

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

022526Orig1s000

PHARMACOLOGY REVIEW(S)

Final Comments on NDA 22-526 flibanserin

Date 8/11/15

From: A Jacobs AD

1. Comments on approvability: There are no outstanding pharm/tox issues.
2. The hepatocellular neoplasms in male mice were considered to be drug-related. The increase of mammary neoplasms in female mice is also considered to be drug related (incidences: combined controls: 1/140; low dose: 3/70; mid dose: 3/70; mid-high dose: 6/70; and high dose: 7/70). The relevance to humans is unknown but cannot be excluded. I have conveyed other comments to the supervisor, and they have been addressed.

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/s/

ABIGAIL C JACOBS
08/12/2015



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

Division of Bone, Reproductive, and Urologic Products

Date: August 3, 2015

Reviewer: Lynnda Reid, Ph.D.
Supervisory Pharmacologist/Toxicologist

NDA: 22-526

Sponsor: Sprout Pharmaceuticals, Inc.

Drug Product: ADDYI (Flibanserin; BIMT 17 BS)

Indication: Treatment of Premenopausal women with Hypoactive Sexual Desire Disorder (HSDD)

Drug History: Flibanserin was originally developed to treat depression. However, in Phase 2 depression trials, it was noted that unlike other serotonin antidepressants, flibanserin was not associated with sexual dysfunction. Rather, it appeared to have a positive response in women regarding sexual function. On November 10, 1009, Boehringer Ingelheim submitted NDA 22-526 for the treatment of women with hypoactive sexual desire disorder. At the time of the original submission, Boehringer Ingelheim submitted a complete nonclinical package to support their new drug application. Following review of this package by the primary reviewer, Dr. Alexander Jordan, Ph.D., the submission was deemed approvable from a Pharmacology/Toxicology perspective. This submission was given a complete response (CR) by DBRUP based on clinical deficiencies. After receiving a complete response, the NDA was sold to Sprout Pharmaceuticals, Inc.

Sprout Pharmaceuticals resubmitted the NDA on March 29, 2013. This submission also received a CR based on clinical deficiencies. The current resubmission was filed on February 14, 2015.

No new nonclinical data was submitted with the Sprout submissions. Dr. Jordan's recommendation for approval based on nonclinical data remains unchanged.

Related IND: (b) (4)

Nonclinical data supporting approval of NDA 22-030:

Pharmacology: The therapeutic mechanism of action for flibanserin in HSDD is unknown. In vitro binding studies demonstrated a high affinity for 5-HT_{1A} and 5-HT_{2A}. Flibanserin has a moderate affinity for 5-HT_{2B}, 5-HT_{2C}, and dopamine D₄ receptors. In vivo studies in female rats demonstrated that flibanserin functions as a 5-HT_{1A} agonist and a 5-HT_{2A} antagonist, decreasing extracellular serotonin and increasing extracellular norepinephrine in the medial prefrontal cortex compared to controls. Dopamine concentrations were variable.

In a rat model for female sexual behavior, flibanserin induced a dose-dependent increase in solicitations on days 15 and 22 but not on days 1, 8 or 29. There were no other effects of sexual motivation such as ear wiggles or hops and darts. Similarly, there was no effect on lordosis behavior (posture indicating receptivity or readiness to allow copulation).

Toxicology Studies:

Acute Toxicity: Following administration of acute doses of flibanserin at 64 mg/kg [human equivalent dose (HED) ~620 mg], adverse neurological effects were characterized by decreased locomotor activity, hypothermia, hindlimb abduction, reduced grip strength and head weaving. At 128 mg/kg [HED ~625 mg] two mice were cataleptic.

Repeat-Dose Toxicity: In the 6 month toxicology study in rats at oral doses of 20, 100 and 400 mg/kg, flibanserin administration produced hepatic hypertrophy in males at 100 and 400 mg/kg and in females at 400 mg/kg. In females there was also a mild increase in hepatic lipid content. No other histopathological changes were noted. This study showed very little toxicity in rats at exposures up to approximately 20 times the human exposure (2080 ng·h/ml) in women taking 100 mg.

In dogs flibanserin administered orally at doses of 3, 15 and 100 mg/kg/day for 13 weeks produced significant adverse clinical signs, lower body weights, and increases in intraocular tension in the eyes of dogs treated with 100 mg/kg. In the 6 month study animals were dosed at 3, 15 and 75 mg/kg/day. There were significant adverse neurological findings at 15 and 75 mg/kg/day (~8 and 70 times clinical exposures based on AUC). One dog at 75 mg/kg was sacrificed moribund on week 7. Salivation and ataxia occurred on isolated occasions at 15 mg/kg. At 75 mg/kg salivation occurred after dose administration, animals became recumbent, vocalized, and were ataxic or convulsive from about 1 hour after drug administration until beyond 6 hours after administration. Dogs also exhibited dry eyes and various behavioral traits such as biting the metal drinking bowls or feeding racks, standing with head forward, and forcing head or limbs between the bars of the door or the enclosure. Symptoms were seen from 2 hours after administration onwards beyond 6 hours. In the course of the study the number of tremor and vocalization episodes decreased and the duration of vocalization became shorter. There was also a moderate increase in heart rate at all doses.

One dog at 15 mg/kg and 7 dogs at 75 mg/kg demonstrated dose-related ophthalmological and/or histopathological changes of the eyes characterized by discrete, focal, smoky opacity or by focal facet (focal thickening of the cornea epithelium) in the central or peripheral region of the cornea. There was no increase in intensity with time and two of the opacities disappeared with time and did not reappear. Similar ocular findings were seen in the 13 week dog toxicity studies where 2/12 dogs at 100 mg/kg and 4/6 dogs at 100/75 mg/kg were affected.

Similar to observations in rats, there was an increase in the frequency and intensity of fatty liver which appeared to be dose and possibly duration related. These changes were characterized as mild to marked with an increase in intensity in animals dosed at 15 and 75 mg/kg/day. Fat droplets were present in the periportal and midzonal areas of the liver.

Genotoxicity and Carcinogenicity:

Genotoxicity: Based on the weight of evidence, flibanserin is not genotoxic. It was negative in the Ames assay, mammalian cell culture (CHO), and Comet assay; and positive in the in vitro chromosomal aberration study. It was also negative in the in vivo rat bone marrow micronucleus study.

Carcinogenicity: Carcinogenicity potential was evaluated in 2-year bioassays: rats were dosed at 10, 30 and 100 mg/kg/day and mice at 10, 80, 200, 1000 (M), 1000/1200 (F) mg/kg/day. Protocols and final reports were reviewed by ECAC (see Appendix).

Rat Study: There were no statistically significant tumor findings in the rat study. The incidence of hepatocellular carcinomas was slightly higher at 100 mg/kg/day in males. Two males in the 100 mg/kg group died due to intra-abdominal hemorrhage from liver tumors in weeks 90 and 86. There were no effects in females. Exposure multiples at 30 and 100 mg/kg/day are roughly 3 and 8, and for males 3 and 5, respectively, using a human AUC of 2080 ng·h/ml (steady state exposures with the recommended 100 mg once daily clinical dose).

Mouse Study: In the mammary gland of female mice, there was a clear increase in adenocarcinomas (incidence was 0, 1 in the two controls and 3, 3, 5, 5 in the treated groups). When combined with malignant adenoacanthomas (also called adenosquamous carcinoma; a variation of adenocarcinoma), the controls had 0 and 1, and the treated groups had 3, 3, 6, and 7 total malignant mammary tumors which is higher than the historical controls for any one group. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes.

Female mice also had a statistically significant increase in combined hepatocellular carcinomas and adenomas. In male mice, there was a statistically significant increased trend in hepatocellular carcinomas (1 and 6 in male controls, and 7, 8, 9, 11, in the treated groups) which was also significant in the pairwise test at 1000 mg/kg/day for

hepatocellular carcinomas, but not when combined for total hepatocellular adenomas and carcinomas.

To evaluate a potential hormonal mechanism related to the induction of the mammary gland tumors, prolactin levels were evaluated in female mice. There were no effects on prolactin levels at either 14 or 34 weeks at doses up to 1200 mg/kg, exposures approximately 10 times the human exposures following doses of 100 mg/day.

Prolactin levels were also evaluated in the rat 6-month toxicology study. Prolactin was measured 2 hours post-dosing in weeks 8 and 23, and 24 hours post-dosing in week 15 of the study. There was a dose dependent increase in serum prolactin in both males and females at 80 and 400 mg/kg/day in week 8 and week 23. No change was seen after 24 hours at 15 and 80 mg/kg/day and a slight decrease was seen at 400 mg/kg/day in week 15.

Reproductive and Developmental Toxicity

Rat Fertility: There were no effects on female fertility (copulation index, fertility index, gestation index). There were no effects on litter parameters (implantations, resorptions, viable fetuses).

Rat Embryo/Fetal Development (EFD): Pregnant rats were administered doses of 20, 80 and 400 mg/kg during the period of organogenesis. Maternal toxicity was observed at doses ≥ 80 mg/kg/day. Within 2-3 hours of administration, dams treated at 80 mg/kg showed somnolence, sedation, and timidity after the first treatment through the 9th treatment. At 400 mg/kg there were severe maternal clinical signs including somnolence, reduction of spontaneous activity, sedation, prone position, timidity, catalepsy, and others.

Developmental abnormalities were observed at 400 mg/kg. One dam in the high dose group had total embryo resorptions. The NOAEL (no observable adverse effect level) was considered to be 80 mg/kg (~15 X human exposure). There was one runt (fetuses weighing less than 65% of the weighted control mean values) in the 80 mg/kg and 10 in the 400 mg/kg groups. Skeletal and visceral abnormalities were seen in controls and treated animals with equal distribution. In the 400 mg/kg group, there were a greater number of fetuses with reduced ossification of fore limbs and a greater number with lumbar ribs. Malformations were seen only in the treated groups and included cleft (2 at 80 mg/kg, 1 at 400 mg/kg) or fused vertebrae (1 at 400 mg/kg) and a single malformation (agnathia) at the 20 mg/kg. In the 400 mg/kg group, there were 2 fetuses with anophthalmia and one with hydrocephalus, all within one litter.

Rat Pre- and Post-natal Development (PPND): Dams were dosed at 20, 80, and 200 mg/kg. The 200 mg/kg group had significantly reduced numbers of implantations and newborns, due primarily to post implantation loss. In the 200 mg/kg dose group viability rate was decreased to 49.3% and weaning rate to 85.5%. In the 80 mg/kg dose group the effect on viability was 82%. All offspring survived after weaning. At delivery the body

weights of the F1 pups from the 80 and 200 mg/kg groups were significantly lower than the control and below the mean historical control mean. Body weight gain of these groups remained significantly decreased during lactation.

Rabbit Embryo/Fetal Development: Rabbits were doses with 20, 40, and 80 mg/kg/day during organogenesis. An increase in fetal resorptions was observed at 80 mg/kg. There were no teratology findings.

Summary and Conclusions: The pharmacologic mechanism of flibanserin in the treatment of premenopausal women with hypoactive sexual desire disorder (HSDD) is unknown. Effects in animal models of sexual behavior were modest at best.

Safety issues in animals were primarily related to adverse effects on the central nervous system, some severe at high doses but not unexpected for a centrally acting drug. CNS effects appeared to be less tolerated in pregnant rats. CNS effects included abnormal behavior including timidity and discomfort, somnolence, increased vocalization, tremors, ataxia, and convulsions.

In dogs there were dose-related ophthalmologic and histopathologic changes of the eyes characterized by discrete, focal, smoky opacity or by focal thickening of the corneal epithelium. This effect was not seen in any study in rats. To address this potential adverse finding, ophthalmologic endpoints were included in a 24 week, randomized, double blind placebo controlled clinical trial with an open-label extension (no adverse ocular effects were reported).

Also in dogs, flibanserin produced fatty changes in the liver of males and females after 26 and 52 weeks. This effect seemed both dose and duration related with an increase in severity and numbers of dogs affected. There was an increase in fatty accumulation in the myocardium of dogs of both sexes without an increase in severity or number of dogs affected from 26 to 52 weeks. The mechanism for the fat deposition is unknown but it did not produce measurable changes in EKG patterns measured throughout the study.

Hepatocellular fatty changes were also seen in the 13 and 26 week rat toxicity studies. However, there were no increases in myocardial fat in rats. Mild elevations in blood pressure and heart rate were observed but with no effect on ECG parameters.

Flibanserin was not considered to be mutagenic. When administered orally to mice and rats for two years, there was no statistically significance increase in tumorigenicity in male and female rats. In female mice there was a clear dose-related, statistically significant increase in mammary gland adenocarcinomas and malignant adenoacanthomas. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes. There was a statistically significant increase in hepatocellular carcinomas in male and female mice treated at ≥ 1000 mg/kg/day.

In reproduction studies in rats, flibanserin administration had no effect on female fertility. When given during the period of organogenesis, maternal and fetal toxicity was observed at doses ≥ 80 mg/kg/day (>15 times the clinical exposures at 100 mg/day). Flibanserin produced some visceral and skeletal variations in rat fetuses at the 400 mg/kg dose (approximately 42 times human exposure) and there were a small number of various malformations in pups from all treated groups without a discernable drug-related pattern. There were resorptions in rabbits treated with 80 mg/kg (26 times human exposure) but there was no increase in fetal variations or malformations.

In a pre- and postnatal development study in rats, flibanserin produced maternal toxicity in all groups with a significant reduction in number of implantations and newborns from dams treated with 200 mg/kg (~ 30 times human exposures based on body surface area). Dams treated at 200 mg/kg showed poor maternal care leading to failure to thrive and high mortality. There were some development delays in these pups but fertility and the F2 generation seemed unaffected. In general, flibanserin administration produced no clear signal for adverse effects on fetal development. In both the embryo/fetal and the pre- and post-natal development study, flibanserin administration resulted in pups with anophthalmia. In the rat embryo/fetal development study, two pups from one litter from the 400 mg/kg had anophthalmia and in the post-natal study there were two pups in the 200 mg/kg group with anophthalmia. There was one fetus with hydrocephalus in both treated rats (42 times human exposure) and rabbits (8 times human exposure).

Safety Issues

Neurologic and Ophthalmologic Effects: Potential adverse neurological and ocular effects have been adequately evaluated in humans.

Carcinogenicity: The ECAC concluded that there was a drug induced increase in malignant mammary gland tumors in female mice. Although the increase was small, the increases were dose-related and statistically significant. The ECAC asked the sponsor to evaluate a potential mechanism of induction based on increases in prolactin levels. There was no increase in prolactin levels in mice (and only slight increases 2-hours post dosing in rats) and therefore the mechanism of induction is unlikely to be hormonal. It is also unlikely that tumor induction is via mutation as the standard battery was considered to be negative for genotoxicity.

A dose-related increase in malignant mammary tumors at low multiples of the human exposures (~ 3 fold) is particularly noteworthy given the targeted population and indication for this drug: relatively healthy, premenopausal females with low sexual desire. The clinical significance of the mammary tumors in mice is unknown. Nonetheless, Nonclinical recommends that women be advised of this animal finding under the Warnings and Precautions section of labeling because the finding may be an important consideration for some healthcare providers and women when deciding whether or not to use flibanserin. Many patients and health care practitioners may miss this finding if it is only reported in the Animal Toxicology section.

Developmental: The NOAEL for maternal and fetal toxicity was considered to be 80 mg/kg/day. Although cleft vertebrae was observed in 3 litters (2 in the 80 mg/kg and 1 in the 400 mg/kg groups) this finding was not considered to be drug induced. Cleft vertebrae is one of the most common malformations seen in Chbb: (b) (4) rats and there was no dose-response. Based on the animal data, it would appear that there is a low probability that the inadvertent use of flibanserin during pregnancy would result in fetal harm. Findings were minimal and at high multiples of exposure. Additionally they were only observed in the presence of significant maternal toxicity that should not occur in women.

Conclusion: I concur with the primary Pharmacology/Toxicology reviewer, Dr. Alexander Jordan, that from a nonclinical perspective this NDA may be approved.

Recommended Labeling

Established Pharmaceutical Class (EPC): Since it is unknown how flibanserin increases sexual desire, no EPC should be used in Highlights.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no studies of ADDYI in pregnant women to inform a drug-associated risk. In animals, fetal toxicity only occurred in the presence of significant maternal toxicity including reductions in weight gain and sedation. Adverse reproductive and developmental effects consisted of decreased fetal weight, structural anomalies, and increases in fetal loss at exposures greater than 15 times the clinical exposure (b) (4) the recommended human dosage [see Data]. Animal studies cannot rule out the potential for fetal harm (b) (4).

8.2 Lactation

Flibanserin is excreted in rat milk. It is unknown whether flibanserin is present in human milk, whether ADDY has effects on the breastfed infant, or whether ADDYI affects milk production. Because of the potential for serious effects adverse reactions including sedation in a breastfed infant, breastfeeding is not recommended during treatment with ADDYI.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

A two-year carcinogenicity study was conducted in CD-1 mice with dietary administration of 0, 10, 80, 200 and 1000/1200 mg/kg/day of flibanserin. Statistically significant increases in combined mammary tumors (adenocanthomas and adenocarcinomas) were observed in female mice administered flibanserin at doses of 200 and 1200 mg/kg/day (exposures, based on AUC, were 3 and 10 times the clinical exposures at the

recommended clinical dose). No increases in mammary tumors were observed in male mice. Statistically significant increases were also seen for combined hepatocellular adenomas/carcinomas in female mice treated with flibanserin 1200 mg/kg/day (exposures, based on AUC, were 10 times clinical exposure at the recommended clinical dose) and for hepatocellular carcinomas in male mice treated with flibanserin 1000 mg/kg/day (exposures, based on AUC, were 8 times the clinical exposure at the recommended clinical dose).

There was no significant increases in tumor incidence in a two-year carcinogenicity study conducted in Wistar rats with dietary administration of flibanserin at 0, 10, 30 and 100 mg/kg/day.

Mutagenesis

Flibanserin was negative for mutagenesis in vitro in *Salmonella typhimurium* (Ames test) and in Chinese hamster ovary cells. (b) (4) was positive for chromosomal aberrations in cultured human lymphocytes but was negative for chromosomal aberrations in vivo in the rat bone marrow micronucleus assay (b) (4) for DNA damage in rat liver in the Comet assay.

