated analytical method and validation data for (b) (4) with an appropriate lower detection limit to meet the total daily exposure requirement, as well as updated batch results to show that the exposure to this impurity is not more than (b) (4) ug per day.

Additionally, we have some concern because these 7 impurities share a common structural alert for genotoxicity (they are all (b) (4)). Therefore, we recommend that you evaluate whether these 7 impurities can be controlled at levels lower than the currently proposed levels to reduce the overall patient exposure to this class of potentially genotoxic impurities. We also recommend that you provide any information that is available on the levels of each of these 7 impurities in the batches of drug substance used for the carcinogenicity studies in rats and mice, as well as any other information that might be relevant regarding the carcinogenic potential of these impurities".

In response to our recommendation the Sponsor has adequately addressed the issues of 7 genotoxic (b) (4) impurities (submission on 11/04/10):

- Regarding impurity (b) (4) the specification was lowered from NMT (b) (4) ppm (i.e., NMT (b) (4) as an unspecified impurity) to NMT (b) (4) ppm (i.e., NMT (b) (4) ug/day human exposure).
- Regarding the other 6 (b) (4) impurities: They have lowered specs for (b) (4) and (b) (4) each from NMT (b) (4) ppm to NMT (b) (4) ppm (this submission); and will evaluate whether the 4 others can also be lowered to reduce the overall patient exposure (to be submitted in their 1st annual report).
- They have also noted that the batches used in the mouse (EE 77485) and rat (EE 77485 and EE 479) 2-year carcinogenicity studies were manufactured using Process 1, which resulted in the formation of (b) (4) at levels higher than the currently-proposed limit of NMT (b) (4) ppm (i.e., present in each batch at amounts between (b) (4) ppm [the LOQ] and (b) (4) ppm), but not in the formation of any of the other potentially-genotoxic impurities. This information may be helpful, but probably will not be necessary; because they have 1) lowered the spec for (b) (4) (to NMT (b) (4) ug/day) and 2) further lowered (or are attempting to lower) the specifications for each of the other 6 (b) (4) impurities to individual limits even lower than NMT (b) (4) ug/day.

11 Integrated Summary and Safety Evaluation

Pharmacology:

Vilazodone is a selective serotonin reuptake inhibitor and 5-HT_{1A} receptor partial agonist. It binds with high affinity to the cloned human serotonin transporter ($K_i = 0.1$ nM) but with considerably lower affinity to the human norepinephrine ($K_i = 56$ nM) and dopamine ($K_i = 37$ nM) transporters. In rat brain synaptosomes vilazodone potently and selectively inhibits reuptake of 5-HT ($IC_{50} = 0.2$ nM) but not NE ($IC_{50} = 60$ nM) or DA ($IC_{50} = 90$ nM). Vilazodone also binds with high affinity to 5HT_{1A} receptors ($IC_{50} = 2.1$ nM – cloned human receptors; $IC_{50} = 0.5 - 1.6$ nM – rat brain homogenate) and acts as 5HT_{1A} receptor partial agonist.

Vilazodone showed functional activity following oral administration in a dose range of 1 – 10 mg/kg. Its presence in the brain (*ex vivo* rodent studies) was demonstrated by similar occupancy of 5-HT reuptake binding sites and rat cortical 5-HT_{1A} receptors (postsynaptic) in this dose range (approximately 50% occupancy at 1-3 mg/kg; 90-100% occupancy at 10 mg/kg).

In several functional studies *in vitro*, vilazodone has been shown to be 5-HT_{1A} receptor partial agonist. Depending upon the assay system, estimates of agonist efficacy (intrinsic activity) ranged from 43% to 98% of the reference full agonist (usually 5-HT) indicating that vilazodone is an intermediate to high efficacy partial agonist. In treatment naïve rat brain slices, vilazodone showed partial agonist efficacy similar to buspirone and gepirone (5-HT_{1A} receptor partial agonists), but less than 8-OH-DPAT (full 5-HT_{1A} receptor agonist) in dorsal raphe (presynaptic site) and in the hippocampus (postsynaptic site).

Agonist-like efficacy of vilazodone was also demonstrated in functional studies that include reductions of electrically-elicited twitch in isolated guinea pig ileum, inhibition of dorsal raphe 5-HT neuronal firing, and increases in 5-HT levels in dorsal raphe brain slices. These effects were similar to those of 8-OH-DPAT and blocked by the 5-HT_{1A} receptor antagonist WAY 100635. In contrast to other 5-HT_{1A} agonists, vilazodone did not produce a “serotonin syndrome” in rats, but actually dose-dependently blocked the serotonin syndrome produced by the full agonist 8-OH-DPAT. Moreover, vilazodone did not produce the hypothermia in rats, another effect typically seen with 5-HT_{1A} receptor agonists. Therefore the partial agonist functional profile of vilazodone seems to be complex and not fully understood at present.

Depression has been recognized to be attributable, at least in part, to deficient serotonergic neurotransmission in the CNS, and therefore, an agent which augments 5-HT transmission in the brain would be predicted to be an effective antidepressant. The mechanism of vilazodone’s antidepressant effect is not fully elucidated, but is thought to be related to its enhancement of serotonergic neurotransmission in the CNS through selective inhibition of 5HT reuptake. The microdialysis studies *in vivo* demonstrated

that vilazodone augments extracellular 5-HT levels in rat frontal cortex and hippocampus similar to SSRI. Vilazodone also has partial agonist activity at 5-HT_{1A} receptors which may contribute to the increased serotonergic activity in discrete brain regions affected in the pathology of depression. Evaluation of vilazodone in behavioral animal models using various routes of drug administration demonstrated potential antidepressant-like efficacy in forced swimming test (in rats and mice) and in mouse tail suspension test. Vilazodone also showed anti-anxiety activity in animal models. Buspirone, another 5-HT_{1A} partial agonist, is marketed for the treatment of anxiety.

Several metabolites of vilazodone have affinity for 5HT reuptake sites, but considerably lower affinity than vilazodone; vilazodone is at least 35-fold more potent than M 11 metabolite, the most potent among metabolites at the rat 5-HT reuptake transporter. The affinity of M 11 metabolite for 5HT_{1A} receptor (rat hippocampus membranes) was 70 times less potent than that of vilazodone.

Safety pharmacology: Vilazodone is a CNS (as well as peripherally) acting drug which was active in animal models predictive of antidepressant and anti-anxiety activity. Vilazodone induced behavioral stimulation in mice and rats, and significant hyperthermia in mice but not rats. Body weight was reduced at 360 mg/kg in mice but not in rats at the same dose. In anti-seizure tests in mice, vilazodone (36 – 360 mg/kg) did not affect pentylentetrazole-induced convulsions but showed tendency to protect mice from convulsions induced by the electroshock with a maximum effect of 50% at 360 mg/kg.

Vilazodone did not demonstrate abuse liability or dependence potential in several models of behavioral dependence in mice or rats.

In cardiovascular studies, vilazodone (0.01 – 10.0 µM) was not active in the hERG channel assay (< 20% inhibition at all concentrations) and had no activity indicative of potential proarrhythmic effects in guinea-pig papillary muscles. Oral doses of vilazodone (30, 100 mg/kg) did not affect blood pressure or heart rate in spontaneously hypertensive rats. Vilazodone at doses of 0.1, 1, and 3 mg/kg, IV had no effect on hemodynamic parameters in anaesthetized, normotensive pigs.

Overall, the safety pharmacology studies conducted with vilazodone at doses up to 360 mg/kg (87 times the MRHD of 40 mg/day) did not reveal any significant areas of concern.

Pharmacokinetics: Vilazodone was absorbed after oral administration in all nonclinical species and in humans. Low oral bioavailability was observed in rats (~5% in males and ~28% in females), dogs (< 16%), and monkey (~27%). In humans, oral bioavailability of vilazodone was 72% - 81% when taken with food; bioavailability was considerably lower without food [labeling recommends that vilazodone be taken with food]. In general, the low oral bioavailability of vilazodone seen in rats and dogs was considered to be partly due to poor absorption (low water solubility) and partly due to extensive first-pass metabolism of absorbed drug.

Vilazodone exhibited > 96% serum protein binding in all species tested including humans. Vilazodone is widely distributed throughout the body and in 70% to 90% of the oral dosing total radioactivity distributed to tissues in rats was present as unchanged vilazodone. The highest tissue concentrations of radioactivity in rats were found in organs of absorption and elimination (GI tract, liver, kidney), and in the adrenal, pancreas, and lung. Concentrations in brain tissue were generally comparable to, or slightly lower than, plasma concentrations. It should also be noted that substantial depositions of crystalline material, presumably the drug, were observed in several organs in rat toxicology studies (see discussion below).

After single-dose administration of ¹⁴C-vilazodone to pregnant or lactating rats, < 1% of radioactivity was found in fetuses or in suckling pups, indicating that very little vilazodone crossed the placental barrier of rats and that little was secreted into the milk of lactating rats.

Vilazodone was extensively metabolized in all nonclinical species and humans (*in vivo*) and the metabolic profiles were similar across species. Twenty seven metabolites were identified in plasma, urine, bile or feces. The major route of metabolism of vilazodone was hydroxylation at various positions in the molecule, followed by glucuronidation (primary) or sulfation (secondary).

Two major human metabolites, M10 and M17, each circulating at greater than 10% of total drug-related exposure, were identified late in this review cycle [human mass balance study data were submitted on 8/31/10; N-0014] during this review cycle. These metabolites were found in urine of dogs and rats. M10 was found in plasma of rats and dogs; however, M17 was found in plasma of dogs, but was apparently not detected in plasma of rats to date. We have communicated to the Sponsor that they will need to address the safety of metabolites M10 and M17 as assessed in nonclinical studies; we referred them to ICH Guidance: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (2010); and the CDER Guidance: Safety Testing of Drug Metabolites (2008). The decision about whether additional studies will be needed to qualify these major human metabolites can not be made at the time this review is being finalized.

The primary mode of elimination of vilazodone and its-related material (total radioactivity) is fecal. In mice, rats and dogs, excretion of vilazodone is primarily via hepatic metabolism, with a pronounced first-pass effect. Excretion patterns are similar after oral and IV routes of drug administration in animals. In humans, as in the nonclinical species, fecal excretion accounted for most of the administered dose of vilazodone, but unlike the nonclinical species very little of the dose recovered in the feces was unchanged vilazodone. This difference is likely related to the higher oral bioavailability of vilazodone in humans compared to the nonclinical species. Much of the unchanged vilazodone recovered in the feces of the animal species may represent unabsorbed drug.

General toxicology: Vilazodone was adequately tested in acute and repeat oral dosing toxicology studies in mice, rats, and dogs. Its acute toxicity was observed at very high doses in mice ($LD_{50} \geq 2500$ mg/kg) and rats ($LD_{50} > 5000$ mg/kg) of both genders.

After chronic oral dosing of vilazodone, significant toxicity was only seen at the highest doses: hyperemia and impairment of general condition in a few rats (at 75 mg/kg/d; 26-week study) and convulsions and death in dogs (at 40 mg/kg; 52-week study). The NOAEL in rats was 75 mg/kg and NOAEL in dogs was 10 mg/kg (19-fold and 12-fold margin of safety based on exposure [AUC], respectively for MRHD of 40 mg/day).

Although, no limiting toxicity was observed in the 26-week rat study (3, 15, 75 mg/kg/day), a limiting drug “overload phenomena,” was seen at higher doses in shorter studies. In the 13-week rat oral toxicity study when much higher doses were used (100, 300, and 1000 mg/kg) vilazodone had no effect on mortality or body weight (BW) loss but toxicity related to an “overload phenomena” evidenced by; mild to massive deposition of crystalline material (drug/metabolites) in the intestinal mucosa, associated with reactive histiocytosis, granulocyte infiltrates and enlarged villi, and an immune response in mesenteric lymph nodes in ~one third of rats, were observed at ≥ 300 mg/kg/d dose. Similarly, mice exposed to vilazodone at ≥ 270 mg/kg/d (13-week study) exhibited histiocytosis in multiple organs caused by crystal deposition and associated degenerative and inflammatory reactions along with bone marrow myeloid hyperplasia and splenic erythropoiesis. These findings were consistent with the PK data of low oral bioavailability observed in rodents.