Impairment of fertility

(b) (4)

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The mechanism of action of ADDYI in the treatment of premenopausal women with hypoactive sexual desire disorder is not known.

12.2 Pharmacodynamics

Receptor Binding:

In vitro, flibanserin demonstrated high affinity for the following serotonin receptors: agonist activity at 5-HT_{1A} and antagonist activity at 5-HT_{2A}. Flibanserin also had moderate (b) (4) 5-HT_{2B}, 5-HT_{2C}, and dopamine D₄ receptors. (b) (4)

Appendix: ECAC Minutes

Executive CAC

Date of Meeting: May 19, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Dan Mellon, Ph.D., OND IO, Alternate Member
Lynnda Reid, Ph.D., DRUP, Supervisor
Alex Jordan, Ph.D., DRUP, Presenting Reviewer

Author of Draft: Alex Jordan

The following information reflects a brief summary of the Committee discussion and its recommendations.

IND #: (b) (4)

Drug Name: Flibanserin

Sponsor: Boehringer Ingelheim

Background: Flibanserin is a 5-HT_{1A} agonist / 5-HT_{2A} antagonist intended for the treatment of premenopausal women with hypoactive sexual desire.

Mouse Carcinogenicity Study Protocol and Dose Selection

This was a two year study of flibanserin given orally in the feed. Doses were 10, 80, 200 and 1000/1200 mg/kg/day. The high dose was initially 1000 mg/kg and increased to 1200 mg/kg on drug week 23 because of lack of toxicity.

Rat Carcinogenicity Study Protocol and Dose Selection

This was a two year study of flibanserin given orally in the feed. Doses were 10, 30 and 100 mg/kg/day.

Executive CAC Recommendations and Conclusions:

Rats:

The Committee concurred that the study was adequate, noting prior Exec CAC approval of the protocol on Dec. 23, 1997.

The Committee concurred that there were no clearly drug-related neoplasms in rats

Mice:

The Committee concurred that the study was adequate, noting prior Exec CAC approval of the protocol on July 14, 1998.

The Committee concurred that the increased incidences of hepatocellular carcinomas plus adenomas (combined) in female mice and mammary adenocarcinomas plus adenoacanthomas (combined) were drug related in female mice.

The Committee recommends inclusion of the positive findings of hepatocellular carcinomas and mammary carcinomas in mice in the label. Currently the Sponsor is investigating the possible role of elevated prolactin concentrations as an explanation for the increased incidence of mammary tumors in mice. If the data reveal an increase in the serum concentration of prolactin in mice without a similar increase in women, the Committee feels that the tumors in mice would be of less concern for women and this information should be included in the labeling.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\

- /Division File, DRUP
- /Lynnda Reid, PhD., Team leader, DRUP
- /Alex Jordan, PhD., Reviewer, DRUP
- /Project Manager, DRUP
- /ASeifried,

Linked Applications	Sponsor Name	Drug Name
IND (b) (4)	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	BIMT 17 BS (b) (4)

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/s/

ADELE S SEIFRIED
05/29/2008

DAVID JACOBSON KRAM
05/29/2008

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/s/

LYNNDA L REID
08/10/2015



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22526
SERIAL NUMBER: 001
DATE RECEIVED BY CENTER: Complete Response: February 14, 2015
PRODUCT: Addyi (flibanserin)
INTENDED CLINICAL POPULATION: Women with hypoactive sexual desire disorder
SPONSOR: Sprout Pharmaceuticals Inc.
DOCUMENTS REVIEWED: ECT
REVIEW DIVISION: Division of Bone, Reproductive and Urological Products
PHARM/TOX REVIEWER: Alex Jordan, PhD
PHARM/TOX SUPERVISOR: Lynnda Reid, PhD
DIVISION DIRECTOR: Hylton Joffe, MD
PROJECT MANAGER: Charlene Williamson

Date of review submission to DARRTS: July 14, 2015

APPEARS THIS WAY ON ORIGINAL

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EXECUTIVE SUMMARY

I. Recommendations

Recommendation on approvability: I recommend approval of flibanserin (Addyi) for women with hypoactive sexual desire disorder.

Recommendation for nonclinical studies: none

Recommendations on labeling

These recommendations have not been finalized.

8.1 Pregnancy

Data

Animal Data

Pregnant rats were administered flibanserin at doses of 0, 20, 40 and 400 mg/kg/day during organogenesis. The highest dose was associated with significant maternal toxicity as evidenced by severe clinical signs and marked reductions in weight gain during dosing. Decreased fetal weights, (b) (4) decreased ossification of the forelimbs and increased (b) (4) and two fetuses with anophthalmia (b) (4). The no adverse effect level for (b) (4) embryofetal toxicity was 80 mg/kg/day (15 times clinical exposure based on AUC).

Pregnant rabbits were administered flibanserin at doses of 0, 20, 40 and 80 mg/kg/day during organogenesis. Marked decreases in maternal body weight gain (>75%), abortion and complete litter resorption were observed at 40 and 80 mg/kg/day indicating significant maternal toxicity at these doses. Increases in resorptions and decreased fetal weights were observed at ≥ 40 mg/kg/day. No treatment related teratogenic effects were observed in fetuses at any dose level. The no adverse effect level for maternal and embryofetal effects was 20 mg/kg/day (3-4 times clinical exposure based on AUC).

Pregnant rats were administered flibanserin at doses of 0, 20, 80 and 200 mg/kg/day from day 6 of pregnancy until day 21 of lactation to assess for effects on peri- and postnatal development. The high dose was associated with clinical signs of toxicity in pregnant and lactating rats. All doses resulted in sedation and decreases in body weight gain during pregnancy. Flibanserin prolonged gestation in some dams in all dose groups and decreased implantations, number of fetuses and fetal weights at 200 mg/kg/day (>15 times clinical exposure). Dosing dams with 200 mg/kg also decreased pup weight gain and viability during the lactation period and delayed opening of the vagina and auditory canals. Flibanserin had no effects on learning, reflexes, fertility or reproductive capacity of the F1 generation. The no adverse effect level for maternal toxicity and peri/postnatal effects was 20 mg/kg/day (3-4 times therapeutic exposures). [see Nonclinical Toxicology (13.1)].

8.2 Lactation

Risk summary

Flibanserin is excreted in rat milk.

(b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

A two-year carcinogenicity study was conducted in CD-1 mice with dietary administration of 0, 10, 80, 200 and 1200/1000 mg/kg/day flibanserin. Statistically significant increases in combined malignant mammary tumors (adenoacanthomas and adenocarcinomas) were observed in female mice administered flibanserin at doses of 200 and 1200 mg/kg/day ((b) (4) exposure based on AUC). No increases in mammary tumors were observed in male mice. Statistically significant increases were also seen for combined hepatocellular adenomas/carcinomas in female mice treated with flibanserin (b) (4) mg/kg/day (b) (4).

A two year carcinogenicity study was conducted in Wistar rats with dietary administration of 0, 10, 30 and 100 mg/kg/day flibanserin. (b) (4)

Mutagenesis

Flibanserin was negative for mutagenesis in vitro in *Salmonella typhimurium* (Ames test) and in Chinese hamster ovary cells. Flibanserin was positive for chromosomal aberrations in cultured human lymphocytes but negative for chromosomal aberrations in vivo in the rat bone marrow micronucleus assay and negative for DNA damage in rat liver in the Comet assay.

Impairment of Fertility

Female and male rats were administered flibanserin 14 and 28 days before mating, respectively, to assess for potential effects on fertility and early reproductive performance. Flibanserin slightly increase the duration of the estrus cycle but had no adverse effects on fertility or early embryonic development at doses up to 200 mg/kg/day (b) (4).

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings: In rats, chronic administration of flibanserin resulted in an increase in weights of the thymus, adrenal and liver with a reduction in fat deposition in the liver of males and an increase in hepatocellular hypertrophy in females treated with 400 mg/kg daily (approximately 29 times human exposure). In dogs, chronic flibanserin administration produced corneal opacities, increased heart rate and an increase in the frequency and intensity of fatty liver and heart which seemed dose and duration dependent. The fatty accumulation in the heart occurred only in dogs at exposure levels in females of approximately 5 times the exposure in women taking 100 mg. In the adrenal, there was an increase in fat accumulation which was dose but not duration dependent. There was a degeneration of the mucous membranes of the trachea in dogs treated with 15 or 75 mg/kg/day (5 to 53 times human exposure). In mice, there was a dose-related statistically significant increase in malignant mammary tumors. This was not seen in rats. The weight of evidence indicates that flibanserin is not genotoxic. Flibanserin had no significant effects on fertility in male or female rats. When flibanserin was given to rats during embryogenesis, there was an increase in fetal variations at the high dose and there were sporadic non dose-related fetal malformations. There were no adverse fetal effects in rabbits other than an increase in resorptions at the high dose. In a pre-and postnatal development study in rats, flibanserin reduced the number of implantations and newborns from dams treated with the HD (200 mg/kg or roughly 30 times human exposure). Dams treated with the HD showed poor maternal care leading to failure to thrive and high mortality. There were some development delays in these pups but fertility and the F₂ generation seemed unaffected.
- B. Pharmacologic activity: Flibanserin acts on the serotonin receptor and functions as a 5-hydroxytryptamine (5-HT)_{1A} agonist 5-HT_{2A} antagonist that decreases extracellular serotonin and increases norepinephrine in rat prefrontal cortex, medial preoptic area and nucleus accumbens. There are also regional, dose-related effects on dopamine. These effects are believed to be related to its effect on increased sexual desire in females.
- C. Nonclinical safety issues relevant to clinical use: The major issue is the increased incidence of mammary tumors in mice. Flibanserin is a new molecular entity with a neurochemical mechanism indicated for hypoactive sexual desire disorder in women. Mammary tumors are not particularly common in mice so there is some concern about an increased risk of breast cancer. However, flibanserin does not seem to be genotoxic and there was no increased incidence of mammary tumors in rats. The incidence of mammary tumors in mice while significantly elevated compared to concurrent controls, was only somewhat higher than the historical control maximum, thus the risk, if any, for breast cancer in women seems slight.

[Please limit to 1-3 pages]

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-526

Review number: 1

Sequence number/date/type of submission: EDR

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Sprout Pharmaceuticals

Manufacturer for drug substance: (b) (4)

Reviewer name: Alex Jordan, PhD

Division name: Reproductive and Urological Products

HFD #:

Review completion date:

Drug:

Trade name:

Generic name: flibanserin

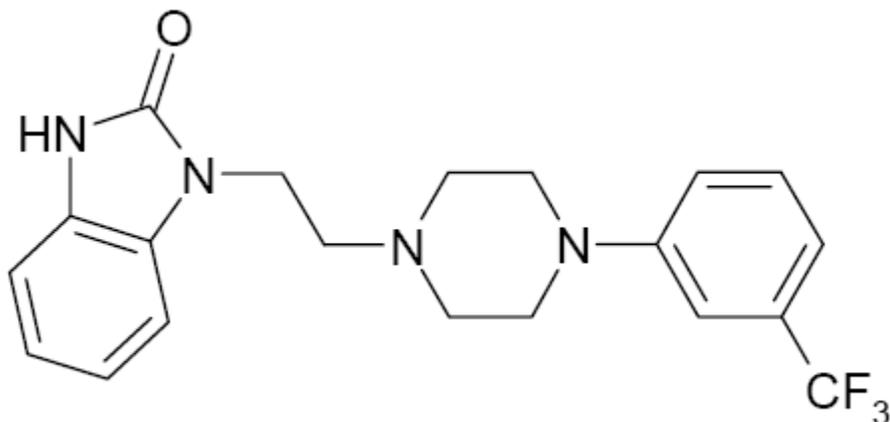
Code name: BIMT 17 BS

Chemical name: 2H-benzimidazol-2-one, 1,3-dihydro-1-[2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl].

CAS registry number:

Molecular formula/molecular weight: 390.4 g/mol

Structure:



Relevant INDs/NDAs/DMFs: IND (b) (4)

Drug class: Serotonin modulator

Intended clinical population: Women with hypoactive sexual desire disorder

Clinical formulation:

Table 1 Qualitative and quantitative composition of flibanserin film-coated tablets, 100 mg

Ingredient	mg per Tablet	Function	Reference to Standards
Flibanserin	100.000	Active ingredient	Company Standard
Lactose monohydrate (b) (4)	(b) (4)	(b) (4)	NF/Ph. Eur./JP
Microcrystalline cellulose (b) (4)			NF/Ph. Eur./JP
Hypromellose (b) (4)			USP/Ph. Eur./JP
Croscarmellose sodium			NF/Ph. Eur./JP
Magnesium stearate (b) (4)			NF/Ph. Eur./JP
(b) (4)			USP/Ph. Eur./JP
(b) (4) Pink (b) (4)			Company Standard
Total			347.0

Route of administration: oral

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Flibanserin acts preferentially as a post-synaptic 5-hydroxytryptamine (HT) [serotonin]_{1A} receptor agonist and a 5-HT_{2A} receptor antagonist. Flibanserin had high affinity for human 5-HT_{1A} recombinant receptors ($K_i = 6.59$ nM) and was a full agonist on 5-HT_{1A} receptors when receptors were expressed in Chinese hamster ovary (CHO) cells. Flibanserin had high affinity ($K_i = 15.3$ nM) and was a full antagonist for human 5-HT_{2A} receptors when expressed in CHO cells. Flibanserin also had moderate affinity for 5-HT_{2B}, 5-HT_{2C} and dopamine (DA) D₄ receptors. Flibanserin does not have physiologically significant affinity for human monoamine transporters, does not inhibit reuptake of these neurotransmitters into synaptosomes expressing rat monoamine transporters and does not inhibit monoamine oxidase type A or B in rat brain.

Flibanserin was originally developed to treat depression. In Phase 2 depression trials, flibanserin was not associated with sexual dysfunction, which is unusual for serotonergic antidepressants. A multi-dimensional measure of sexual dysfunction, the Arizona Sexual Experiences Scale (ASEX), was included in the four Phase 2b depression studies for comparison of flibanserin, standard antidepressants, and placebo. In one of these trials, flibanserin was superior to both the positive comparator and placebo on the ASEX scale, mainly on the "sex drive" item in women.

Hypoactive sexual desire disorder (HSDD) is defined in the DSM-IV-TR as a deficiency or absence of sexual fantasies and desire for sexual activity that causes marked distress or interpersonal difficulty and is not due to the physiological effects of a substance or general medical condition. The biological causes of HSDD are only poorly understood and to date there are no approved pharmacological treatments in the US. Clinical development and off-label usage has focused on hormone products including a testosterone transdermal system that has been approved for surgically postmenopausal women in Europe.

The therapeutic mechanism of action of flibanserin in HSDD is unknown but there is evidence for the involvement of serotonin. Serotonin reuptake inhibitors (SSRIs) produce a number of sexual side effects with the most common being anorgasmia or delayed orgasm. There are also reports of spontaneous orgasm in conjunction with fluoxetine treatment and reports of hypersexual effects following treatment with fluoxetine or the serotonin releaser, fenfluramine suggesting that both inhibited and enhanced sexual responses to serotonergic agents may be produced. Flibanserin has been shown to stimulate 5-HT_{1A} receptors while blocking 5-HT_{2A} receptors and has moderate affinity to 5-HT_{2B,2C} receptors. These 5-HT receptor mediated mechanisms directly affect serotonergic neurotransmission but also have some indirect effects on dopaminergic and noradrenergic neurotransmitter signaling. Flibanserin decreases extracellular serotonin levels and increases the extracellular dopamine concentration in the medial prefrontal cortex in rats. This effect is completely abolished by the selective 5-HT_{1A} antagonist WAY100.635.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Flibanserin binding studies to various receptors in vitro revealed a high affinity for 5-HT_{1A} ($K_i = 6.59$ nM) and 5-HT_{2A} ($K_i = 15.3$ nM). Flibanserin has a moderate affinity for 5-HT_{2B} ($K_i = 89.3$ nM), 5-HT_{2C} ($K_i = 88.3$ nM), and dopamine D₄ receptors ($K_i = 167$ nM). Flibanserin does not have physiologically significant affinity for monoamine transporters 5-HT: ($K_i = 2.63$ μ M), DA: ($K_i = 2.63$ μ M), NA ($K_i = 2.63$ μ M), and does not inhibit monoamine A and B oxidase.

In human post mortem studies, flibanserin significantly reduced the activity of forskolin-stimulated adenylyl cyclase postsynaptically (prefrontal cortex), and in the hippocampus, but had no effect in the raphe nuclei (presynaptic level).

Flibanserin acts as a full agonist on 5-HT_{1A} receptors in reducing forskolin-stimulated adenylyl cyclase activity. This was observed in all tissues in the human dorsal raphe (largest serotonergic nucleus where some antidepressants are believed to act) which contrasts with other 5-HT_{1A} receptor agonists, which are inactive in reducing forskolin-stimulated AC activity in the cortex.

Flibanserin 5-HT_{1A} agonist activities result in many of its pharmacological properties in vivo. Flibanserin decreases firing rates of 5-HT neurons in the rat dorsal raphe nucleus and alters firing rates in the hippocampus. Flibanserin also reduces neuronal cortical activity and these decreases can be inhibited by 5-HT_{1A} antagonists.

Drug activity related to proposed indication:

Effect of acute and chronic treatment with flibanserin 15 and 45 mg/kg po on extracellular serotonin, norepinephrine, dopamine, GABA and glutamate in the medial prefrontal cortex and in nucleus accumbens shell of freely moving female rats.

This study was conducted at (b) (4) under study key 191 (parts A and B). Rats (280-350 g) were from (b) (4). Drug was flibanserin batch 05071202M.

Female Wistar rats in the nonreceptive stage (not in estrous) were anesthetized and placed in a stereotaxic frame and I shaped probes were inserted in the medial prefrontal cortex (mPFC) and Nucleus Accumbens shell (NAcc).

The rats were orally dosed with 0, 15 or 45 mg/kg flibanserin two times a day for 21 days. On the day of the experiment, the probes were connected with flexible PEEK tubing to a microperfusion pump and perfused with artificial CSF at a flow rate of 1.5 ul/min. Microdialysis samples were collected at 30 min intervals. After the experiment the rats were sacrificed and the brains removed and the position of each probe was verified histologically.