In the 52-week dog study (2.5, 10, 40 mg/kg/day), four high dose dogs (3/5 males and 1/5 females) did not survive to the scheduled sacrifice (deaths on Days 68, 175, and 355). In 3 dogs, death was preceded by convulsions (described as epileptiform). Convulsions were not witnessed in the fourth dog (euthanized on Day 130) but physical evidence suggested convulsions occurred prior to death. No convulsions or deaths were observed in the 26-week study with the same doses of vilazodone; and most seizures (3/4 dogs) occurred in the first 6 months of the 52-week study. The Sponsor provided the following explanation regarding the seizures: “It is conceivable that EMD 68 843 lowers the threshold for the induction of seizures in beagle dogs and that convulsions can be precipitated in dogs with a certain predisposition. Primary genetic or idiopathic (breed-related inherited) epilepsy has been documented in Beagles (Podell, 1996). This form of epilepsy is mainly seen in dogs aged 1-5 years. This age relationship would also be in line with the type of convulsions seen in this study.” Although the dogs for these two studies were provided by the same breeder, the studies were conducted two years apart. Therefore, the apparent difference in seizure sensitivity at 40 mg/kg dose could reflect an inherent difference in the dogs used in these two studies.

Clinical findings of fearfulness, salivation, hypo/hyperkinesia, sexual arousal and mydriasis, which were considered pharmacological effects rather than toxicity were observed at ≥ 10 mg/kg/day at the beginning of treatment and completely resolved by Week 4. Decreased body weights as a consequence of less food consumption were

also observed during the first 2-3 weeks of treatment. Blood glucose and serum cholesterol levels were increased by 20% and 47%, respectively, in both sexes throughout treatment, returning to normal (glucose) or nearly normal (cholesterol) during the recovery period. Corneal opacities were transiently seen at mid and high dose and correlated with decreased tear production at that time (first 3 weeks of treatment); corneal opacities were not seen late during dosing and no cataracts were found.

The NOAEL for seizures was the mid dose of 10 mg/kg (52-week study) or the high dose of 40 mg/kg (26-week study), which provides a 3-fold or 9-fold margin of safety, respectively based on (AUC) exposure for the MRHD of 40 mg/day.

Genotoxicity: Vilazodone was adequately tested and found to be negative for mutagenicity (\pm metabolic activation) in bacterial cells in an Ames test and in an *in vitro* V79/HGRPT mammalian cell forward mutation assay. Vilazodone was considered clastogenic without or with metabolic activation, in V79 Chinese hamster lung cells *in vitro*, but only at cytotoxic concentrations. Vilazodone was also clastogenic in the human lymphocyte assay at cytotoxic concentrations in the presence of metabolic activation, but not without the activation system. However, vilazodone was negative for clastogenic activity in two *in vivo* studies, in a rat bone marrow chromosome aberration assay and in a micronucleus test in rats, at single oral doses up to 2000 mg/kg. Vilazodone was also negative in an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes.

Carcinogenicity: Carcinogenicity studies were conducted in rats and mice for 2 years. Dose levels used in mice (15, 45, and 135 mg/kg/day; 1.8, 5.5, and 16.5 times the MRHD, respectively) and rats (7.5, 25, 75, and 150 mg/kg/day; 1.8, 6, 18, and 36 times MRHD of 40 mg/day) had been approved by Executive CAC. MTDs were reached or exceeded in the 2-year studies, based on increased mortality in vilazodone treated mice and significant histiocytosis in multiple organs caused by crystal deposition (possibly test article) and associated degenerative and inflammatory reactions along with bone marrow myeloid hyperplasia and splenic erythropoiesis in both rats and mice.

The toxicokinetic assessment indicated that both rats and mice were exposed to the test article throughout the study period. Exposure (AUC_{0-24}) increased with repeated dosing in mice (Day 1 to Week 52) and rats (Day 1 to Week 26) and was not different between genders in mice but higher in female rats than in males. Moreover, tissue concentration in the liver was ~ 40 and ~ 350 times higher than plasma concentration in male and female rats, respectively, (when measured at week 98). In view of these high tissue concentrations and poor solubility of vilazodone in aqueous medium, it seems likely that crystalline deposits observed at necropsy and histopathology represented deposits of test article.

In rats, there were no biologically relevant, drug-related increases in incidences of neoplasms. Non-neoplastic findings were limited to increases in incidence/severity of fibrohistiocytic granulomas in mesenteric and mediastinal lymph nodes in both males and females at the high dose of 150 mg/kg/day.

In mice, biologically relevant, drug-related increases in incidences of neoplasms were limited to hepatocellular carcinomas in males at the high dose of 135 mg/kg/day and mammary gland adenocarcinomas in females at 45 and 135 mg/kg/day.

Hepatocellular adenomas and carcinomas were present in male and female mice in all groups including control. Although statistical significance was noted at some doses of vilazodone in both sexes, there was no clear dose response relationship. Carcinomas were significantly increased at high dose males only.

The incidence of mammary gland tumors was increased similarly at mid and high doses in female mice. This finding was associated with increases in serum prolactin in vilazodone treated mice (in a separate mechanistic study), suggesting a hormonally mediated mechanism for the mammary gland findings. The hypothesis that the increase in mammary tumors is caused by increases in prolactin is supported by a substantial body of published literature showing this relationship in rodents including drugs acting directly or indirectly on activation of 5-HT receptors similar to vilazodone. The relevance of these findings to humans is not known at present. However, in one clinical trial with vilazodone (20, 40, or 80 mg; study: GPP-007-CLN-CP1-1997-232) serum prolactin was measured and no treatment-related effects were observed. Even if an increase in prolactin would occur in humans, there are experimental data suggesting that prolactin does not play the same role in mammary tumorigenesis in humans as it does in rodents.

An increased incidence of tumor findings in thyroid glands of mice, noted by the Sponsor, was not considered biologically relevant by the Executive CAC or this Reviewer. A supporting immunohistological and morphometric study was conducted on paraffin sections of pituitaries (obtained from dosed female mice) which indicated a correlation between TSH-positive pituitary lesions and thyroid neoplasms in both control and vilazodone treated mice. Moreover, in the 3-month mice study, treatment with vilazodone resulted in small, but statistically significant increase in TSH. Taken together, these data suggest that overstimulation of the thyroid may have contributed to the hyperplasia and adenomas observed in 2-year carcinogenicity study in mice. The relevance of these findings to humans is unknown.