Concentrations of 5-HT, NA, DA, GABA and Glu were determined by HPLC separation and electrochemical and fluorometric detection.

Effects of chronic treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle

	5-HT	NA	DA	GABA	Glu
mPFC (15 mg/kg)	Decrease +	Increase +	----	----	----
NAcc (15 mg/kg)	----	----	----	----	----
mPFC (45 mg/kg)	Decrease +	Increase +	Increase +	----	----
NAcc (45 mg/kg)	Decrease +	----	(Decrease +)	----	----

---- no statistical significance
(significant only at a single time point)
+ statistically significant

Sponsor concludes: At an oral dose of 15 and 45 mg/kg, extracellular serotonin and norepinephrine were significantly changed in the medial prefrontal cortex compared to controls.

At an oral dose of 45 mg/kg extracellular dopamine in the medial prefrontal cortex and serotonin in the nucleus accumbens were significantly changed compared to vehicle.

Basal absolute values of the neurotransmitters vary between the doses given. This variation is an effect of the compound when given chronically and is dose dependent in the prefrontal cortex with respect to dopamine and norepi.

Effects of acute and chronic treatment with flibanserin on extracellular serotonin, norepinephrine, dopamine, GABA and Glutamate in the Medial Preoptic Area of freely moving female rats.

Nonreceptive female Wistar rats were anesthetized and placed in a stereotaxic frame and I shaped probes were inserted into the medial preoptic area (MPOA).

Rats were given flibanserin orally twice a day for 21 days. On the day of the experiment, the probes were connected to flexible PEEK tubing and perfused with artificial CSF. On the day of the experiment the animals were challenged with flibanserin at different doses depending on the pretreatment dose. Animals treated with vehicle were challenged with vehicle on the first day. On the second day they received an acute dose of 45 mg/kg flibanserin.

Effect of chronic treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle.

	5-HT	NA	DA	GABA	Glu
MPOA (15 mg/kg)	Decrease +	Increase +	Decrease (+)	----	----
MPOA (45 mg/kg)	Decrease +	Increase +	----	----	----

---- not statistically significant
 + statistically significant
 (+) significant only at a single time point

Effect of acute treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle.

	5-HT	NA	DA	GABA	Glu
MPOA (45 mg/kg)	Decrease +	Increase +	Increase (+)	----	----

---- not statistically significant
 + statistically significant

Flibanserin reduced 5-HT release in the mPFC, mPOA and Nacc, but not in the hippocampus. Flibanserin increased extracellular DA in the mPFC and in the mPOA of the hypothalamus, but not in the Nacc or hippocampus. Repeated treatment of flibanserin induced selective effects. In rats treated twice daily for 15 or 45 mg/kg for 21 days, flibanserin administration decreased serotonin and increased NA in the mPFC and mPOA. DA levels were increased only in the mPFC (45 mg/kg) and not in the mPOA or Nacc. Serotonin was decreased in the Nacc (45 mg/kg) but no changes in NA or DA were observed. Repeated treatment increased basal levels of DA and NA in the mPFC but only NA was increased in the Nacc.

Animal model for female sexual behavior

The bilateral chamber test is used to examine sexual incentive motivation. The chamber consists of an upper and lower level which allows the female to escape the male and thus pace mating. Female Long-Evans rats (13 per group) were ovariectomized and rendered sexually receptive with injections of estradiol and progesterone. Flibanserin was administered orally at doses of 5, 15 and 45 mg/kg for 28 days. Sexual behavior was noted during 30 minute period tests on days 1, 8, 15, 22 and 29. Proceptivity was defined as gestures such as solicitations (headwise orientation toward the male followed by abrupt runaway terminated by the assumption of a crouching posture), hops and darts and ear wiggings. Receptivity or readiness to allow copulation is represented by lordosis.

Flibanserin induced a dose-dependent increase in solicitations on days 15 and 22 but not on the other days. There were no other effects of sexual motivation such as ear wiggles or hops and darts. Similarly, there was no effect on lordosis behavior.

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects:

In mice, flibanserin administration at an oral dose of 64 mg/kg resulted in a significant reduction in locomotor activity and hypothermia. Four of eight mice exhibited hindlimb abduction, one exhibited flaccid abdominal tone, two had reduced grip strength and one showed grasping in the traction test. After 128 mg/kg flibanserin, a complete inhibition of locomotor activity, an increase in hot plate reaction time and hypothermia were observed. All treated animals had marked hindlimb abduction and head weaving. One mouse showed straub-tail (erect tail often seen with opiate treatment), three had ptosis, six had reduced grip strength, six exhibited grasping in the traction test and two mice were cataleptic.

Cardiovascular effects:

Arterial blood pressure, heart rate and ECG (lead II) were measured in two male and two female Beagles which had previously been chronically implanted with telemetry transmitters. Following a predose recording for 30 minutes, the animals were orally dosed with 3, 10 or 30 mg/kg flibanserin or vehicle control.

Flibanserin treatment induced mild but statistically significant elevations in mean arterial blood pressure at the high dose. Time to peak effect was approximately 1-2 hrs after dosing and these effects had abated after about 5 hrs.

Mean heart rate was mildly elevated approximately 3 hrs after dosing at 30 mg/kg.

Flibanserin produced no adverse changes to the ECG rhythm or waveform morphology at any dose. There were treatment-related changes in the RR, PR and uncorrected QT interval which would be anticipated from mild elevation of heart rate at the high dose. There was no evidence of QT prolongation at doses (3, 10 and 30 mg/kg) that produced geometric overall mean plasma concentrations at one hour of 250, 549 and 1190 ng/ml, respectively.

Maximum changes in arterial pressure in comparison with mean pre-dose values and the approximate times of peak effect

Treatment & dose (mg/kg)	Maximum increase from pre-dose values at time measured, for:					
	Systolic (mmHg)		Diastolic (mmHg)		Mean (mmHg)	
	Pre*	Change	Pre*	Change	Pre*	Change
Vehicle	137	+14 (1 hr)	87	+15 (1 hr)	104	+15 (1 hr)
Flibanserin (3 mg/kg)	139	+17 (3.17 hr)	91	+14 (4.17 hr)	107	+14 (3.17 hr)
Flibanserin (10 mg/kg)	138	+23 (1 hr)	89	+18 (1 hr)	105	+20 (1 hr)
Flibanserin (30 mg/kg)	134	+27 (1.17 hr)	87	+24 (2 hr)	103	+26 (2 hr)

Flibanserin was shown to block hERG-mediated potassium current in HEK293 cells with an IC₅₀ of 1.18 µM.

Fraction of hERG current (I/I₀) at four different concentrations of flibanserin

Concentration	individual experiment			mean	SD
0.1 µM	0.90	0.87	0.93	0.90	0.03
0.3 µM	0.61	0.73	0.64	0.66	0.06
1.0 µM	0.46	0.44	0.58	0.49	0.08
3.0 µM	0.37	0.31	0.27	0.32	0.05

Flibanserin has no effect on the action potential configuration in guinea pig papillary muscles at concentrations up to 10 µM. Increasing the concentration up to 30 µM produced a shortening of the action potential.

Pulmonary effects:

Flibanserin was investigated for effects on respiratory rate, tidal volume and minute volume following a single oral administration to rats at doses of 20, 100 and 250 mg/kg.

The low dose of 20 mg/kg had no effect on any parameter. At 100 mg/kg and above, a moderately delayed decrease in respiratory rate and moderate to marked delay of the normal decrease in tidal volume were observed.

Renal effects: not done

Gastrointestinal effects: not done

Abuse liability:

Effects of flibanserin and amphetamine in the self-administration paradigm in male Wistar rats

Flibanserin was compared to amphetamine and saline in the self-administration paradigm in male rats. Rats were trained to lever-press in the operant chambers with sucrose pellets as reinforcement, on a fixed ratio (FR)-one schedule of reinforcement.

Operant chambers were equipped with two levers – one designated active, the other inactive. Drug delivery was accomplished via a jugular vein catheter through an injector mounted on a swivel-arm apparatus, which was connected to a syringe pump delivering a 10 uL drug infusion upon active lever responding. Each drug delivery was followed by a 20 second time-out period in which lever pressing produced no consequence.

During the acquisition/maintenance phase, all animals were trained to lever press for infusions of their assigned drug (amphetamine, flibanserin or saline) on an FR-1 schedule of reinforcement to establish self-administration behavior.

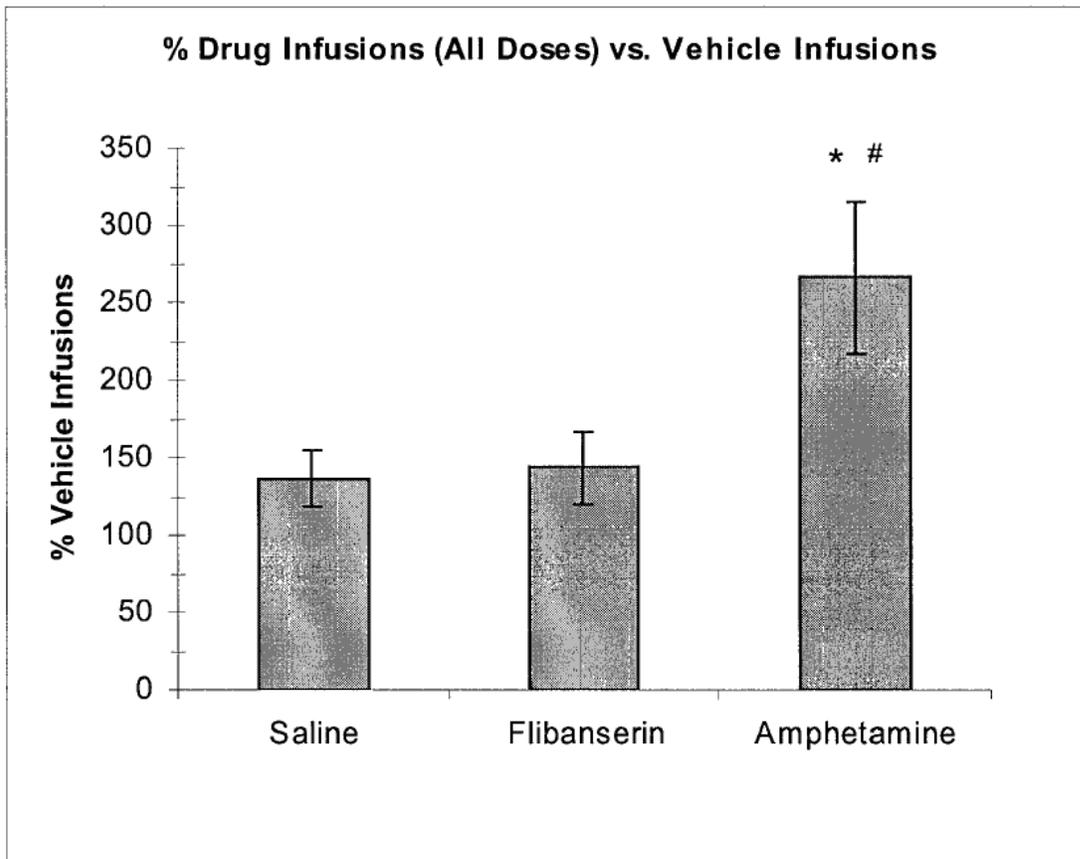
Flibanserin was dissolved in 25% polyethylene glycol and administered at doses of 0, 25, 50 and 100 ug/infusion. D-amphetamine was given at doses of 0, 5, 10 and 20 ug/infusion.

Dosing Schedule for Dose-Response Self-Administration Phase

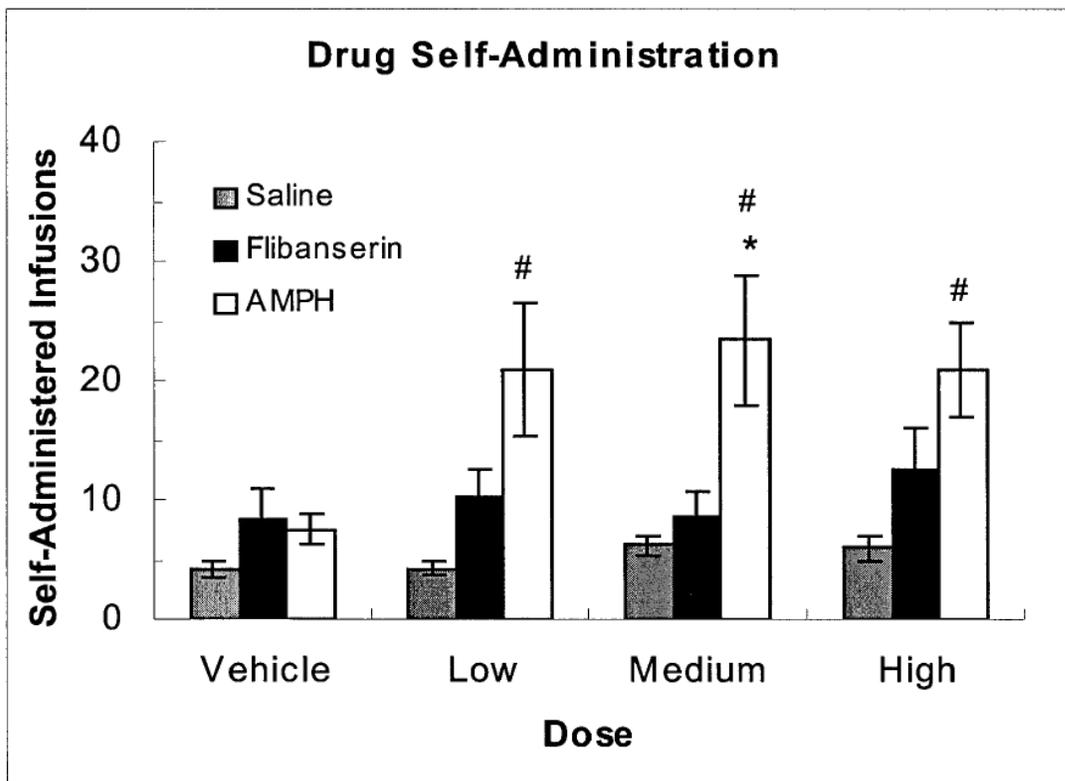
Dose-Response Test Days	Amphetamine Group	Flibanserin Group	Saline Group
Day 1-4	0.5 mg/mL (5 µg/infusion)	2.5 mg/mL (25 µg/infusion)	0.9% saline
Day 5-8	1 mg/mL (10 µg/infusion)	5.0 mg/mL (50 µg/infusion)	0.9% saline
Day 9-12	2 mg/mL (20 µg/infusion)	10 mg/mL (100 µg/infusion)	0.9% saline
Day 13-15	0 mg/mL (0 µg/infusion)	0 mg/mL (0 µg/infusion)	0.9% saline

- The total duration of the Dose-Response Phase was 15 days.
- Testing of the vehicle (0 µg/infusion) was limited to 3 days, rather than 4, to reduce the likelihood of extinction of self-administration behaviour.
- Animals responded on an FR-1 schedule of reinforcement.
- One priming infusion of the appropriate drug commenced all sessions
- Infusion volume for all reinforcers was 10 µL, with a 20 second time-out (during which lever pressing produced no consequence) following each infusion

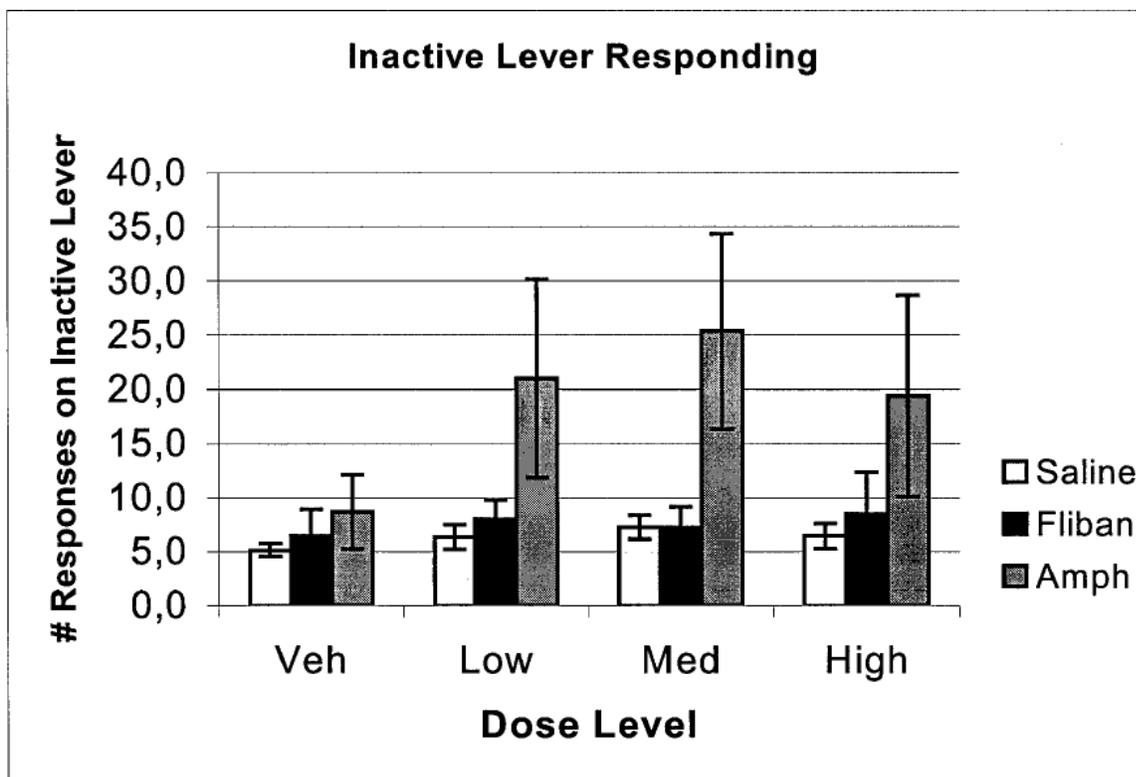
Sample sizes were amphetamine n=6, flibanserin n=5 and saline n=4.



Bars represent the mean (\pm SEM) number of infusions delivered for each dose group, expressed as a percentage of the mean number of vehicle administrations. Flibanserin did not differ significantly from saline.