Reproductive toxicity: Effects of vilazodone on fertility and early embryonic development were assessed in rats with males and females treated in separate studies. Male rats treated with vilazodone (at 5, 25, or 125 mg/kg/d for 4 weeks prior to pairing with untreated females) showed a reduced fertility index (76% versus 96% in controls) with no effect on mating, at HD (30 times the MRHD of 40 mg/day); there were no changes in weight and no microscopic findings in male sexual organs that could explain the reduced fertility. Treatment of female rats with vilazodone (at doses up to 125 mg/kg/day for 2 weeks prior to pairing with untreated males) had no effect on their fertility or reproductive function.

In embryo-fetal development studies, vilazodone was not teratogenic in rats, up to the high dose of 200 mg/kg/day, or in rabbits, up to the high dose of 50 mg/kg/day (35.8 mg/kg, actual dose).

Pregnant rabbits were dosed orally on GD 6-18 at 2, 10, and 50 mg/kg/day (however, the actual doses were 1.6, 7.8, and 35.8 mg/kg, respectively). The high dose exceeded the MTD for maternal toxicity based on deaths (3 deaths and 2 spontaneous abortions) and reductions in body weight gain and food consumption. Other findings were limited to the high dose, including increased post implantation loss and reduced fetal weight at birth and delayed skeletal ossification. However, no malformations were observed in rabbits at any dose up to the actual high dose of 35.8 mg/kg, which is 17 times the MRHD of 40 mg/day (based on body surface area conversion).

Pregnant rats were dosed orally at 8, 40, and 200 mg/kg/day during the period of organogenesis (GD6 to GD17). Maternal toxicity was limited to slight transient hyperemia of the furless skin, reduced food consumption, and reduced body weight gain at the high dose. Fetal findings were also limited to the high dose; fetal weight was reduced and this finding correlated with delayed skeletal ossification. The NOEL for developmental toxicity was at the mid dose of 40 mg/kg/day. The LOEL for developmental toxicity was the high dose of 200 mg/kg/day, based on lighter fetuses and weaker skeletal ossification. The NOEL for developmental toxicity was the mid dose of 40 mg/kg/day. The LOEL for developmental toxicity was the high dose of 200 mg/kg/day, based on lighter fetuses and weaker skeletal ossification. However, no malformations at any dose up to 48 times the MRHD of 40 mg/day (based on body surface area conversion) were observed in rats.

In the rat pre- and post-natal development study, vilazodone was administered to pregnant female rats from implantation (GD 6) through pregnancy, parturition and lactation to weaning of progeny (postnatal Day 21) at oral doses of 5, 25, and 125 mg/kg/day. Toxicity in the maternal generation (F0) consisted of reduction in body weight gain and food consumption (mid and high dose); reduced number of live born pups and fewer females with live pups at weaning (high dose); increased implantation loss and number of stillborn (high dose); increased number of cannibalized pups (dose-dependent, at mid and especially high dose); decreased probability of survival, based on decreased birth index, decreased viability index on Day 4 and decreased weaning index (at LD21) at mid and high dose.

The NOAEL for maternal toxicity was the low dose of 5 mg/kg/day based on reduced body weight and reduced food consumption at higher doses.

F₁ generation pups showed decreased body weight from birth to weaning (mid and high dose); and developmental delays, namely a longer time to show surface righting reflex and pinna detachment (all doses); delayed vaginal opening (but not preputial separation) and delayed traction reflex (mid and high dose); and longer time to fur appearance and deficit in chimney test (high dose only).

The reproductive performance of F1 rats showed decreased fertility index (high dose), both genders treated with vilazodone, but no effect on mating. Body weight gain was decreased from weaning to sexual maturity at mid and high dose; and body weight was still slightly (~5%) lower than in controls at sexual maturity (PND84) in both groups of rats. Caesarean section data showed lower number of corpora lutea, implantations, and live fetuses (F₂ generation), along with reduced weight of pregnant uteri at the high

dose compared to controls. The NOAEL for offspring toxicities was the LD of 5 mg/kg based mainly on reduced body weight from birth to weaning and from weaning to sexual maturity.

Based on another study, neither vilazodone nor its metabolites crossed the placenta of rats or were excreted into the milk of lactating rats as evidenced by lack of fetal/pup exposure.

It should be noted that body weight gain was reduced more in pregnant animals, rats and rabbits as compared to male and non-pregnant female rats, and non-pregnant rabbits. In male and non-pregnant female rats treated with vilazodone, a slight increase rather than decrease in body weight gain was observed at higher doses.

Impurities: Seven vilazodone HCl impurities were identified with structural alerts for genotoxicity which were not evaluated in non-clinical studies. The limits for these impurities had originally been set at the level of (b) (4) ppm (except (b) (4)) to allow a human exposure of (b) (4) µg/day for each. An exposure of (b) (4) µg /day equates to (b) (4) ppm for vilazodone HCl at the MRHD dose of 40 mg/day.

(b) (4)) and (b) (4)) impurities were assessed for genotoxicity using standard test systems.

(b) (4) was negative in the Ames test for mutagenicity and was considered not clastogenic in chromosome aberration assay with Chinese hamster lung cells *in vitro*. The proposed specification of NMT (b) (4) as unspecified impurity is acceptable for (b) (4)

(b) (4) was found to be mutagenic in *Salmonella typhimurium* strain TA 1535 (Ames test – *in vitro*) and was clastogenic in V79 cells (*in vitro*) without and with metabolic activation (S-9 mix) but was not genotoxic in an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay in rat liver and was negative for clastogenicity in mammalian micronucleus test system *in vivo*.

The Sponsor considered (b) (4) impurity as having negligible potential for the risk of genotoxicity based on negative findings in two *in vivo* assays (bone marrow micronucleus assay and induction of hepatic unscheduled DNA synthesis in rats). Based on the positive *in vitro* findings for mutagenicity (Ames test) and clastogenicity (chromosomal aberration test in V79 Chinese hamster cells) (b) (4) impurity is considered to be genotoxic by this Reviewer. Therefore, (b) (4) needs to be controlled in the drug substance at the level of not more than (b) (4) µg/day of clinical exposure.

Following communication with the Sponsor, the specification for (b) (4) was lowered from NMT (b) (4) ppm (i.e., NMT (b) (4), as an unspecified impurity) to NMT (b) (4) ppm (i.e., NMT (b) (4) µg/day human exposure). In addition the Sponsor lowered specifications for other (b) (4) impurities (for (b) (4) each from NMT (b) (4) ppm to NMT (b) (4) ppm) and agreed to evaluate whether the 4 others can also be lowered to reduce the overall patient exposure (to be submitted in their 1st annual report).