Mean \pm SEM of number of self-administered infusions of each test drug at each dose levels. None of the test doses of flibanserin differed significantly from saline.



Relative to vehicle, animals had a significantly higher number of self-administered infusions of amphetamine than flibanserin or saline in the first days of the experiment. However, amphetamine treated rats had a higher number of inactive lever pulls than controls, indicating that amphetamine increased overall activity such that the self administration of amphetamine may be a response to hyperactivity and not a signal of self-reward.

All data are highly variable and amphetamine showed an overall tendency to be self-administered, the results with flibanserin seem to indicate that this compound may not be self-administered, even if no definitive conclusions can be drawn due to the small sample size and the failure of the positive control to clearly elicit self-administration behavior.

2.6.2.5 Pharmacodynamic drug interactions

Flibanserin was active in several animal models sensitive to antidepressants and anxiolytics but it did not elicit depression- or anxiety-like behavior. Flibanserin had antinociceptive activity in two models of acute pain which was antagonized by naloxone and potentiated by morphine. Flibanserin has a low affinity for opioid receptors so the action may be indirect. Flibanserin did not impair learning and memory in a water maze test in rats.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

In male and female rats, absorption was relatively fast after oral administration of a suspension with a Tmax of approximately 1 h. Absolute bioavailability (F) was 35% in males and 65% in females despite almost complete absorption suggesting a moderate first pass effect. After IV administration the clearance (CL) was 27 ml/min/kg in males and 18 ml/min/kg in females indicating a fast clearance of parent drug. Plasma $t_{1/2}$ of parent was approximately 1-2 hrs at steady state. In contrast, the $t_{1/2}$ for radioactivity in plasma was 66 h after oral dosing indicating the presence of long-lived metabolite(s). In humans, the $t_{1/2}$ is 10-11 hrs for parent drug and 66 hrs for radioactivity.

In a pilot study in the cynomolgus monkey, drug bioavailability was only about 4% resulting in low blood levels and the decision not to use cynomolgus monkeys for toxicity studies. The $AUC_{0-\infty}$ for flibanserin in monkeys following oral administration of 3 mg/kg of flibanserin was 100 ng.h/ml compared to 2100 ng.h/ml in dogs following a similar dose.

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Absorption in humans

Pharmacokinetics of flibanserin in healthy women following a single dose. Study no. 511.97
U07-1869

Table 11.5.2.2: 1 Comparison of geometric mean (gCV in %) key pharmacokinetic parameters of Flibanserin by treatment

Flibanserin		1 x 25 mg (F1_25) (N=22)	2 x 25 mg (F2_25) (N=22)	1 x 50 mg (F1_50) (N=22)	2 x 50 mg (F2_50) (N=22)	1 x 100 mg (F1_100) (N=20)
AUC _{0-∞}	[ng·h/mL]	515 (44.9)	1140 (38.6)	1140 (39.2)	2250 (34.2)	2320 (32.8)
AUC _{0-∞,norm}	[ng·h/mL/mg]	20.6 (44.9)	22.8 (38.6)	22.8 (39.2)	22.5 (34.2)	23.2 (32.8)
%AUC _{tz-∞}	[%]	5.74 (42.5)	3.36 (67.5)	3.58 (60.3)	2.78 (63.5)	2.48 (59.6)
AUC ₀₋₁₂	[ng·h/mL]	403 (39.4)	831 (32.9)	827 (31.5)	1610 (29.6)	1670 (33.7)
AUC _{0-12,norm}	[ng·h/mL/mg]	16.1 (39.4)	16.6 (32.9)	16.5 (31.5)	16.1 (29.6)	16.7 (33.7)
AUC ₀₋₂₄	[ng·h/mL]	472 (41.4)	999 (35.0)	1000 (34.6)	1960 (31.4)	2020 (32.9)
AUC _{0-24,norm}	[ng·h/mL/mg]	18.9 (41.4)	20.0 (35.0)	20.0 (34.6)	19.6 (31.4)	20.2 (32.9)
C _{max}	[ng/mL]	140 (34.8)	282 (35.8)	253 (27.2)	483 (38.6)	540 (36.5)
C _{max,norm}	[ng/mL/mg]	5.59 (34.8)	5.64 (35.8)	5.07 (27.2)	4.83 (38.6)	5.40 (36.5)
t _{max} ¹	[h]	0.750 (0.500-2.03)	0.750 (0.500-1.50)	0.750 (0.500-2.00)	0.750 (0.500-2.00)	0.750 (0.500-3.00)
t _{1/2}	[h]	8.22 (24.5)	9.95 (27.4)	9.77 (25.7)	10.1 (21.0)	10.5 (16.1)

¹ median and range

The AUC_{0-∞} for flibanserin in women taking a single dose of 100 mg was 2320 ng.h/ml, C_{max} was 540 ug/ml and the T_{1/2} was 10.5 hrs.

Human pharmacokinetics in patients (single dose). Study no. 511.105 U07-1871

Single dose	50 mg q.d. (N=30)		100 mg q.d. (N=28)		25 mg b.i.d. (N=33)		50 mg b.i.d. (N=32)	
	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]
AUC₀₋₁₂ [ng·h/mL]	685	36.4	1150	52.4	391	50.5	745	45.9
AUC_{0-12,norm} [ng·h/mL/mg]	13.7	36.4	11.5	52.4	15.7	50.5	14.9	45.9
AUC₀₋₂₄ [ng·h/mL]	817	39:4	1420	54.7	---	---	---	---
AUC_{0-24,norm} [ng·h/mL/mg]	16.3	39:4	14.2	54.7	---	---	---	---
AUC_{0-tz} [ng·h/mL]	816	39.3	1420	54.6	391	50.5	744	45.8
AUC_{0-∞} [ng·h/mL]	904	43.5	1630	54.6	474	53.2	919	52.0
AUC_{0-∞,norm} [ng·h/mL/mg]	18.1	43.5	16.3	54.6	19.0	53.2	18.4	52.0
%AUC_{tz-∞} [%]	8.34	56.5	11.0	59.8	16.4	36.8	17.3	42.5
C_{max} [ng/mL]	217	40.8	336	50.7	136	41.9	250	40.0
C_{max,norm} [ng/mL/mg]	4.33	40.8	3.36	50.7	5.45	41.9	4.99	40.0
t_{max}¹ [h]	0.875	0.500-3.00	1.00	0.500-3.00	0.750	0.500-3.00	1.00	0.500-3.00
t_{1/2} [h]	8.45 ²	23.32	9.33	27.8	5.93 ³	24.8 ³	6.06	27.0
MRT_{po} [h]	8.46	29.2	10.0	35.4	6.47	24.6	6.79	30.2
CL/F [mL/min]	922	43.5	1020	54.6	878	53.2	907	52.0
V_Z/F [L]	676	33.2	827	62.6	457	58.2	476	43.7

Source Data: Section 15, [Table 6.3: 1](#), [Table 6.2.1: 1](#) and [Table 6.2.1: 15](#)

¹ median and range

² N=31; ³ N=34

AUC_{0-∞} was 1630 ng·h/ml in patients following a single dose of 100 mg

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Human pharmacokinetics in patients (steady state) Study no. 511.105 U07-1871

Steady state	50 mg q.d. (N=30)		100 mg q.d. (N=29)		25 mg b.i.d. (N=33)		50 mg b.i.d. (N=31)	
	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]
AUC_{τ,ss} [ng·h/mL]	991	47.7	2080	46.6	653	60.1	1400	46.9
AUC_{τ,ss,norm} [ng·h/mL/mg]	19.8	47.7	20.8	46.6	26.1	60.1	28.1	46.9
R_{A,AUC} []	1.20 ²	19.3 ²	1.44 ³	63.5 ³	1.70 ⁴	26.4 ⁴	1.84	21.4
C_{max,ss} [ng/mL]	234	41.2	469	42.7	168	50.9	346	34.4
C_{max,ss,norm} [ng/mL/mg]	4.68	41.2	4.69	42.7	6.72	50.9	6.91	34.4
R_{A,Cmax} []	1.09 ²	23.5 ²	1.36 ³	58.0 ³	1.28 ⁴	40.1 ⁴	1.37	31.4
t_{max,ss} ¹ [h]	1.00	0.417-4.00	1.00	0.500-3.00	1.00	0.500-3.00	0.750	0.500-3.00
t_{1/2,ss} [h]	10.1	23.3	11.4	24.3	11.9	25.5	11.6	21.4
MRT_{po,ss} [h]	9.44	34.5	11.3	27.8	11.1	30.2	12.1	29.5
CL/F_{ss} [mL/min]	841	47.7	803	46.6	638	60.1	593	46.9
V_{Z/F,ss} [L]	734	33.9	795	53.9	655	46.8	594	38.4

Source data: Section 15 [Table 6.3.1: 1](#)

¹ median and range

² N=28; ³ N=27; ⁴ N=32

The steady state drug level (AUC_τ) in patients taking 100 mg was 2080 ng.h/ml. This is the figure I have used for all cross-species comparisons of drug exposure.

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Pharmacokinetic parameters for various species following a single dose

Route	Parameter	Unit	Mouse (m)	Rat (m/f)	Rabbit (f)	Dog (m&f)	Cynomolgus Monkey (m&f)	Man ^{a, d} (m)
	Dose	mg/kg	5	5 / 5	3	3.0 p.o. 3.3 i.v.	3	50 mg p.o. 20 mg i.v.
oral	C(max)	µg/mL	0.17	0.42 / 0.61	nd	0.64	0.057	0.25
	t(max) ^b	h	1.0	0.67 / 1.0	nd	0.5	1.0	0.5
	AUC(0-∞)	(µg/mL)·h	1.1	1.1 / 3.0	nd	2.1	0.1	0.75
	MRT	h	7.0	2.7 / 3.8	nd	3.3	1.7	5.9
	t(½)	h	5.5	nd / 2.5	nd	4.4	0.73	6.8
	MAT	h	6.1	1.2 / 1.0	nd	0.17	-0.1	nd
	F(a)	%	nd	119 ^c / 111	>72 ^c	105 ^c	nd	90
F	%	84	35 / 65	nd	44	4.1	33	
i.v.	AUC(0-∞)	(µg/mL)·h	1.3	3.1 / 4.7	3.5	5.1	2.4	0.91
	MRT	h	0.92	1.5 / 2.8	3.0	3.2	1.8	6.3
	t(½)	h	0.75	0.9 / 2.1	3.5	2.9	2.0	7.2
	CL	(mL/min)/kg	62	27 / 18	15	11	21	366 mL/min
	V(ss)	L/kg	3.4	2.3 / 3.0	2.6	2.0	2.3	134 L
<i>in vitro</i>	F(b)	%	98	97 / nd	98	98	98	98

^a body weight = 72 to 86 kg

^b median

^c from urinary data

^d geometric means reported for human except of t(max) (median) and plasma protein binding (arithmetic mean)

2.6.4.4 Distribution

Flibanserin exhibited a high volume of distribution (approximately 2-3 L/kg) in all species examined. Thus, there is a species independent distribution of flibanserin from plasma into tissues.

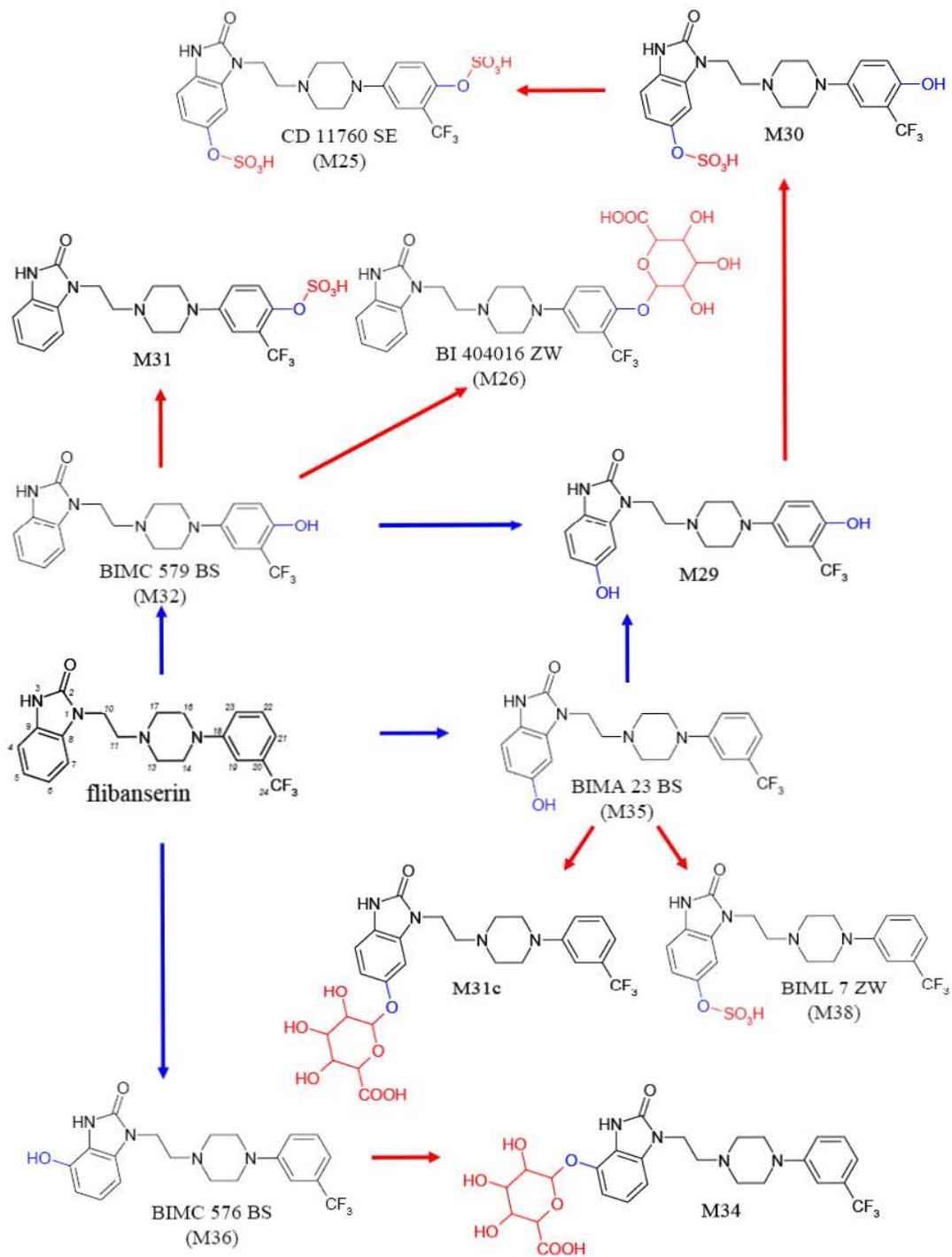
Tissue distribution was investigated in rats by whole body autoradiography. After repeated dosing, radioactivity was present in high concentrations in the liver, kidney, pancreas and brown fat. Pigmented rats showed higher concentrations of radioactivity in skin and eyes in comparison with non-pigmented ones, suggesting melanin binding of flibanserin or its metabolites.

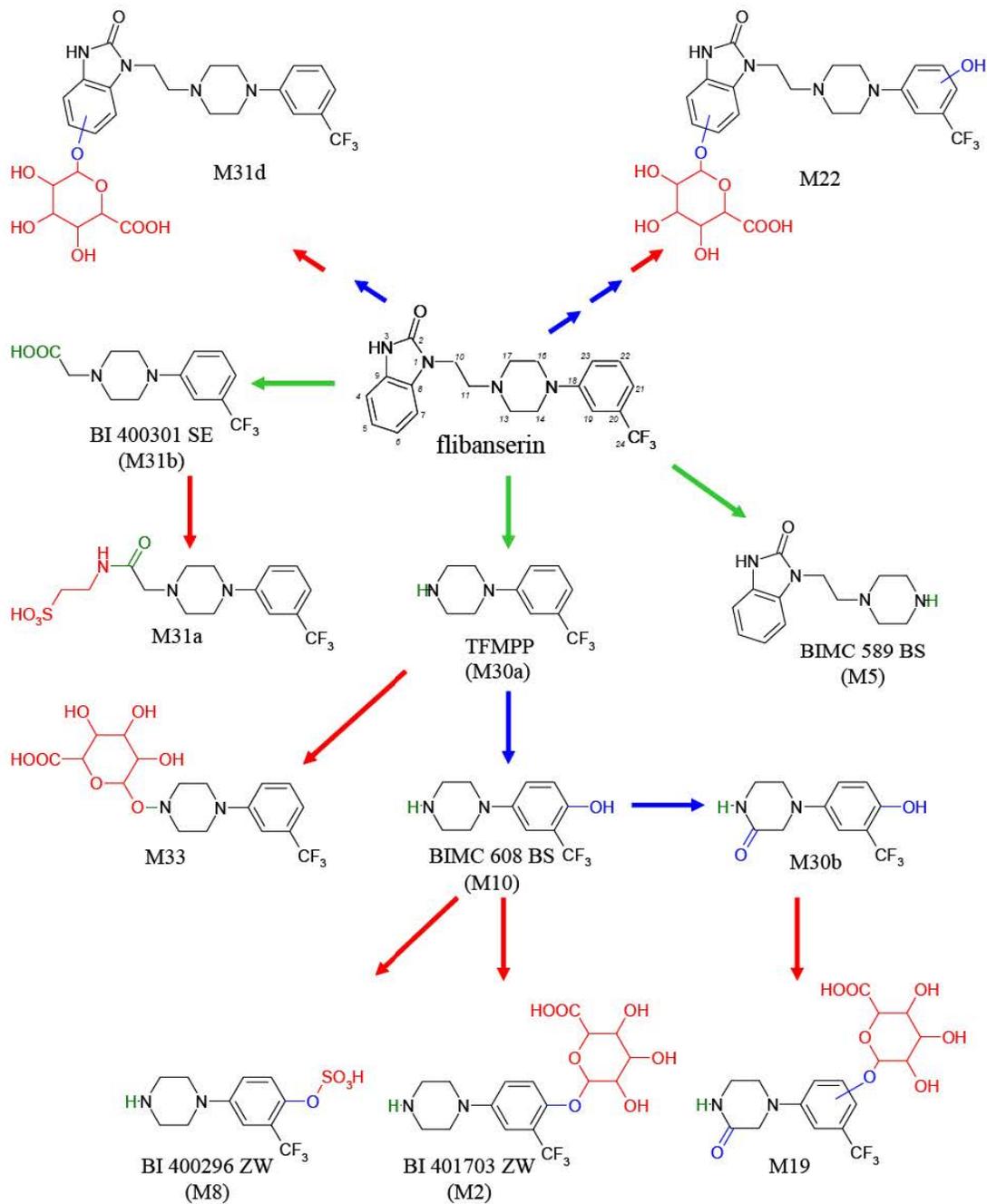
Flibanserin and its active minor metabolite M30a crossed the blood brain barrier to a large extent, whereas the active metabolites M35 and M38 did not.