Safety evaluation:

Vilazodone binds with high affinity to the serotonin transporter site ($K_i = 0.1$ nM), but not to the norepinephrine ($K_i = 56$ nM) or dopamine ($K_i = 37$ nM) transporter sites. Vilazodone inhibits reuptake of serotonin ($IC_{50} = 0.5 - 1.6$ nM) and binds to 5HT_{1A} receptors with similar affinity ($IC_{50} = 2.1$ nM) and is 5HT_{1A} receptor partial agonist. Vilazodone showed functional activity following oral administration (dose range of 1 – 10 mg/kg); its presence in the brain (*ex vivo* rodent studies) was demonstrated by occupancy of 5-HT transporter binding sites and 5-HT_{1A} receptors (50% occupancy at 1-3 mg/kg; 90-100% occupancy at 10 mg/kg). Vilazodone was shown to increase level of serotonin in the brain (microdialysis study in freely moving rats) and to produce functional desensitization of presynaptic (inhibitory) 5-HT receptors in electrophysiological studies. Although the mechanism of vilazodone's antidepressant activity has not been fully elucidated, it is thought to be related to its role in enhancement of serotonergic neurotransmission in the CNS through selective inhibition of 5HT reuptake and activity at 5-HT_{1A} receptors.

Safety pharmacology studies with vilazodone in nonclinical species revealed no areas of concern. Vilazodone was absorbed after oral administration in animals and in humans. High oral bioavailability was observed in humans (when taken with food), but not in animal species and higher exposure to vilazodone was observed in female rats than in males and no sex difference in another species. The elimination of vilazodone was primarily fecal in animals (40-60% was unabsorbed drug) and in humans. Vilazodone was extensively metabolized in all species including humans. Metabolites generated *in vitro* by human microsomes were also generated by rat microsomal enzymes indicating the appropriate range of metabolites was likely present during *in vitro* genotoxicity testing.

Two major human metabolites, M10 and M17, each circulating at greater than 10% of total drug-related exposure, were identified late in this review cycle (human mass balance study submitted to this NDA [N-0014] on 31-08-10). These metabolites were found in urine of dogs and rats. M10 was found in plasma of rats and dogs; however, M17 was found in plasma of dogs, but was apparently not detected in plasma of rats to date. We have communicated to the Sponsor that they will need to address the safety of metabolites M10 and M17 as assessed in nonclinical studies; we referred them to ICH Guidance: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (2010); and the CDER Guidance: Safety Testing of Drug Metabolites (2008). The decision about whether additional studies will be needed to qualify these major human metabolites can not be made at the time this review is being finalized.

Vilazodone's toxicity in rats was mainly related to an "overload phenomena" evidenced by; mild to massive deposition of crystals of unabsorbed drug in the intestinal mucosa, associated with reactive histiocytosis, granulocyte infiltrates and enlarged villi, and an immune response in mesenteric lymph nodes at high doses. Convulsions (and convulsion-related deaths) were seen in dogs at the high dose of 40 mg/kg in the 12-

month study (but not in the 6-month dog study with the same dose); the AUC at this high dose is 12 times the AUC in humans at MRHD of 40 mg/day. The NOAEL for seizures was the mid dose of 10 mg/kg (52-week study) or the high dose of 40 mg/kg (26-week study), which provides a 3-fold or 9-fold margin of safety, respectively based on (AUC) exposure for MRHD of 40 mg/day.

The corneal opacities were transiently seen in these two dog studies (2-3 weeks of treatment) that correlated with decreased tear production at that time, but no cataracts were found in dogs (after up to 52 weeks of exposure) or in other animal species.

Vilazodone was not mutagenic without or with metabolic activation (S9 mix) in the Ames test or in the *in vitro* V79/HGRPT mammalian cell forward mutation assay. Vilazodone was clastogenic in V79 Chinese hamster lung cells (\pm S9 mix) and in human lymphocytes with S9 mix (but not without S9 mix) and only at cytotoxic concentrations in both of these *in vitro* assays. However, vilazodone was not clastogenic in two *in vivo* studies, rat bone marrow chromosome aberration assay and in micronucleus test in rats at single oral doses up to 2000 mg/kg. Vilazodone was also negative in an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes.

In the carcinogenicity studies conducted in rats and mice for 2 years, the only biologically relevant, drug-related increases in incidences of neoplasms were limited to hepatocellular carcinomas in male mice (at 16.5 times the MRHD) and mammary gland adenocarcinomas in female mice (at 5.5 and 16.5 times the MRHD, on mg/m² basis). No biologically relevant, drug-related increases in incidences of neoplasms were found in rats at doses up to 36 times the MRHD of 40 mg/day.

Vilazodone caused reduced fertility in male but not in female rats at 30 times, but not at 6 times the MRHD of 40 mg, on a mg/m² basis. Vilazodone was not teratogenic in rats and rabbits, however, it did increase post-implantation loss in rabbits and decreased fetal weights and delayed ossification in rats and rabbits (48 and 17 times the MRHD of 40 mg on a mg/m² basis). When pregnant rats were treated with vilazodone at doses up to 30 times the MRHD of 40 mg/day) during the period of organogenesis and throughout pregnancy and lactation, the number of live born pups was decreased. There was an increase in early postnatal pup (F₁) mortality, and decreased body weight among surviving pups, delayed maturation, and decreased fertility in adulthood. Neither vilazodone nor its metabolites crossed the placenta of rats or were excreted into the milk of lactating rats as evidenced by lack of fetal/pup exposure.

Vilazodone was absorbed after oral administration with plasma exposure in all animal species, sufficient for assessing targeted toxicities and toxicological margins of safety as summarized in the following table:

Toxicity	Species	NOAEL (mg/kg) M/F	Safety Margin Based on AUC*
General	Rat (6 month)	75/75	19 (16 M/23 F)
	Dog (12-month)	10/10	3
Carcinogenicity	Dog (6-month)	40/40	9
	Rat	150/150	16 M/56 F
	Mouse	45/15	9 M/3.3 F
Reproductive & Developmental			
Fertility	Rat	25/125	6 M**/30 F**
Embryo/fetal development	Rat	40	10**
	Rabbit	10 (maternal); 2 (fetal)	3.5 (maternal) Not determined
Pre- and postnatal development	Rat	5 (maternal – F0) 5 (offspring – F1)	1.2**

*AUC in human: 1645 ng•hr/ml at 40 mg/day.