2.6.4.5 Metabolism

After oral administration, flibanserin is almost completely metabolized in mice, rats, rabbits, dogs and humans to at least 35 metabolites. In humans, flibanserin is extensively metabolized by CYP3A4 and to a lesser extent by CYP2D6.

Flibanserin is mainly metabolized by aromatic hydroxylation to several mono- and di-alcohols of flibanserin. In addition, three cleavage pathways exist.





Metabolite Peak No.	BI-Code	mouse (m)	rat (m)	rabbit (f)	dog (m/f)			man (m)	
		5 mg/kg 2 h	5 mg/kg 2 h	3 mg/kg 2 h	3 mg/kg 2 h (m)	3 mg/kg 2 h (f)	5.5 h (f)	50 mg 1 h	50 mg 4 h
M25/M26 ^a	BI 404016 ZW CD 11760 SE	55	72	826	235	219	682	940	139
M38 ^b	BIML 7 ZW	22	168	29	39	34	81	373	50
M39	flibanserin	237	161	274	1198	1318	230	308	166
M31b	BI 400301 SE	17 ^c	458 ^c	199 ^c	99 ^c	93 ^c	73 ^c	159	69
M8	BI 400296 ZW	nd	nd	nd	nd	nd	nd	138	89
M2	BI 401703 ZW	25	98	307	11	7	nd	23	132
M21		6	nd	nd	nd	nd	15	69	33
M1a		nd ^d	nd ^d	nd ^d	26	39	46	13	39
M30 ^e		6	20	25	nd	13	9	39	7
M1		52	128	110	124	139	66	13	39
M22		9	11	146	7	6	26	36	35
M41		78	43 ^f	144	377 ^f	750 ^f	571 ^f	13	31
M35	BIMA 23 BS	47	15	6	131	162	90	29	27
radioactivity		318	1447	2865	2791	3432	2333	2240	925

^a M25 and M26 could not be separated in plasma samples. Investigations by LC-MS showed that M25 dominated in animals, whereas M26 dominated in man

^b originally reported as M37 or M38, but shown to be only one metabolite (M38 = BIML 7 ZW)

^c in animal samples, M31 could not be separated from M31a, M31b, M31c, M31d

^d might be included in M1

^e in animal samples, M30 could not be separated from M30a (TFMPP) and M30b

^f M41/42

nd = not detected

Table 7.2.1: 3 Overview of geometric mean (gCV) key pharmacokinetic parameters of flibanserin and relevant metabolites after single oral administration of 100 mg flibanserin to healthy female subjects and HSDD patients (Datasets 1 + 3)

100 mg single dose	Flibanserin		BIMA 23 BS		BIML 7 ZW		TFMPP	
	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])
C_{max} (ng/mL)	100	399 (47.8)	51	30.6 (55.0)	51	452 (89.7)	50	5.47 (63.6)
AUC₀₋₁₂ (ng·h/mL)	81	1380 (44.8)	51	153 (45.7)	51	1000 (63.7)	50	36.7 (63.2)
AUC_{0-∞} (ng·h/mL)	100	2050 (47.6)	51	258 (49.3)	51	1460 (62.4)	50	58.6 (75.3)
RAUC_{0-∞,Met} ()	NC	NC	51	0.137 (34.7)	51	0.647 (80.0)	50	0.0530 (74.4)

NC = not calculated

Table 7.2.1: 4 Overview of geometric mean (gCV) key pharmacokinetic parameters of flibanserin and relevant metabolites after multiple oral administration of 50 mg flibanserin twice daily to healthy female subjects and HSDD patients (Datasets 1 + 3)

50 mg b.i.d.	Flibanserin		BIMA 23 BS		BIML 7 ZW		TFMPP	
	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])
$C_{max,ss}$ (ng/mL)	79	298 (41.7)	79	31.1 (37.9)	79	268 (48.4)	79	5.19 (75.8)
$AUC_{\tau,ss}$ (ng·h/mL)	79	1320 (46.6)	79	206 (45.8)	79	897 (34.2)	79	36.7 (82.9)
$C_{pre,ss}$ (ng/mL)	292	48.4 (81.0)	292	10.2 (75.7)	292	26.8 (54.5)	277	1.82 (93.2)
$RAUC_{\tau,ss,Met}$ ()	NC	NC	79	0.150 (42.7)	79	0.544 (53.5)	79	0.0470 (84.0)

NC = not calculated

Based on pharmacological tests including a receptor screen, three metabolites were considered potentially active, M35, M38 and M30_a (TFMPP). Considering the human plasma exposure at therapeutic doses and the brain penetration in rat in addition to the receptor binding data of flibanserin and its metabolites, sponsor concludes that flibanserin in the CNS active substance at therapeutic doses.

The metabolite M8 (BI 400296 ZW) was detected in humans but not in the species used in the toxicology studies. The toxicity of the metabolite was investigated in a 2 week IV dose range finding study, a 4 week GLP study and in in vitro and in vivo genotoxicity assays.

Flibanserin was administered IV to 10 male and 10 female Chbb: (b) (4) rats at daily doses of 0, 0.4, 4.0 and 40 mg/kg for four weeks.

There was no drug related mortality or clinical signs at any dose.

There were no clear drug-related effects on body weight or food consumption, ophthalmology, clinical chemistry and urinalysis. There was a dose related decrease in WBC (mainly lymphocytes) in males only but it was mild and not considered biologically significant (no effect in females).

There were no drug related organ weight changes or test item related microscopic changes.

The NoAEL was considered to be 40 mg/kg corresponding to a mean C_{max} of 64.2 ug/ml in males and 58.9 ug/ml in females. Corresponding mean AUC (0-24h) values were 48.9 and 44.5 ug.h/ml, respectively.

Based on a receptor screen, M8 was considered inactive. M8 was negative in the Ames test at doses up to 5000 ug/plate and the rat micronucleus assay in vivo when given at 40 mg/kg IV for

4 weeks. Plasma protein binding of the metabolite was fairly low (~50%) in both rats and humans.

Human exposure, plasma protein binding, receptor binding and brain/plasma ratios of flibanserin and metabolites

Code	Human C(max)ss at 100 mg q.d. [nM]	PPB man [%]	lowest K _i [nM]	C(max)/lowest K _i	Brain/plasma ^a ratio
Flibanserin	1200	98%	6.59	182	3.71
BIML 7 ZW	1320	97%	207	6.38	0.02
CD 11760 SE ^c	1050 ^b	nd	>10000	<0.105	nd
BI 401703 ZW	282	nd	>10000	<0.028	nd
BI 400296 ZW	281	52%	1760	0.160	nd
BI 400301 SE	159 ^c	nd	>10000	<0.016	nd
BIMA 23 BS	105	95%	7.65	13.7	0.08
BI 404016 ZW	36.6	nd	>10000	<0.004	nd
TFMPP	27.8	73%	32	0.869	2.80

PPB plasma protein binding in man

nd not determined

a based on total radioactivity data in tissues at the end of a 4h i.v. infusion of the respective radiolabelled compound to rats

b C(max) after single oral administration of 100 mg flibanserin to man [U09-1167, Module 5.3.5.3]

c based on C(max) after single oral dosing of 50 mg [¹⁴C]-flibanserin to man determined from pooled 1 h and pooled 4 h plasma samples [U99-1776]

In the above table, it can be seen that the drug concentration (Cmax) divided by the potency (K_i) gives essentially an activity ratio with a higher number denoting greater relative activity. Take into consideration the ability to penetrate the blood brain barrier and it can be seen that flibanserin is basically the only active substance.

Plasma protein binding of flibanserin at a concentration of 0.1 ug/ml was 97% in rat and 98% in mouse, rabbit, dog, monkey and human. Most of the binding was to serum albumin with some binding to α₁-acid glycoprotein.

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2.6.4.6 Excretion

Flibanserin excretion is primarily via feces in mice, rats and dogs with most of the rest through the urine. It is 50/40 feces/urine in humans.

Parameter	Units	Mouse p.o.	Rat p.o.	Rat i.v.	Rabbit p.o.	Dog p.o.	Dog i.v.	Man ^a p.o.	Man ^a i.v.
N/Gender		5m/5f	4m/4f	4m/4f	3f	2m/2f	2m/2f	6m	6m
Dose	mg/kg	5	5	5	3	3	3	50 mg	20 mg
Bile	% of dose	nd	nd/25.3	57.0/39.9	nd	nd	nd	nd	nd
Urine	% of dose	21.6/21.4	19.1/32.5	16.0/18.6	71.9	15.1	15.0	44.1	40.7
Feces	% of dose	70.8/50.0	73.3/70.0	79.1/78.3	19.9	82.4	80.9	50.9	56.0
Recovery	% of dose	92.5/71.4	92.5/105.4	95.2/98.4	92.4	98.7	97.6	95.5	97.0

^a geometric mean nd = not determined

Bile sampling period: male rat (0-5h), female rat (0-6h)

Urine sampling period: mouse (0-48h); male rat (0-96h); female rat, rabbit, dog (0-168h);
man (p.o. 0-264h, i.v. 0-192h)

Feces sampling period: mouse (0-48h); male rat (0-96h); female rat, rabbit, dog (0-168h);
man (p.o. 0-384h, i.v. 0-288h)

2.6.4.7 Pharmacokinetic drug interactions

See biopharmaceutics review

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

See final discussion

2.6.4.10 Tables and figures to include comparative TK summary

See above

02.6.5 PHARMACOKINETICS TABULATED SUMMARY

See above

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

See final discussion

Genetic toxicology:

See final discussion

Carcinogenicity:

See final discussion

Reproductive toxicology:

See final discussion

Special toxicology:

See final discussion

2.6.6.2 Single-dose toxicity

Single dose studies were conducted in mice and rats. Oral flibanserin was given at doses up to 4000 mg/kg in mice and rats. When given IV, the high doses were 80 mg/kg in mice and 90 mg/kg in rats. Additionally, an acute oral toxicity study was conducted in dogs at 50 and 100 mg/kg.

Oral doses resulted in significant mortality at 2000 mg/kg in mice and greater than 4000 mg/kg in rats. After IV administration, significant mortality was seen at 50 mg/kg for mice and 70 mg/kg for rats. Dogs had significant clinical signs after both 50 and 100 mg/kg.

2.6.6.3 Repeat-dose toxicity

Sponsor performed a number of toxicity tests in rats, mice and dogs. In a 13 week toxicity test in mice with a single dose of 1000 mg/kg/day, flibanserin treatment resulted in an increase in liver weight and hepatic lipidosis. Mean plasma flibanserin AUC_{0-24h} values for the mice were 18 ug.h/ml for males and 12 ug.h/ml for females. In a 13 week oral dose ranging study with doses of 20, 100 and 400 mg/kg (raised to 1200 and 2400 mg/kg in drug weeks 6 and 11 due to lack of toxicity), oral administration of flibanserin to CD-1 mice produced hepatocellular hypertrophy in MD and HD animals of both sexes with fatty accumulation.

In a 13 week toxicity study, rats were dosed with 20, 100 and 400 mg/kg which resulted in significant neurological signs at the HD with a reduction in body weight gain at all doses in males only. There was slight but significant decrease in RBC's with an increase in MCH in males. There was an increase in liver and ovary weights of females with reversible peri-acinar hypertrophy in the liver of the MD (males) and HD (males and females) at week 13. Mild reversible fatty changes were seen in livers of HD females.

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In the 13 week rat study, serum prolactin levels were measured

PROLACTIN (ng/ml)				
	Dose mg/kg	MEDIAN	Interquartile Range	NI
CONTROL		52.45	27.05 - 67.41	10
BIMT17 BS	20	65.37	40.76 - 79.52	10
BIMT 17 BS	100	98.27 *	83.42 - 137.43	10
BIMT17 BS	400	174.00 **	142.27 - 275.93	10

* P < 0.05
** P < 0.01

In a 6 month toxicity study in rats at oral doses of 20, 100 and 400 mg/kg flibanserin administration produced hepatic hypertrophy. This change occurred in males at the two higher doses and in females at the highest dose only. In females at the highest dose, there was a mild increase in hepatic lipid content. No other histopathological changes were noted.

Tab. A: Geometric mean C_{max} - and AUC-values and median t_{max} -values (data of both sexes are combined).

Day	1			9			last		
	t_{max} h	C_{max} ng/ml	$AUC_{0-\infty}$ ng/ml*h	$t_{max,ss}$ h	$C_{max,ss}$ ng/ml	AUC_{ss} ng/ml*h	$t_{max,ss}$ h	$C_{max,ss}$ ng/ml	AUC_{ss} ng/ml*h
20	1.5	552	2663	1	520	3321	1	521	3334
100	2	2791	50523	2	1617	17464	2	2321	20662
400	8	7315	- *	2	2755	31712	2	3069	41386

* not calculable due to the late C_{max}

This study showed very little toxicity in rats at exposures up to approximately 20 times the human exposure (2080 ng.h/ml) in women taking 100 mg.

In a 13 week study in 3 male and 3 female beagles per group, flibanserin given orally at doses of 3, 15 and 100 mg/kg/day produced significant clinical signs and lower body weight in males and females of the highest dose group. There was an increase in intraocular tension in the eyes of 3 of 6 dogs treated with 100 mg/kg. No other significant findings were noted.

Study title: 6-month oral toxicity study in the rat

Key study findings: Minimal toxicity

Study no.: Internal study no. I48

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim KG, Ingelheim, Germany.

Date of study initiation: Nov. 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: FIMT 17 BS, batch 9403-P, purity not stated

Methods

Doses: 15, 80, 400 mg/kg/day

Species/strain: Chbb: ^{(b)(4)} strain rats

Number/sex/group or time point (main study): 20/sex/gp

Route, formulation, volume, and infusion rate: oral gavage, flibanserin suspended in 0.5% Methocel-solution, 10 ml/kg.

Satellite groups used for toxicokinetics or recovery: none

Age: 57 days

Weight: 174-258 gg

Sampling times:

Unique study design or methodology (if any): None

Results

Mortality:

Group Sex	1 (control)		2		3		4	
	m	f	m	f	m	f	m	f
Died	1	1	0	1	1	0	2	0
Sacrificed	0	0	0	0	0	1	1	0
Total	1	1	0	1	1	1	3	0
%	5	5	0	5	5	5	15	0

The eight animals that died or were sacrificed all had congestion of the parenchymal organs, pulmonary edema and emphysema, intraorbital hemorrhages (one animal) and indications of gavage error (one animal). The cause of death was not determined.

Clinical signs:

Group Sex	1 (control)		2		3		4	
	m	f	m	f	m	f	m	f
Observation								
Ataxia	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20
Convulsions	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20
Dyspnoea/altered respiration	0/20	0/20	0/20	0/20	1/20	0/20	1/20	0/20
Lateral recumbency	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20
Sternal recumbency	0/20	0/20	0/20	0/20	0/20	0/20	2/20	9/20
Phobia	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Resistance to administration	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Sedation	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Poor condition/ruffled coat	0/20	0/20	0/20	0/20	1/20	0/20	2/20	2/20

Body weights:

Males

Week	Group			
	1	2	3	4
13	163	167 (102)	149 (92)	122 (75)
26	223	215 (97)	193 (86)	145 (65)

Females

Week	Group			
	1	2	3	4
13	68	72 (105)	77 (113)	84 (124)
26	89	94 (105)	100 (113)	105 (118)

() = percent of control body weight

For males, the decrease in body weight gain was statistically significant for the mid and high dose. In females, flibanserin treatment resulted in a significant increase in body weight in mid dose animals at weeks 10 and 11 and a significant increase in body weight in high dose rats from week 5 onward.

Food consumption: Animals of the two higher dosed groups demonstrated a statistically significant tendency to consume more food than the controls.

Ophthalmoscopy: No drug induced lesions could be determined with slit-lamp exams.

Hematology: From week 6 onwards high dose males and females showed slightly decreased RBC counts and hemoglobin values.

Clinical chemistry:

GPT: Females in the mid and high dose had a slight but significant increase in GPT (ALT) values during the entire treatment phase. There was no dose dependency.

AP: There was a slight reduction in enzyme activity in mid and high dose males during the entire treatment.

Triglycerides: Moderate to marked dose dependent decrease in serum triglycerides was seen in the mid and high dose males and females during the entire treatment.

Cholesterol: Slight elevations of serum levels were seen in high dose females during the entire treatment and in mid dose females during weeks 6 and 26 and, as a tendency, in low dose females at the end of the study.

Bilirubin: Minimal reductions in serum values in the high dose animals were within the normal range.

Glucose: Slight decreases in mid and high dose animals of both sexes.

Inorganic phosphate: Mid and high dose females showed a slight, dose dependent increase in serum levels. There was also a small increase in low dose females at the end of the study.

Prolactin: Levels were measured in all animals 2 hours after drug administration in weeks 8 and 23 of the study. In week 15 prolactin was determined 24 hours after dosing. There was a dose dependent increase of serum prolactin 2 hours after dosing in both males and females of the mid and high dose groups in week 8 and week 23. No change was seen after 24 hours in the low and mid dose groups and a slight decrease was seen in the high dose group.

Dose mg/kg	Male Rats	Week 8 2 h p. Appl.	Week 15 24 h p. Appl.	Week 23 2 h p. Appl.
Control	Median NI	65.43 20	66.32 20	93.09 19
15	Median NI	91.01 20	60.86 20	97.50 20
80	Median NI	**134.82 20	39.32 20	**173.98 20
400	Median NI	**198.99 20	**9.94 19	**249.14 17

Dose mg/kg	Female Rats	Week 8 2 h p. Appl.	Week 15 24 h p. Appl.	Week 23 2 h p. Appl.
Control	Median NI	103.86 19	15.24 19	48.63 18
15	Median NI	59.31 20	10.30 20	89.09 19
80	Median NI	132.67 19	10.60 19	**202.08 18
400	Median NI	290.11 20	<4.40 20	**335.22 18

** = significantly different from the control group (p < 0.01)

Urinalysis: There were no biologically relevant differences between control and treated animals.