** On a mg/m² basis (TK data not available)

12 Appendix/Attachments

12.1 Table 1: Affinity of Vilazodone, Ipsapirone, Buspirone, 8-OH-DPAT and Fluoxetine for Receptor and Ion Channel Binding Sites (IC₅₀, nM)

Receptor binding	Vilazodone	Ipsapirone	Buspirone	8-OH-DPAT	Fluoxetine
5-HT _{1A} serotonin	0.5; 1.6	6	30	0.9	>10000
5-HT _{1A} serotonin (h)	2.1			0.68	
5-HT _{1B} serotonin	3300				
5-HT _{1D} serotonin	726; 4000	3000	>10000	300	
5-HT _{2A} serotonin	4300	2600	920	>10000	
5-HT _{2A} serotonin (h)	14300				
5-HT _{2B} serotonin (h)	78; (Ki = 34)				
5-HT _{2C} serotonin	2000			>10000	600
5-HT _{2C} serotonin (h)	4720				
5-HT ₃ serotonin	10000				
5-HT ₃ serotonin (h)	>30000				
5-HT ₄ serotonin	100; 196				
5-HT _{5A} serotonin (h)	18900				
5-HT ₆ serotonin (h)	7430				
5-HT ₇ serotonin (h)	1880				
Sigma	17	856	79	985	
D ₁ dopamine	>10000				
D ₁ dopamine (h)	16100				
D ₂ dopamine	550	1200	270	3700	>10000
D ₂ dopamine (h)	760; 2040				
D ₃ dopamine (h)	140; 360				
D ₄ dopamine	5200				
D ₅ dopamine (h)	17500				
α ₁ norepinephrine	1000	500	5000	>10000	
α ₂ norepinephrine	6000	1000	>10000	800	
β ₁ - β ₂ - β ₃ - norepinephrine (h)	>10000				
M ₁ /M ₂ muscarinic Ach	>10000		>10000	>10000	
M ₁ muscarinic Ach (h)	5240				
M ₂ muscarinic Ach (h)	3750				

Receptor binding	Vilazodone	Ipsapirone	Buspirone	8-OH- DPAT	Fluoxetine
M ₃ muscarinic Ach (h)	28000				
M ₄ muscarinic Ach (h)	5030				
GABA _A	>10000				
κ - opiate	>10000				
κ - opiate (h)	4640 (K _i =2320)				
δ - opiate	3000				
δ - opiate (h)	20400 (K _i =10700)				
μ-opiate	800				
μ-opiate (h)	500 (K _i =179)				
H ₁ histamine	690	77	838	8700	
H ₂ histamine	>10000				
AMPA, Kainate, NMDA - glutamate	>10000				
PCP/NMDA	>10000	>10000	>10000	>10000	
Glycine site glutamate	>10000				
NK ₁ (h)	27300 (K _i =4710)				
NK ₂ (h)	18000 (K _i =14000)				
NK ₃ (h)	>10000				
Batrachotoxin- sensitive Na ⁺ channel	4030 (K _i =3630)				

Data source from studies: GPP-007-NCD-PCL-2000-049; -195-046; -1999-065; -2000-066; -2002-117)

12.2 Table 2: Affinity of Vilazodone and Metabolites in Selected Receptor Binding Assays (IC₅₀, nM)

Assay	Vilazodone	M11	M10	EMD 80 546
5-HT _{1A} serotonin	0.5 1.6	35	200	20
Human (h) 5-HT _{1A} serotonin	2.1			> 1000
5-HT _{1B} serotonin		>10000		
5-HT _{1D} serotonin	726 4000	>1000	>10000	>10000
5-HT _{2A} serotonin	4300		>10000	>10000
5-HT _{2A} serotonin (h)	14300	20000		
5-HT _{2C} serotonin	2000	3000	>10000	>10000
5-HT ₃ serotonin	10000		>10000	4300
5-HT ₃ serotonin (h)	>30000	9700		
5-HT ₄ serotonin	100 196	700	2000	>10000
5-HT ₆ serotonin (h)	7430	6000		
D ₁ dopamine	>10000	>10000		
D ₂ dopamine	550		>10000	>10000
D ₂ dopamine (h)	700 2040	20000		
D ₃ dopamine (h)	140 360	680	6300	>10000
D _{4,2} dopamine (h)	3400	27000	>10000	>10000
α ₁ norepinephrine	1000	>10000	>10000	>10000
α ₂ norepinephrine	6000	>10000	>10000	1000
M ₁ /M ₂ acetylcholine	>10000	>10000	>10000	>10000
α ₃ β ₄ nicotinic		>10000		
α ₄ β ₂ nicotinic		>10000		
α ₇ nicotinic		>10000		
μ opiate	800	2000	>10000	8000
κ-opiate	>10000	>10000		
δ opiate	3000		4000	2000
σ ₁ receptor		630		

Assay	Vilazodone	M11	M10	EMD 80 546
σ_2 receptor		1400		
σ receptor	17		>10000	>10000
H ₁ histamine	690	>10000	>10000	>10000
H ₂ histamine	>10000	>10000		
Benzodiazepine site (GABA _A receptor)	>10000	>10000		
NMDA glutamate	>10000	>10000		
PCP/NMDA site (glutamate)	>10000	>10000		
Glycine site (glutamate)	>10000	>10000		

Note: The affinity of M17 metabolite was at IC₅₀ >10000 nM in any receptor assay included in Table 2.

12.3 Reproductive toxicology dose-range finding studies:

Study title: Preliminary embryotoxicity and teratogenicity study with EMD 68 843 administered by oral gavage in albino rabbits

Study no: GPP-007-NCD-TOX-1997-181
 Study report location: Merck KGaA, Frankfurter Strasse 250, D-64293 Darmstadt, Germany

Conducting laboratory and location:

(b) (4)

Date of study initiation: April 14, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: EMD 68 843, lot# EE 77185, 99.4% purity

Key Study Findings

Oral administration of vilazodone at doses of 5, 10, and 50 mg/kg/d to pregnant New Zealand White rabbits (5/group) resulted in maternal toxicities that included clinical signs of reduced defecation, diarrhea and reduced water consumption at ≥ 10 mg/kg and decreases in BW ($\leq 7.5\%$) and food consumption during the treatment period (significant at 50 mg/kg). No developmental toxicities were observed.