Gross pathology: No biologically significant findings.

Organ weights (specify organs weighed if not in histopath table):

In males there was an increase in absolute thymus weights in all dosed groups and a significant increase in relative thymus weights in the low and high dose groups only. Group 2, 3 and 4 mean absolute values were 20, 18, and 30% higher than control values.

There was an increase in adrenal weights in the mid and high dose groups of males only. Group 2, 3 and 4 mean absolute values were 2, 14 and 19% higher than control.

In males, there was an increase in the relative liver weights in the high dose group. No change in absolute weight.

In females there was an increase in both absolute and relative liver weights in the mid and high dose groups. Group 2, 3 and 4 mean absolute weights were 9, 17 and 44% higher than control values.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (x), no ()

Only changes were seen in the liver of the high dose group. There was a reduction of the regularly occurring fat deposition with the lobuloperipheral hepatocytes in males which may explain the decrease in relative liver weights in males. In contrast, high dose females demonstrated a slight to mild hypertrophy of the lobulocentrally located hepatocytes which probably correlates with the increased liver weight.

Liver changes	G1		G2		G3		G4	
	0 mg/kg		15 mg/kg		80 mg/kg		400 mg/kg	
	M	F	M	F	M	F	M	F
Fatty change	18/20	20/20	20/20	8/20	19/20	14/20	4/20	17/20
Hepatocellular hypertrophy	0/20	0/20	0/20	0/20	0/20	0/20	0/20	20/20

There was no increase in myocardial fat deposition in rats at any dose.

Toxicokinetics

Geometric mean; males and females combined; n = 7-10.

Week	2			26		
	T _{max} h	C _{max} ng/mL	AUC _{ss} ng/mL*h	T _{max} h	C _{max} ng/mL	AUC _{ss} ng/mL*h
15	1.0	722	3820	1.1	791	3840
80	1.4	2020	17700	1.1	2690	22300
400	2.8	6040	61800	1.3	7100	59800

Exposures are 10 fold and 29 fold the human exposure (2080 ng.h/ml at 100 mg) at the mid and high doses, respectively.

Study title: **52-week oral (gavage) toxicity study in beagle dogs with interim autopsy after 26 weeks.**

Key study findings: Severe neurological signs in HD with clear animal suffering, mild toxicities in LD and MD dogs

Study no.: U99-1576; internal study no. I49

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim KG, Ingelheim, Germany.

Date of study initiation: Dec. 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403-P & 9605-P, purity not stated.

Methods

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

Species/strain: Beagles

Number/sex/group or time point (main study): 3/sex/gp in the 6 month part of the study and 4/sex/gp in the 12 month part.

Route, formulation, volume, and infusion rate: oral gavage, flibanserin suspended in 0.5% Methocel-solution.

Satellite groups used for toxicokinetics or recovery: none

Age: 6-18 months

Weight: 8.9 – 14.5 kg

Unique study design or methodology (if any): None

Results

Mortality: One HD group 4 dog, sacrificed moribund on week 7.

Clinical signs: Salivation and ataxia in group 3 (15 mg/kg) on isolated occasions. In group 4 dogs salivation occurred after dose administration, the animals became recumbent, vocalized and were ataxic or convulsive from about 1 hour after drug administration until beyond 6 hours after administration. Dogs also exhibited dry eyes and various behavioral traits such as biting the metal drinking bowls or feeding racks, standing with head forward, and forcing head or limbs between the bars of the door or the enclosure. Symptoms were seen from 2 hours after administration onwards beyond 6 hours. In the course of the study the number of tremor and vocalization episodes decreased and the duration of vocalization became shorter.

Extreme aggression was seen in one high dose dog that also suffered from salivation, vocalization, sedation, ataxia, convulsions, refusal to eat, no defecation, and poor general condition. The dog was sacrificed on humane grounds.

Isolated cases of reduced locomotion, absence of feces or abnormal feces and miosis were also seen in the high dose group.

There was an increase in neurological signs (compulsive gnawing) with dose.

The continued treatment of the high dose dogs seems to me to be clear animal abuse. I'm not sure why the animal welfare monitor did not demand lowering the dose for these animals.

Body weights: Significant decrease BW gain in group 2 and 3 males but not in females. Group 4 males and females also gained less weight than control dogs but the difference for females was not significant.

Food consumption: No drug related effects.

Ophthalmoscopy: One MD dog and 7 HD dogs demonstrated dose-related ophthalmological and/or histopathological changes of the eyes. They were characterized by discrete, focal, smoky opacity or by focal facet (focal thickening of the cornea epithelium) in the central or peripheral region of the cornea. There was no increase in intensity with time and two of the opacities disappeared with time and did not reappear.

Similar ocular findings were seen in the 13 week dog toxicity studies where 2/12 dogs at 100 mg/kg and 4/6 dogs at 100/75 mg/kg were affected.

EKG: There was an increase in heart rate in both male and female dogs of all treated groups at different time points during the study (mostly 3 hours after drug administration). From 18-58% in the low dose group to a mean maximum increase of 72 beats/minute (120%) in the male HD group. In general, males seemed more affected than females.

PQ-, QRS-, QT-intervals were within the normal historical range but there were increases in blood pressure in males and females of groups 2 and 3, 3 and 24 hours after administration in week 26 and 24 hours after administration in weeks 39 and 52 and in females of group 4, 24 hrs after administration in week 52. There was no dose response and the relationship to treatment is questionable.

Hematology: Individually reduced RBC counts and hemoglobin values in treated dogs without a dose response. Platelet counts were increased slightly, biologically and statistically significantly in group 4 dogs at all weeks tested. Thrombin and partial thrombin times were slightly reduced in these animals.

There were no differences in bone marrow smears from group 4 and control animals.

Clinical chemistry: Alkaline phosphatase values were slightly but significantly increased in group 4 dogs during the entire treatment phase.

Prolactin levels for groups 3 and 4 were elevated both for males and females. The increase was slight and not consistent, being less pronounced in males but not in females during later stages of the study.

There was a slight increase in cortisol values in both sexes of group 4 during the course of the study.

There was a slight increase in the α_2 -globulin fraction in group 4 animals which was accompanied by a corresponding change in the albumin-globulin ratio.

Urinalysis: There were no biologically relevant differences between control and treated animals.

Gross pathology: there was an increase in findings in treated groups but no particular lesion seemed significant. 2/14 high dose dogs had pale livers, two had some kidney changes (pale areas, abnormal brown yellowish color) compared to no liver or kidney effects in control dogs.

Organ weights (specify organs weighed if not in histopath table):

There were no significant changes in organ weights in treated animals compared to controls.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (x), no ()

Liver, fatty change in males after 26 and 52 weeks

Group:	Incidence	Males 26WK	Incidence	Males 52WK
Dose (mg/kg/d)	26WK	Animal No./Fatty Changes	52WK	Animal No./Fatty Changes
<i>controls:</i>	2/3	102, mild	4/4	104, mild
0		103, mild		105, very mild
				106, mild
				107, very mild
<i>low dose:</i>	0/3	none	4/4	204, very mild
3				205, moderate*
				206, mild to moderate
				207, very mild
<i>mid dose:</i>	3/3	301, mild	4/4	304, mild to moderate
15		302, moderate		305, moderate*
		303, moderate to marked*		306, mild to moderate
				307, mild
<i>high dose:</i>	2/2	401, moderate to marked*	4/4	403, moderate to marked*
75		402, moderate		404, mild
		(405 premature decedent)		406, mild to moderate*
				407, mild to moderate

Liver, fatty change in females after 26 and 52 weeks

Group:	Incidence	Females 26WK	Incidence	Females 52WK
Dose (mg/kg/d)	26WK	Animal No./Fatty Changes	52WK	Animal No./Fatty Changes
<i>controls:</i>	1/3	152, mild	1/4	157, very mild
0				
<i>low dose:</i>	1/3	253, moderate*	1/4	254, mild
3				
<i>mid dose:</i>	3/3	351, mild to moderate	2/4	355, mild
15		352, moderate*		356, mild to moderate*
		353, moderate		
<i>high dose:</i>	2/3	451, moderate	4/4	454, mild
75		452, mild		455, very mild
				456, very mild
				457, very mild

* Macroscopical findings consisted in some kind of discoloration of the liver.

Frequency and intensity of fatty liver seemed dose and possibly duration related. It was characterized as mild to marked with an increase in intensity in the mid and high dose groups. The fat droplets were present in the periportal and midzonal areas of the liver and did not coalesce into larger droplets.

Hepatocellular fatty change has also been observed in a 13 week toxicity study in rats which may have been an adaptive change perhaps due to the induction of metabolizing enzymes. Flibanserin is not an enzyme inducer in dogs.

Heart, fatty changes in males and females after 26 and 52 weeks

Dose Group	Weeks	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
control	26	0/3	no	0/3	no
control	52	0/4	no	0/4	no
low	26	0/3	no	0/3	no
low	52	0/4	no	0/4	no
mid	26	1/3	303, very mild	0/3	no
mid	52	1/4	306, mild	2/4	355, mild 356, moderate
high	26	1/3	402, mild	0/3	no
high	52	3/4	403, mild to moderate 404, very mild 407, mild to moderate	3/4	454, moderate 456, moderate to marked 457, moderate

There was a fine, focal or multifocal lipid droplet accumulation in the myocardium of the left ventricle. The frequency and severity seemed dose and duration dependent. Sponsor attributed the fatty disposition to a relative hypoxia due to the increased heart rate and/or the reduced physical condition (I assume due to the CNS toxicity). The no effect dose for fat deposition in the heart is 3 mg/kg or approximately 0.6 times the exposure of women taking 100 mg.

APPEARS THIS WAY ON ORIGINAL

Trachea, degeneration of mucous membrane in males and females after 26 and 52 weeks

Study Group (mg/kg/d)	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
controls	0/4	no	0/4	no
3	0/4	no	0/4	no
15	3/4	304, moderate 305, mild 307, moderate	1/4	355, mild
75	4/4	403, mild 404, moderate 406, moderate 407, mild	3/4	454, moderate 455, moderate 456, moderate

The tracheal degeneration consisted of decreased size and number of goblet cells, mild and diffuse granulocytic infiltration in the superficial mucosal layers and in the lamina submucosa, loss of cilia and regenerative changes of the respiratory epithelium. The changes were drug and dose dependent without gender differences. The mechanism and relevance for humans for this effect is unknown.

Thymus, accelerated involution in males and females after 52 weeks.

Study Group (mg/kg/d)	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
controls	0/4	no	0/3	no
3	1/4	207, moderate	2/3	256, mild 257, moderate
15	3/4	304, moderate to marked 305, moderate 307, moderate to marked	1/4	357, moderate
75	3/4	403, mild 406, moderate to marked 407, moderate to marked	2/4	454, mild 455, moderate

There was thymic involution in animals of all groups including the controls. It was accelerated in animals of the dosed groups and considered by the sponsor to be a nonspecific stress response.

Histology conclusion: Administration of flibanserin for one year to dogs resulted in a dose and time dependent, mild increase of hepatocellular fatty change in all dose groups.

A dose-related focal or multi-focal, very mild to marked myocardial fatty change in the left ventricle in the mid and high dose groups.

A dose-related degeneration of the mucous membrane of the trachea in the mid and high dose groups.

A mild to marked, accelerated thymus involution in all dosed groups.

A dose-related, but not time dependent, focal thickening of the corneal epithelium.

APPEARS THIS WAY ON ORIGINAL

Toxicokinetics:

Geometric C_{max}; AUC_{0-24h} and median T_{max} values in the male dogs (n = 3-7)

Gender	Week	Dose	[mg/kg]	3	15	75
male	1	T _{max}	[h]	1	1	6
		C _{max}	[µg/mL]	0.5	2.9	9.0
		AUC	[µg/mL·h]	1.3	12.1	114.0
	14	T _{max}	[h]	1	1	2
		C _{max}	[µg/mL]	0.4	3.2	10.2
		AUC	[µg/mL·h]	1.0	11.9	77.3
	25	T _{max}	[h]	1	1	3
		C _{max}	[µg/mL]	0.5	3.5	13.2
		AUC	[µg/mL·h]	1.5	14.9	135.0
	51	T _{max}	[h]	1	1	2.5
		C _{max}	[µg/mL]	0.6	3.3	13.2
		AUC	[µg/mL·h]	1.2	15.9	108.0
female	1	T _{max}	[h]	1	2	10
		C _{max}	[µg/mL]	0.3	0.8	5.9
		AUC	[µg/mL·h]	0.8	4.8	72.0
	14	T _{max}	[h]	1	2	3
		C _{max}	[µg/mL]	0.4	1.6	9.7
		AUC	[µg/mL·h]	1.1	6.6	73.9
	25	T _{max}	[h]	1	1	3
		C _{max}	[µg/mL]	0.5	2.8	14.1
		AUC	[µg/mL·h]	1.3	13.6	140.0
	51	T _{max}	[h]	1	2	2
		C _{max}	[µg/mL]	0.6	2.9	15.1
		AUC	[µg/mL·h]	1.9	13.7	161.0

Plasma AUC is nonlinear for dose and seems to be more pronounced at weeks 25 and 51. Human AUC_{0-24h} for women taking 100 mg is 2080 ng.h/ml.

Aside from the moderate increase in heart rate, the NoAEL for this study was 3 mg/kg.

2.6.6.4 Genetic toxicology

Study title: **Study of the capacity of the test article to induce gene mutations in strains of Salmonella typhimurium**

Key findings: Study was negative

Study no.: (b) (4) no. 910474; U93-0573

Volume #, and page #:

Conducting laboratory and location: (b) (4)

Date of study initiation: Nov. 1991

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 CL, lot and purity not stated

Methods

Strains/species/cell line: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100

Doses used in definitive study: 5, 15, 50, 150, 500 ug/plate

Basis of dose selection: Initial study used doses of 1, 10, 100, 1000 and 5000 ug/plate. Doses above 100 ug/plate were toxic

Negative controls: DMSO 0.1 ml/plate

Positive controls: Hydrazine sulfate 500 ug/plate, 9-aminoacridine 40 ug/plate, 2-nitrofluorene 2.5 ug/plate, doxorubicine 4 ug/plate, 2-aminofluorene 5 ug/plate

Incubation and sampling times: 72 h incubation

Results

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

Plates run in triplicate in two experiments. If the number of colonies reverted was at least double the number of spontaneously reverted colonies the test was considered positive.

Study outcome:

Toxicity was apparent from 100-300 ug/plate without S9 and 500-800 ug/plate with S9.

No mutagenic effect at concentrations up to 500 ug/plate with or without S9

Positive controls were active in all experiments

Study title: **Mutagenicity study in the V79 (HPRT) forward mutation assay**

Key findings: Study was negative

Study no.: U95-2191

Volume #, and page #:

Conducting laboratory and location: Dept Exp Path Tox, Dr. (b)(4) Gmbh, Boehringer Ingelheim

Date of study initiation: March, 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9402-P, purity not stated

Methods

Strains/species/cell line: V79 Chinese hamster cell line

Doses used in definitive study: 10, 20, 30, 40, 50 ug/ml

Basis of dose selection: Dose range finding study from 5-500 ug/ml without metabolic activation. Concentrations above 50 ug/ml had no cells surviving. In the activation system, cellular toxicity was evident (59.6% survival) at a dose concentration of 40 ug/ml.

Negative controls: DMSO

Positive controls: ethylmethane sulfonate (EMS); methyl-N-nitro-nitrosoguanidine (MNNG); 7,12-dimethylbenz(a)anthracene (DMBA)

Incubation and sampling times: Cells were treated with the control and test article for 3 hrs. Experiments consisted of one negative control (DMSO), one positive control (EMS, MNNG or DMBA) and four compound-treated cultures. In the activation system the S9 was added during the incubation procedure.

Results

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

Positive response is defined as a reproducible and concentration dependent increase in mutant frequency for a minimum of two successive concentrations.

COMPOUND ($\mu\text{g/ml}$)	% SURVIVORS		HPRT-MUTANTS/ 1 MIO. SURVIVORS	
	EXP. 1	EXP. 2	EXP. 1	EXP. 2
CONTROLS NEGATIVE DMSO	100.0	100.0	7.9	1.0
POSITIVE EMS 500 MNNG 0.2	99.9 -	- 43.8	161.6* -	- 155.0*
BIMT 17 BS				
10	96.4	96.0	6.9	0.4
20	86.4	94.6	7.1	0.6
30	53.4	57.8	7.5	5.0
35	-	0.6	-	-
40	0	-	-	-

COMPOUND ($\mu\text{g/ml}$)	% SURVIVORS		HPRT-MUTANTS/ 1 MIO. SURVIVORS	
	EXP. 1	EXP. 2	EXP. 1	EXP. 2
CONTROLS NEGATIVE DMSO	100.0	100.0	2.5	4.0
POSITIVE DMBA 5	32.8	84.1	117.1*	170.9*
BIMT 17 BS				
10	91.6	88.0	5.1	8.8
20	104.0	83.5	0	5.6
30	87.3	-	3.6	-
40	59.6	69.8	1.7	12.4*
50	-	14.1	-	0

P: PRECIPITATION

HISTORICAL DATA FOR MUTANT FREQUENCY: MEAN 6.0/1 MIO. (RANGE 0-35.2)

* SIGNIFICANT INCREASE ($P \leq 5\%$)

Study outcome: Study was negative. The positive at 40 $\mu\text{g/ml}$ in the activated experiment was considered not treatment related mainly because it fell within the historical control range. Sponsor also stated that there was no consistent, dose-related or reproducible increase in the mutant frequency compared to vehicle control. True, but since the next highest dose was toxic with very low % survival, it would be hard to detect a dose-response. Nevertheless, the dose is in the toxic range (% survivors are decreased) and the result is within historical control range so I would agree with sponsor on their interpretation.