Based on the results of this study, doses of vilazodone at 2, 10, and 50 mg/kg/d were selected for the main study.

Methods

Doses: 0, 5 (LD), 10 (MD), and 50 (HD) mg/kg/d
 Frequency of dosing: Once a day
 Dose volume: 5 ml/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: 1% aqueous carboxymethyl cellulose
 Species/Strain: Rabbit/New Zealand White
 Number/Sex/Group: 5 females/group
 Satellite groups: N/A
 Study design: Female rabbits were artificially inseminated with semen samples from donor bucks or mated with male rabbits. Does were treated with vilazodone or vehicle from GD6 to GD18 of gestation inclusive.

Deviation from study protocol: Not reported

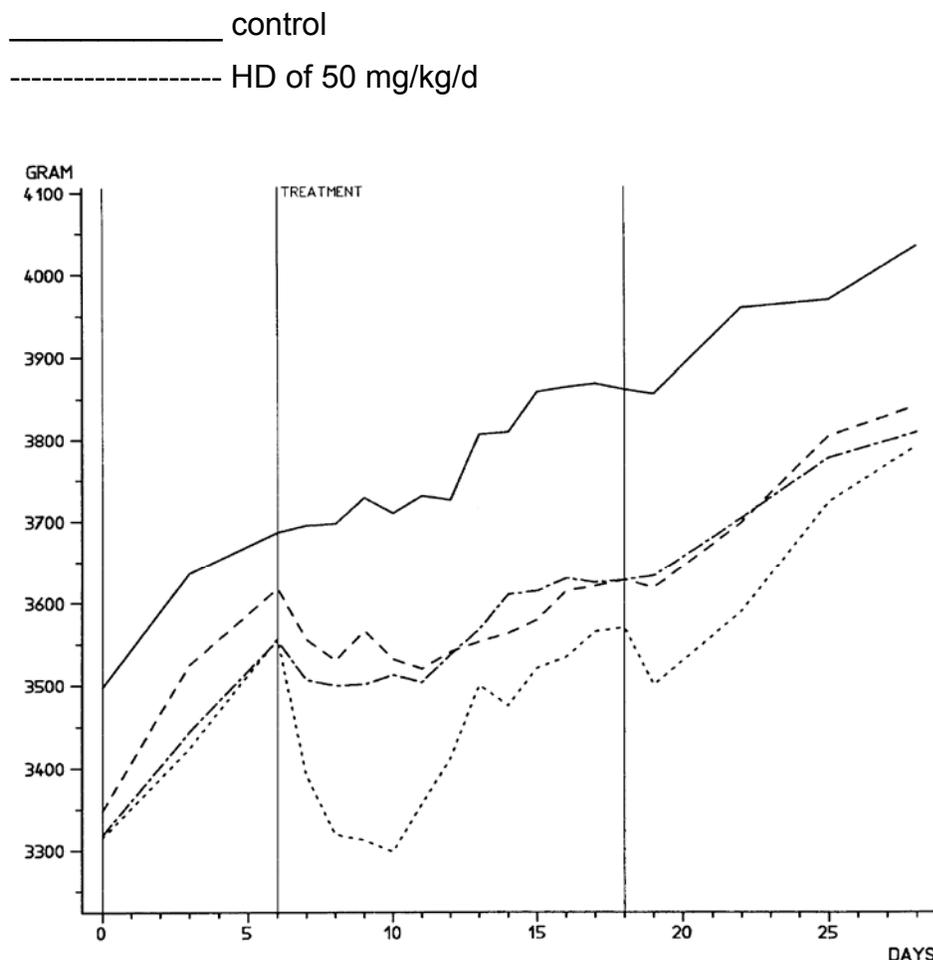
Observations and Results

Mortality, clinical observations, body weights and food consumption were recorded. Dams were sacrificed on G28. Uteri were weighed and contents were examined. Reproductive indices were calculated. Fetuses were examined macroscopically for external, internal and skeletal malformations and were weighed and sexed.

The pregnancy rate was 100% (4/4), 80% (4/5), 100% (5/5) and 75% (3/4) at 0, 5, 10 and 50 mg/kg/day, respectively. No test article-related deaths occurred during the study.

Clinical signs consisted of abnormal defecation (decreased number of feces and diarrhea) and reduced water consumption at ≥ 10 mg/kg/day.

Decreased BW was observed in all vilazodone treated dams (significant at HD) as shown on the following graph (similar trends were seen in food consumption).



At necropsy examination, no macroscopic changes were observed. No test article-related findings were seen in reproductive parameters (number of corpora lutea, implantation sites, live or dead fetuses and pre-implantation or post-implantation loss). No early or late resorptions were detected. The fetal sex ratio was altered at LD and HD (increases in number of males) but not at MD. Fetal weights were unaffected and no treatment-related fetal abnormalities or malformations were seen. Macroscopic examination immediately following the caesarian section revealed no treatment related abnormalities.

Study title: Development toxicity study with toxicokinetics: Dose-finding study with oral administration in rats

Study no.:	GPP-007-NCD-TOX-1997-180
Study report location:	Institute of Toxicology, Merck KGaA, 64271 Darmstadt, Germany
Conducting laboratory and location:	Institute of Toxicology, Merck KGaA, 64271 Darmstadt, Germany
Date of study initiation:	August 5, 1996
GLP compliance:	Yes (signed and dated)
QA statement:	Yes (signed and dated)
Drug, lot #, and % purity:	EMD 68 843, lot# EE 77185, 99.4% purity

Key Study Findings

Oral administration of vilazodone at doses of 50, 100, and 200 mg/kg/d to pregnant Wistar rats caused a decrease in BW gain in all females during treatment. With the exception of the slightly lighter fetuses at HD, there was no impairment of reproductive parameters. No malformations were reported in any fetuses.

Methods

Doses:	0, 50, 100, 200 mg/kg/d
Frequency of dosing:	Once a day
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension in 0.25% aqueous carboxymethylcellulose
Species/Strain:	Rats/Wistar, HSD CPB:Wu
Number/Sex/Group:	5 females/group
Satellite groups:	6/group
Study design:	Vilazodone was administered to pregnant rats from GD 6 to GD 15. The fetuses were delivered by c-section on GD 20 and examined

Observations and Results

Mortality: None of the animals had died.