Study title: **Mutagenicity study for chromosomal aberrations in human lymphocytes in vitro**

Key findings: Study was positive

Study no.:

Volume #, and page #:

Conducting laboratory and location: Dr. (b) (4), Boehringer Ingelheim, Biberach an der Riss, Germany

Date of study initiation: January, 1997

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9507-P, purity not stated

Methods

Strains/species/cell line: Human peripheral blood lymphocytes

Doses used in definitive study: Nonactivation: 10, 20, 40, 50 ug/ml

Activation trial 1: 10, 100, 150 (175) ug/ml

Activation trial 2: 10, 100, 150 ug/ml

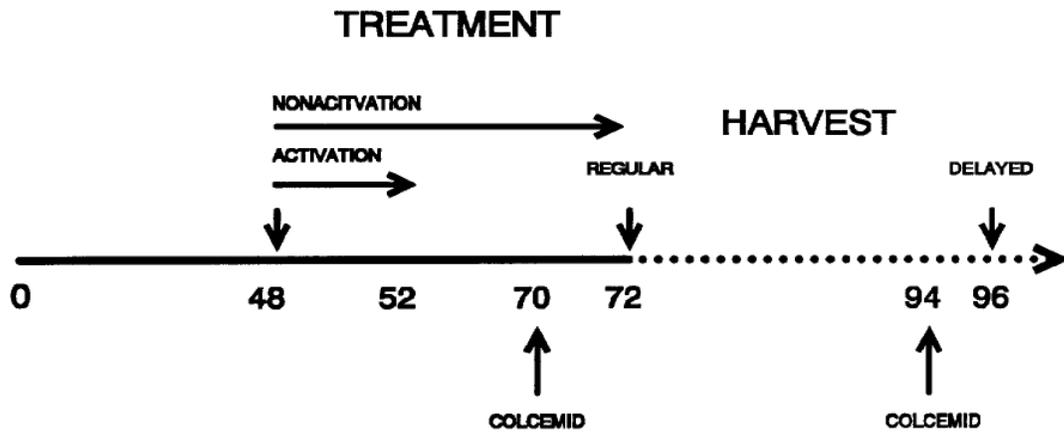
Basis of dose selection: Dose range finding study showed a marked toxicity at a dose concentration range of 50-500 ug/ml (without S9) and 100-500 ug/ml (with S9). Based on this the doses between 10 and 200 ug/ml were selected.

Negative controls: DMSO

Positive controls: Cyclophosphamide (CP), Adriamycin (ADR)

Incubation and sampling times:

Test	Culture Initiation	Treatment	Colcemid	Harvesting	
				Regular	Delayed
- S9	0	48 - 72	70 (94)	72	96
+ S9	0	48 - 52	70 (94)	72	96



Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
Positive study is defined as a reproducible and concentration dependent increase in aberration frequency in the exposed cultures.

APPEARS THIS WAY ON ORIGINAL

Structural chromosome aberrations (% aberrant cells excluding gaps)

Metabolic activation: without

COMPOUND ($\mu\text{g/ml}$)	EXPERIMENT 1	EXPERIMENT 2	
	72. hr	72 hr	96 hr
CONTROLS			
NEGATIVE DMSO	1.0 NT	1.0 NT	1.0 NT
POSITIVE ADR 0.05	23.0 * LT	8.0 * NT	16.0 * NT
BIMT 17 BS			
10	0 NT	2.0 NT	ND
20	3.0 LT	1.0 LT	ND
40	0 ¹ HT	(0 ²) HT	ND
50	0 ¹ HT	(0 ²) HT	1.0 NT

ND: not done

1: <100 metaphases scored (toxic)

2: < 50 metaphases scored (toxic)

NT: no toxicity (MI $75 \geq 100$ %)

LT: low toxicity (MI 50-74 %)

HT: high toxicity (MI ≤ 49 %)

*: Significantly different from the vehicle control ($P \leq 5\%$)

Historical negative control values (% aberrant cells excl. gaps): 0.5 (range 0-4)

APPEARS THIS WAY ON ORIGINAL

Structural chromosome aberrations (% aberrant cells excluding gaps)

Metabolic activation: rat liver S9

COMPOUND (µg/ml)	EXPERIMENT 1	EXPERIMENT 2	
	72. hr	72 hr	96 hr
CONTROLS			
NEGATIVE DMSO	3.0 NT	1.0 NT	1.0 NT
POSITIVE CP 7	27.0 * NT	27.0 * HT	18.0 * LT
BIMT 17 BS			
10	1.0 NT	0 NT	ND
100	7.0 LT	5.0 NT	ND
150	8.5 ¹ LT	3.8 ¹ LT	4.0 LT
175	N.E. HT	ND	ND

¹: < 100 metaphases scored (toxic)

N.E.: Not evaluable

N.D.: Not done

NT: no toxicity (MI 75≥100 %)

LT: low toxicity (MI 50-74 %)

HT: high toxicity (MI ≤49 %)

*: Significantly different from the vehicle control (P≤5%)

Historical negative control values (% aberrant cells excl. gaps): 0.6 (range 0-3.0)

Study outcome: Study was negative without metabolic activation. Slightly increased aberration frequencies were observed in the activation system without dose dependency. Although the difference between treated and negative controls did not reach statistical significance, individual values were outside the historical control range (0-3%).

The effects were reproducible and partly associated with cell toxicity as indicated by mitotic inhibition or poor metaphase quality. Because of the cellular toxicity, the sponsor suggests that the effect may be due to an indirect mechanism and not the result of a direct DNA mutation. This is total speculation and the study met the sponsor's criteria for a positive test.

Study title: **Rat bone marrow micronucleus test after oral administration**

Key findings: Result was negative

Study no.: MUT 265; U96-2432

Volume #, and page #:

Conducting laboratory and location: Dept Exp Path Tox, Boehringer Ingelheim, Ingelheim, Germany

Date of study initiation: Sept, 1994

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403A, purity 99.9%

Methods

Strains/species/cell line: Chbb: ^{(b) (4)} rats

Doses used in definitive study: 600, 1200, 1800 mg/kg

Basis of dose selection: Maximum tolerated dose using same dosing schedule

Negative controls: 0.5% methocel

Positive controls: Cyclophosphamide

APPEARS THIS WAY ON ORIGINAL

Incubation and sampling times:

Doses were administered orally by gavage. Sampling was performed at 24 and 48 hrs after dosing.

Dose	Sampling time	Sex	Animal number
0.5% Methocel solution	24 h	m/f	001-005/051-055
BIMT 17 BS, 1800 mg/kg	24 h	m/f	101-105/151-155
BIMT 17 BS, 1800 mg/kg	48 h	m/f	201-205/251-255
BIMT 17 BS, 1800 mg/kg	--	m/f	301-302/351-352*
BIMT 17 BS, 1200 mg/kg	24 h	m	401-405
BIMT 17 BS, 600 mg/kg	24 h	m	501-505
Cyclophosphamide, 20 mg/kg	24 h	m/f	601-605/651-655

* Additional animals, to substitute animals in the test substance groups, in case of death.

Results

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

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ORIGINAL

Study outcome:

Dose ^a	Sampling time	Sex	Number of rats	Micronucleated PE (‰) Mean ± standard deviation	p-value
Vehicle control	24 h	male	5	1.4 ± 0.9	
		female	5	1.1 ± 0.5	
BIMT 17 BS 1800 mg/kg	24 h	male	5	1.2 ± 0.3	0.758
		female	5	0.5 ± 0	1.000
BIMT 17 BS 1800 mg/kg	48 h	male	5	1.8 ± 0.4	0.262
		female	5	1.3 ± 1.3	0.433
BIMT 17 BS 1200 mg/kg	24 h	male	5	1.2 ± 0.8	0.714
BIMT 17 BS 600 mg/kg	24 h	male	5	1.0 ± 0.6	0.841
Cyclophosphamide 20 mg/kg	24 h	male	5	22.8 ± 7.0*	0.004
		female	5	21.4 ± 3.0*	0.004

^a Vehicle control, 20 ml 0.5% Methocel solution per kg.

* Significantly different from the vehicle control (p<0.05).

PE = polychromatic erythrocytes.

NE = normochromatic erythrocytes.

Study was negative

Study title: **In vivo comet assay for measurement of DNA damage in the liver of rats after repeat oral administration (gavage)**

Key findings: Study was negative

Study no.: 08B162

Volume #, and page #:

Conducting laboratory and location: Non-clinical drug safety, Boehringer Ingelheim, Biberach an der Riss, Germany

Date of study initiation: October, 2008

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 04040593M, 99.9% pure

Methods

Strains/species/cell line: CrI:WI(Han) male rats

Doses used in definitive study: 5 male rats/group were given either 400 or 1500 mg/kg flibanserin 28 and 4 hrs prior to necropsy. Vehicle controls received 0.5% hydroxyethylcellulose and the positive controls were given 200 mg/kg ethyl methanesulfonate, concurrently.

Basis of dose selection: Dose response study, 1800 mg/kg was significantly toxic.

Negative controls: 0.5% hydroxyethylcellulose

Positive controls: Ethyl methanesulfonate/EMS

Incubation and sampling times:

Results

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

Sponsors summary:

At necropsy samples from the target organ liver (right medial lobe) were immersed in chilled tissue buffer (Hank's balanced salt solution containing 20 mmol/L ethylenediaminetetraacetic acid disodium salt (EDTA) and 10 % dimethylsulfoxide (DMSO), pH 7.5).

(b) (4)

(b) (4)

The slides were coded in a random manner to allow an objective evaluation. The coverslipped slides were examined at 200 x magnification using fluorescent microscopy immediately after staining with ethidium bromide. A total of 150 cells (75 cells/slide) was analyzed. Images were visualized by a CCD camera and measured using the Kinetic Imaging software system “Komet 6 GLP”. The head and tail areas of the image were identified and the light intensity of each was quantified and expressed as Olive Tail Moment (OTM) which is calculated automatically and saved to file. $OTM = (Tail.mean - Head.mean) \times Tail\%DNA/100$. Among different parameters including %DNA in tail used for comparison purposes, the OTM is regarded as the most relevant parameter to measure comet induction. Extensively damaged cells or cell clusters, so called “hedgehogs” were excluded from analysis. However, as a sign for toxicity, these “hedgehogs” (necrotic and apoptotic nuclei) were estimated in parallel. Pieces from the livers were taken adjacent to the sites used for comet analysis and fixed in 4 % neutral buffered formaldehyde solution. Since the comet result was negative, these samples were not processed and evaluated.

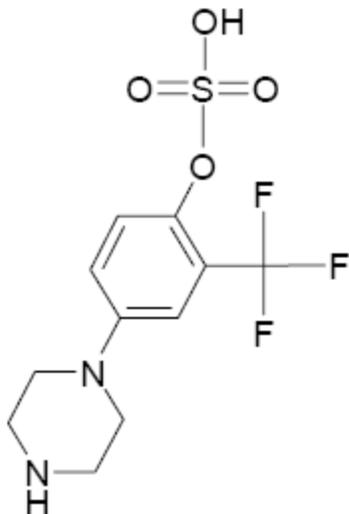
Study outcome:

	Flibanserin (mg/kg)			Pos. Control EMS (mg/kg)
	Control	400	1500	200
OTM:				
	0.18 (0.16-0.21)	0.20 (0.16-0.23)	0.22 (0.17-0.26)	0.41
%DNA in tail:				
	2.36 (2.21-2.65)	2.69 (2.02-2.99)	2.91 (2.50-3.32)	4.11
% Hedgehogs:				
	~2.4	~2.4	~2.4	~4.5

Flibanserin did not induce single strand breaks and alkaline-labile sites in the liver in this assay.

Flibanserin was negative in the in vitro Ames and HPRT assays but induced a non-significant increase in chromosomal aberrations in human peripheral erythrocytes in the presence of a metabolic activation system. However, flibanserin was negative in in vivo assays for clastogenicity (rat bone marrow micronucleus) and for DNA reactivity (Comet) when tested up to toxic doses. Based on the weight of evidence, I consider flibanserin negative for genotoxicity.

Metabolite BI 400296 ZW (M8)



The metabolite was tested for mutagenicity in the Ames assay against TA 1535, TA 1537, TA 98, TA 100 and TA102 with and without metabolic activation (study no. 06B221)

It was negative for all strains at concentrations up to 5000 ug/plate.

APPEARS THIS WAY ON ORIGINAL

Mutagenic activation of M8 in *S. typhimurium* without metabolic activation

Experiment 1 (Plate test)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	10	50	58	131	434
BI 400296 ZW					
100	12	42	63	133	434
300	6	43	75	132	436
1000	7	47	66	128	392
3000	9	45	65	129	422
5000	9	42	63	139	434
Positive Controls					
NaN ₃ 5	<u>1054</u>	-	-	<u>1145</u>	-
9-AA 50	-	<u>558</u>	-	-	-
2-NF 10	-	-	<u>642</u>	-	-
MMC 0.5	-	-	-	-	<u>1160</u>

Experiment 2 (Preincubation)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	7	16	42	88	397
BI 400296 ZW					
100	7	18	44	79	376
300	7	14	45	81	403
1000	6	17	45	84	367
3000	7	13	39	88	381
5000	6	13	43	79	343
Positive Controls					
NaN ₃ 5	<u>761</u>	-	-	<u>1172</u>	-
9-AA 50	-	<u>340</u>	-	-	-
2-NF 10	-	-	<u>526</u>	-	-
MMC 0.5	-	-	-	-	<u>1667</u>

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Historical Range	6 – 22	3 - 30	16 - 68	49 - 150	249 - 458
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Mutagenic activation of M8 in *S. typhimurium* with metabolic activation

Experiment 1 (Plate test)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	12	55	59	139	528
BI 400296 ZW					
100	12	42	53	146	524
300	16	42	60	145	539
1000	12	44	55	156	510
3000	13	46	56	163	533
5000	15	49	61	170	536
Positive Controls					
2-AA 4	<u>133</u>	<u>158</u>	<u>986</u>	<u>1067</u>	-
2-AA 10	-	-	-	-	<u>1148</u>

Experiment 2 (Preincubation)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	10	16	39	107	486
BI 400296 ZW					
100	8	14	36	111	479
300	12	19	34	111	495
1000	9	16	39	108	472
3000	11	18	36	120	505
5000	11	14	37	112	502
Positive Controls					
2-AA 4	<u>162</u>	<u>376</u>	<u>1616</u>	<u>1411</u>	-
2-AA 10	-	-	-	-	<u>668</u>

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Historical Range	12 - 22	3 - 39	20 - 61	74 - 164	279 - 531
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2.6.6.5 Carcinogenicity

Study title: Carcinogenicity study in rats by oral administration (dietary admixture) over a period of 2 years

Key study findings: borderline positive for liver tumors in males

Adequacy of the carcinogenicity study and appropriateness of the test model: Acceptable, doses and methods approved by exec CAC in fax dated 23 December 1997.

Evaluation of tumor findings:

Study no.: 97B090

Volume #, and page #: Electronic submission

Conducting laboratory and location: Dept. non-clinical drug safety, Boehringer Ingelheim Pharm, Biberach, Germany

Date of study initiation: March, 1998

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS (flibanserin), batch 720618, 99.44% pure

CAC concurrence: yes

Methods

Doses: 10, 30, 100 mg/kg

Basis of dose selection (MTD, MFD, AUC etc.):

Species/strain: rat/ Wistar Chbb: (b) (4)

Number/sex/group (main study): 50/sex/gp

Route, formulation, volume: oral in food, admixed in diet

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: 5/sex/gp

Age: 38-42 days

Animal housing: Up to 5/cage

Restriction paradigm for dietary restriction studies: no

Drug stability/homogeneity: stable

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: none

Results

Mortality:

Mortality	Daily dose of BIMT 17 BS [mg/kg]									
	0 (control 1)		0 (control 2)		10		30		100	
	M	F	M	F	M	F	M	F	M	F
Sacrificed	6	14	12	12	6	9	9	10	7	13
Found dead	3	0	3	1	0	3	0	2	4	2
Total	9	14	15	13	6	12	9	12	11	15
% mortality	18	28	30	26	12	24	18	24	22	30

M, F: males, females

Survival rate was equal or greater than 70% for all gps.

Clinical signs:

In parentheses: time period (first to final observation day during study)

Group	control 1		low dose		mid dose		high dose		control 2	
	0		10		30		100		0	
BIMT 17 BS [mg/kg]	M	F	M	F	M	F	M	F	M	F
No. of animals/group	50	50	50	50	50	50	50	50	50	50
No changes observed	18	19	20	19	28	24	25	18	12	23
Eye, milky, opaque	5 (482-722)	4 (279-722)	5 (510-722)	1 (475-722)	2 (412-722)	2 (356-722)	3 (489-723)	0	5 (615-723)	2 (601-723)
Hair loss	1 (700-722)	5 (398-722)	1 (608-671)	9 (447-722)	0	3 (622-722)	1 (447-630)	4 (398-723)	1 (629-723)	3 (398-723)
Pale appearance	0	7 (419-681)	0	6 (608-722)	0	6 (420-712)	0	5 (125-699)	1 (678-684)	2 (548-615)
Rough coat	2 (576-581)	9 (412-728)	0	7 (623-722)	4 (615-666)	10 (293-722)	4 (475-688)	8 (545-708)	4 (454-707)	4 (411-615)
Testes, increased size	1 (664-722)	---	7 (384-722)	---	5 (545-722)	---	6 (307-723)	---	12 (447-723)	---
Thickening, limbs	5 (412-643)	2 (580-663)	4 (405-663)	1 (195-722)	4 (426-722)	0	0	0	3 (384-650)	0
Vaginal discharge, red	---	4 (419-722)	---	7 (384-722)	---	6 (489-722)	---	4 (552-723)	---	1 (538-723)

M, F: males, females

No treatment related clinical signs.