Clinical Signs:

The general condition of rats was unaffected by treatment.

Body Weight:

Rats of all treatment groups lost weight as a reaction to the first dosing which was present until the end of the study. The BW gain during the treatment period was reduced by -37% (LD), -22% (MD), and -35% (HD).

Feed Consumption:

It was not different than control in any treated group of females.

Toxicokinetics:

Blood samples were collected on GD6 and GD15 (day 1 and 10 of treatment) at 1, 3, 6, and 24 h after dosing. The rats of the TK study groups were killed on GD16.

TK data are summarized in the following Sponsor's table:

	group 2: 50 mg/kg		group 3: 100 mg/kg		group 4: 200 mg/kg	
time after administration [h]	GD 6	GD 15	GD 6	GD 15	GD 6	GD 15
1	502	1130	617	1590	998 ^b	1990 ^b
3	819 ^a	1710 ^a	1340	3270	1990	3560
6	1390	1110	1420	1860	2770 ^b	2610 ^b
24	14.0 ^a	35.7 ^a	59.4	82.6	1100	186
C _{max} [ng/ml]	1390	1710	1420	3270	2770	3560
t _{max} [h]	6	3	6	3	6	3
AUC [ng/ml x h]						
0 - 6 h	4890	7590	6410	13200	10600	15800
0 - 24 h	10300	13200	14100	23500	43200	32300
AUC / dose*						
0 - 6 h	97.8	152	64.1	132	53.0	79.0
0 - 24 h	206	264	141	235	216	162

a: N=2 (one animal out of three was found to be not pregnant and was excluded)

b: N=1 (two animals out of three were found to be not pregnant and were excluded)

*: AUC [ng/ml x h]/dose [mg/kg]

Reproduction parameters:

The reproduction data are summarized in the following Sponsor's table:

Dose mg/kg	Dams (n)	Implan-tations	Live fetuses	Resorptions		Fetal loss (%)	Weight in g m/f
				early	late		
0	4	48	47	1	0	2	3.7/3.6
50	4	49	48	1	0	2	3.4/3.2
100	5	65	64	1	0	2	3.7/3.5
200	5	52	50	2	0	0	3.2/3.0

With the exception of the slightly lighter fetuses at HD, there was no impairment of reproductive parameters. No malformations were reported in any fetuses.

In conclusion, the LD in the main study should be < 50 mg/kg (BW loss after the first dosing). 200 mg/kg dose that led to maternal toxicity (loss BW) and mild fetal effects (lighter fetuses) was considered adequate for HD in the main study.

Study title: EMD 68843 Pilot pre- and postnatal toxicity study in rats after oral administration from gestation day 6 until lactation day 7

Study no: GPP-007-NCD-TOX-2001-185
 Study report location: Merck KGaA, Pharma Ethical Preclinical R&D; Frankfurter Strasse 250; 64293 Darmstadt, Germany

Conducting laboratory and location:



Date of study initiation: October 24, 2000
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: EMD 68843, lot#: EE 77485, 99.4% purity

Key Study Findings

Vilazodone at 160 mg/kg/d was administered by oral gavage to Wistar female rats from the gestation day (GD) 6 until lactation day (LD) 7 to determine whether this dose is suitable as the HD for the main study. Decreased BW gain and lower food consumption was observed at this dose.

A postnatal survival was lower and BW of pups was significantly reduced. Based on reduced pup weights and survival index at 160 mg/kg/d, the HD for the main study was selected at 125 mg/kg/d.

Methods

Doses: 0 and 160 mg/kg/d
 Frequency of dosing: Once a day
 Dose volume: 10 ml/kg/d
 Route of administration: Oral gavage
 Formulation/Vehicle: Suspension/aqueous 0.25% hydroxypropyl-methylcellulose
 Species/Strain: Rats/Crl:CD (SD) BR
 Number/Sex/Group: 10/females/group
 Study design: The day a positive indication of mating was observed was designated GD 0. Mortality, clinical observations, BW and food consumption were recorded for dams. BW, sex, survival and L4 viability data were recorded for pups. Dams and pups were sacrificed on LD7. Uteri were examined for implantation sites. Birth indices were calculated. Pups were examined macroscopically for external abnormalities, live/still birth, sex and weight.

Deviation from study protocol: Not reported

Observations and Results

No deaths or clinical signs occurred during this study. In vilazodone treated dams, decreased BW gain was noted from GD6 to GD16 (38%) and from LD 0 to LD 4 (48%) and lower food consumption from GD8 to GD14 (up to 33%). No effect on food consumption was observed during lactation. A recovery was observed from GD16 up to the end of pregnancy.

Pregnancy duration, parturition, implantations/litter, live born pups/litter, post-implantation loss and pup sex ratio were unaffected by treatment. No external abnormalities at birth were found in pups of either group. Although birth index was unaffected, survival probability was significantly lower on LD 4 in pups of treated dams (0.8651 vs. 0.9587 in controls); the viability index was also reduced (87% vs. 96% in controls), although the difference was not statistically significant. The mean BW of these pups was significantly reduced (17-24% in males and females from LD 0 to LD 7).

Based on reduced pup weights and survival index at 160 mg/kg/day, it was determined that the doses to be used in the definitive study were 5, 25 and 125 mg/kg/day.

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/s/

VIOLETTA M KLIMEK
12/22/2010

LINDA H FOSSOM
12/22/2010

See my supervisory memo regarding the qualification of major human metabolites in non-clinical studies.

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

NDA Number: 022567

Applicant: PGxHealth, LLC

Stamp Date: March 23, 2010

Drug Name: Vilazodone HCl NDA Type: Standard

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			The route of administration for the formulation to be marketed is not different from the formulation used in the toxicology studies
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	√		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	√		Yes on face, but it is a subject of review
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		
11	Has the applicant addressed any abuse potential issues in the submission?	√		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____yes____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None at this time

Violetta Klimek, Ph.D.

May 5, 2010

Reviewing Pharmacologist

Date

Linda Fossom, Ph.D.

Team Leader/Supervisor

Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22567	ORIG-1	PGXHEALTH LLC	VILAZODONE HCL

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

/s/

VIOLETTA M KLIMEK
05/10/2010

LINDA H FOSSOM
07/07/2010