Body weights:

Time	Group Dose [mg/kg]	control 1	low dose	mid dose	high dose	control 2
		0	10	30	100	0
Week -1	Males	168.80	171.29 (1.5)	170.93 (1.3)	169.36 (0.3)	171.93 (1.9)
	Females	137.35	140.49 (2.3) ↑	137.45 (0.1)	140.80 (2.5) ↑	138.91 (1.1)
Week 12	Males	428.34	424.97 (-0.8)	418.07 (-2.4)	403.03 (-5.9) ↓	437.71 (2.2)
	Females	267.93	265.09 (-1.1)	254.11 (-5.2) ↓	241.09 (-10.0) ↓	263.52 (-1.6)
Week 52	Males	584.20	566.21 (-3.1)	563.29 (-3.6) ↓	521.94 (-10.7) ↓	575.38 (-1.5)
	Females	313.64	309.81 (-1.2)	300.72 (-4.1) ↓	276.97 (-11.7) ↓	308.72 (-1.6)
Week 80	Males	612.26	597.89 (-2.4)	585.34 (-4.4) ↓	525.63 (-14.2) ↓	612.19 (0)
	Females	317.53	313.99 (-1.1)	300.71 (-5.3) ↓	283.49 (-10.7) ↓	318.93 (0.4)
Week 104	Males	640.72	612.93 (-4.3)	607.78 (-5.1) ↓	525.87 (-17.9) ↓	616.29 (-3.8)
	Females	323.85	321.84 (-0.6)	306.94 (-5.2) ↓	285.44 (-11.9) ↓	327.56 (1.1)

↑, ↓: significantly increased, decreased compared with control 1; p< = 0.05, many-to-one t-test, two-sided

There was a dose related decrease in BW gain that gradually increased over time. For males, the difference in absolute weight in the LD and MD were small, 4-5% when compared to control 1 (heaviest). The HD gp was reduced by ~18%

For females, the decrease in BW was negligible for the LD, 5% for MD and 12% for HD.

Food consumption:

Time	Group Dose [mg/kg]	control 1	low dose	mid dose	high dose	control 2
		0	10	30	100	0
Week -1	Males	150.74	149.51 (-0.8)	150.83 (0.1)	151.57 (0.6)	152.63 (1.2)
	Females	124.17	122.43 (-1.4)	120.57 (-2.9) ↓	119.74 (-3.6) ↓	119.89 (-3.4) ↓
Week 12	Males	204.75	200.84 (-1.9)	203.64 (-0.5)	199.07 (-2.8)	213.34 (4.2)
	Females	152.48	149.22 (-2.1)	141.40 (-7.3) ↓	130.09 (-14.7) ↓	156.50 (2.6)
Week 52	Males	182.77	178.86 (-2.1)	180.17 (-1.4)	177.43 (-2.9)	179.34 (-1.9)
	Females	123.33	123.06 (-0.2)	121.20 (-1.7)	113.94 (-7.6) ↓	132.12 (7.1) ↑
Week 80	Males	165.08	162.20 (-1.7)	164.04 (-0.6)	162.73 (-1.4)	166.78 (1.0)
	Females	120.03	116.49 (-3.0)	111.39 (-7.2) ↓	110.01 (-8.4) ↓	119.46 (-0.5)
Week 104	Males	174.87	164.82 (-5.8)	167.21 (-4.4)	152.98 (-12.5) ↓	173.44 (-0.8)
	Females	125.11	122.61 (-2.0)	117.43 (-6.1) ↓	113.04 (-9.7) ↓	129.73 (3.7)

↑, ↓: significantly increased, decreased compared with control 1; p< = 0.05, many-to-one t-test, two-sided

Non-consistent decrease in FC for both MD and HD males and females.

Hematology: No treatment related changes in hematology.

Organ Weight:

Daily dose of BIMT 17 BS [mg/kg]	0		10		30		100	
Gender (M = male, F = female)	M	F	M	F	M	F	M	F
Number of organs weighed	76	73	44	38	41	38	38	35
Body weight [g]	608.0	307.3	582.8	300.9	577.4	286.7	502.7	265.9
Liver								
Absolute weight [g]	16.0	9.9	16.2	10.2	17.5↑	9.9	15.7	9.7
Liver weight to body weight ratio [%]	2.64	3.23	2.78↑	3.39↑	3.05↑	3.46↑	3.16↑	3.64↑
Liver weight to brain weight ratio [%]	672	460	691	480↑	747↑	465	673	465

↑, ↓: significantly increased, decreased compared with control 1; p< = 0.05, many-to-one t-test, two-sided

Drug related slight increase in relative liver wt in all treated animals. The increase correlates with the histopath findings of centrilobular hepatocellular hypertrophy.

No other organ wt changes were considered toxicologically significant.

Gross pathology:

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
Gender (M = male, F = female)	M	F	M	F	M	F	M	F	M	F
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver:										
Nodule	1	2	1	3	5	2	5	0	1	0
Uterine cervix:										
Enlargement	-	5	-	7	-	13	-	10	-	5
Hard consistency	-	3	-	8	-	14	-	15	-	5

In the liver, there was an increase in nodules in MD and HD males but not in females. In the uterus, the enlargement and hard consistency may be due to fibrosis (see under non-neoplastic histopath)

Histopathology:

Non-neoplastic:

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)	10	30	100	0 (control 2)
Number of animals examined	50	50	50	50	50
Uterine cervix:					
Fibrosis/fibroplasia moderate to severe, with/without necropsy correlate	34 (2.4) 14 3/11	36 (2.7) 23 5/18	37 (2.5) 17 8/9	39 (2.4) 17 13/4	35 (2.3) 13 4/9
Hyperplasia squamous cell	7 (2.6)	8 (2.0)	7 (2.6)	7 (2.6)	2 (1.5)

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
	M	F	M	F	M	F	M	F	M	F
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver										
Hypertrophy hepatoc. centrilobular / slight to moderate	12	1	13	0	24	0	32	4	14	0
Vacuolation centrilobular hepatoc. / moderate to severe	1	0	2	0	6	0	18	1	4	0
	14	2	18	0	26	0	26	0	12	0
	2	0	3	0	9	0	10	0	2	0
Kidneys										
Mineralisation tubular	0	8	0	4	0	1	0	30	1	1
Dilatation of tubules	0	4	1	1	2	3	0	9	1	1
Nephrosis, chronic-progressive	28	5	23	0	22	4	16	1	24	2
Rectum										
Edema	7	5	5	6	8	6	13	3	1	4
Infiltration, inflammatory	9	2	5	2	6	4	13	2	2	0
Spleen										
Hemosiderosis	16	26	10	29	16	30	14	43	15	29
Adrenal gland										
Peliosis / angiectasis	14	43	23	41	25	39	23	38	13	35

Neoplastic:

Group	control 1		low dose		mid dose		high dose		control 2	
	M	F	M	F	M	F	M	F	M	F
Daily dose of BIMT 17 BS [mg/kg]	0		10		30		100		0	
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver										
Carcinoma, hepatocellular	0	0	1	1	4	1	5	0	2	1
Adenoma, hepatocellular	2	2	1	0	1	2	1	0	1	0
Combined adenoma/carcinoma hepatocellular	2	2	2	1	5	3	6	0	3	1
Focus eosinophilic	2	2	2	1	1	0	3	4	3	1
Focus basophilic tigroid	18	10	18	10	11	16	6	2	16	21
Slight or more	10	4	9	3	4	8	0	1	5	10
Focus basophilic diffuse	2	2	4	3	2	3	4	1	4	4
Focus basophilic NOS	14	4	19	5	13	5	9	2	15	4
Focus clear cell	39	13	39	6	43	7	40	11	38	14
Adrenal gland										
Adenoma, cortical	6	1	3	5	3	3	0	4	2	3
Hyperplasia, cortical	32	29	32	28	26	19	20	19	30	28
Moderate to severe	16	8	11	9	5	7	5	7	11	14
Ovary										
Tumor sex cord stromal mixed [B]	-	13	-	7	-	3	-	2	-	8
Hyperplasia sex cord stromal - severe	-	29	-	24	-	23	-	14	-	28
	-	6	-	6	-	7	-	0	-	8

NOS: not otherwise specified

In the liver, the incidence of hepatocellular carcinomas was slightly higher in HD males. Two HD males died due to intra-abdominal hemorrhage from liver tumors in weeks 90 and 86. The carcinogenic finding was not significant when compared to pooled controls. There were no effects in females.

Statistical evaluation of hepatocellular carcinomas (Sponsors statistics) in males

Dose BIMT 17 BS	0 mg/kg (control 1)	0 mg/kg (control 2)	10 mg/kg	30 mg/kg	100 mg/kg	Trend
Examined (N)	50	50	50	50	50	
Incidences	0	2	1	4	5	
p	-	-	0.7460	0.1088	0.0430	0.0116
p1	-	0.2033	0.5116	0.0551	0.0253	0.0044 ^b
p2	-	-	0.9172	0.4162	0.2541	0.0788

p p value including pooled controls
 p1 p value versus control 1
 p2 p value versus control 2
^b significance level $p < 0.005$ for trend

Statistical evaluation of hepatocellular carcinomas and adenomas (combined tumors) in males

Dose BIMT 17 BS	0 mg/kg (control 1)	0 mg/kg (control 2)	10 mg/kg	30 mg/kg	100 mg/kg	Trend
Examined (N)	50	50	50	50	50	
Incidences	2	3	2	5	6	
p	-	-	0.7967	0.2330	0.1166	0.0408
p1	-	0.6543	0.7094	0.2493	0.1165	0.0308
p2	-	-	0.8834	0.4075	0.2895	0.0860

p p value including pooled controls
 p1 p value versus control 1
 p2 p value versus control 2

Other organs/tissues with significant numbers of tumors

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
	M	F	M	F	M	F	M	F	M	F
Gender (M = male, F = female)										
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Adrenal gland Tumor medullary [B]	4	2	4	2	6	8	2	2	5	1
Mammary gland Fibroadenoma	0	2	0	4	0	3	0	9	0	7
Pancreas Infiltration, inflammatory	4	0	6	0	5	4	10	0	2	2
Skin Fibromas ^a	0	1	1	2	0	0	0	3	1	0
Combined fibromas ^a and fibrosarcomas	1	1	1	3	0	0	1	3	2	1
Combined fibromatous tumors ^b	5	1	3	3	3	1	3	5	3	3
Thyroid gland Adenoma, follicular cell [B]	0	0	0	0	0	2	0	1	0	0

^a Including fibroma [B] and fibroma fibromatosis type [B]

^b Including fibroma [B], fibroma fibromatosis type [B], fibrosarcoma [M], fibrous histiocytoma [B], fibrous histiocytoma [M]

Other than possibly the liver, there were no treatment related tumor increases.

Toxicokinetics:

Group	low dose		mid dose		high dose	
	10 mg/kg		30 mg/kg		100 mg/kg	
Dose	Males	Females	Males	Females	Males	Females
Week 13						
AUC(0-24h)	1960	1670	4610	5910	9670	18700
C(6:00 h)	70.6	108	235	322	584	1080
C(16:00 h)	89.2	28.9	145	147	196	400
Week 26						
AUC(0-24h)	1270	2400	4600	5440	8930	22200
C(6:00 h)	60.8	130	276	335	444	1170
C(16:00 h)	43.2	67.7	103	111	294	623
Week 52						
AUC(0-24h)	1330	1800	4300	4440	7520	16500
C(6:00 h)	48.5	91.9	217	202	389	803
C(16:00 h)	59.9	53.1	141	165	231	558
Week 104						
AUC(0-24h)	1780	1760	5960	7190	10000	17100
C(6:00 h)	96.7	108	311	353	516	1050
C(16:00 h)	50.8	36.9	180	245	314	278

	AUC of BIMT 17 BS (ng·h/mL)					
	10 mg/kg		30 mg/kg		100 mg/kg	
	M	F	M	F	M	F
Rats (week 104)						
AUC(0-24h)	1780	1760	5960	7190	10000	17100
Human (steady state)	2 mg/kg (50 mg BID)		4 mg/kg (100 mg BID)			
Geometric mean AUC _{ss}	2170 (2 x 1085)		5188 (2 x 2594)			
Safety margin	10 mg/kg		30 mg/kg		100 mg/kg	
Ratio rat/human (LD)	0.8	0.8	2.7	3.3	4.6	7.9
Ratio rat/human (HD)	0.34	0.34	1.1	1.4	1.9	3.3

Note: Human daily dose: 50 mg BID (LD, low dose), 100 mg BID (HD, high dose); individuals assumed with 50 kg bodyweight; U97-2256

Using 2080 ng.h/ml as the human exposure at a dose of 100 mg, the safety margins of exposure at the two higher doses for females are roughly 3 and 8, and for males 3 and 5, respectively.

Summary of rat study:

This was a 2 year carcinogenicity study in Wistar rats. The doses were 10, 30 and 100 mg/kg which gave multiples of human exposure to female rats of approximately 1, 3 and 8 for women taking 100 mg. These doses produced reductions in final BW of 18% for HD males and 12% for HD females. Decreased food consumption probably accounts for some of the weight decrease.

The doses were agreed to by the exec CAC in a meeting of Dec. 23, 1997.

The only tumorigenic effect was an increase in the incidence of hepatocellular carcinomas in males. Incidence was 0 and 2 in the controls, 1 in LD, 4 in MD and 5 in HD. In a carcinogenicity study in mice, there was an increase in hepatocellular carcinomas in males and malignant mammary tumors in females. In the present study, the incidence of malignant mammary carcinomas was 1 and 2 in controls and 1 in LD, 0 in MD and 1 in HD and the

incidence of mammary fibroadenomas was 2 and 7 in controls and 4, 3 and 9 in LD, MD and HD, respectively.

Historical controls in Wistar rats (hepatocellular carcinomas)

Study No.	Group No.	Start [m/y]	Duration [month]	Strain	Breeder	Males			Females		
						Animals exam.	with lesion	%	Animals exam.	with lesion	%
2	1	11/1984	24	WIST	A	50	2	4.0	50	0	0.0
13	1	05/1984	31	WIST	A	100	1	1.0	100	0	0.0
20	1	09/1986	24	WIST	A	100	4	4.0	100	0	0.0
21	1	06/1986	24	WIST	A	20	0	0.0	20	1	5.0
22	1	06/1986	25	WIST	A	50	3	6.0	50	0	0.0
28	1	02/1989	26	WIST	A	99	5	5.1	100	1	1.0
30	4	02/1989	26	WIST	A	100	2	2.0	100	0	0.0
49	4	05/1989	25	WIST	A	50	2	4.0	49	0	0.0
51	1	02/1990	25	WIST	A	50	0	0.0	50	0	0.0
65	6	02/1990	25	WIST	A	50	3	6.0	50	0	0.0
83	1	04/1993	25	WIST	A	20	2	10.0	20	0	0.0
89	1	09/1992	25	WIST	A	50	2	4.0			
93	1	09/1992	25	WIST	A				50	2	4.0
95	1	02/1989	26	WIST	A	100	8	8.0			
103	1	08/1994	25	WIST	A	50	2	4.0	50	0	0.0
117	1	08/1995	25	WIST	A	50	4	8.0	50	1	2.0
135	1	08/1995	24	WIST	A	20	1	5.0	20	0	0.0
139	1	05/1991	25	WIST	A	50	4	8.0	50	1	2.0
144	1	05/1991	25	WIST	A	20	0	0.0	20	0	0.0
156	1	10/1998	24	WIST	A	50	5	10.0	50	2	4.0
All 20 studies:						1079	50	4.6	979	8	0.8
Range MIN:								0.0			0.0
Range MAX:								10.0			5.0

m/y: month/year

The maximum percent was 10% in the historical controls and 10% for the HD in this study.

This study was borderline for liver tumors in males. Flibanserin increases the incidence and severity of centrilobular hepatocellular hypertrophy probably by inducing hepatic cytochrome P-450 enzymes. Sponsor believes that flibanserin acts similar to Phenobarbital in promoting liver tumors. It's a reasonable explanation since flibanserin was essentially negative in the available genotox studies and is probably not an initiator.

Combined with the data in mice (see below), flibanserin can be considered positive for liver tumors in male rodents.

Study title: **Twenty-four month oral (diet) carcinogenicity study in the mouse.**

Key study findings: mammary and hepatocellular carcinomas

Adequacy of the carcinogenicity study and appropriateness of the test model:

Deemed adequate by exec CAC

Evaluation of tumor findings:

Study no.: 98R003

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim Pharm, Ridgefield, CT

Date of study initiation: 3/1998

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 (flibanserin), lot 720618, purity not stated

CAC concurrence: yes

Methods

Doses: 10, 80, 200, 1000 (M), 1000/1200 (F). Doses for HD females increased from 1000 to 1200 mg/kg in drug week 23.

Basis of dose selection (MTD, MFD, AUC etc.): Doses were recommended by the Exec CAC in a July 14, 1998 meeting with the Division of Neuropharmacology. In a letter to the Sponsor they recommended that the high dose in females should be 1200 mg/kg based on MTD but apparently the sponsor decided initially on 1000 mg/kg and then increased the dose to 1200 mg/kg when little toxicity was apparent.

Species/strain: (b)(4) CrI:CD-1 (ICBR) mice

Number/sex/group (main study): 70/sex/gp

Route, formulation, volume: oral in feed

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: no

Age: 5-6 wks

Animal housing: individual cages

Restriction paradigm for dietary restriction studies: none

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: TK non GLP

Results

Mortality: Survivability similar between all gps including controls. 38-42 males survived and 22-41 females with no dose response.

Group	Flibanserin mg/kg/day	Males	Females
G1 Control I	0	40	22
G2 Control II	0	38	29
G3 Low	10	46	29
G4 Mid	80	42	41
G5 High-mid	200	44	28
G6 High	M: 1000	40	-
	F: 1000/1200	-	32

a Study termination started in Drug Week 105; numbers take into account all early deaths including animals found dead and sacrificed moribund, as well as dead due to accidental trauma.

Clinical signs: Noted mainly in 1000 mg/kg gp males and included distended/dischored abdomens, dermal blanching and hair staining. Hair staining was also seen in 200 mg/kg males. Distended abdomens was observed at wk 85 and increased to 70% at study termination compared to combined controls which had 39%. The abdominal finding was only seen in males at 1000 mg/kg dose level and not in females at any dose or lower dose males.

Body weights: No difference between treated and control males at study end. Mean body wts for 1000/1200 mg/kg females were consistently increased over combined controls from wk 5 to 82 and considered treatment related. From wk 82 on, wts between treated and control females were similar.

BW week	Control 1+2		10 mg/kg		80 mg/kg		200 mg/kg		1000/1200	
	M	F	M	F	M	F	M	F	M	F
104	36.4	31.9	36.0	33.6	36.8	32.7	37.6	32.0	37.3	32.5

Weekly body weight means

Figure 1: 98R003: Carcinogenicity Study in Mice on Flibanserin
Mean Body Weights, Combined Control Groups - Males

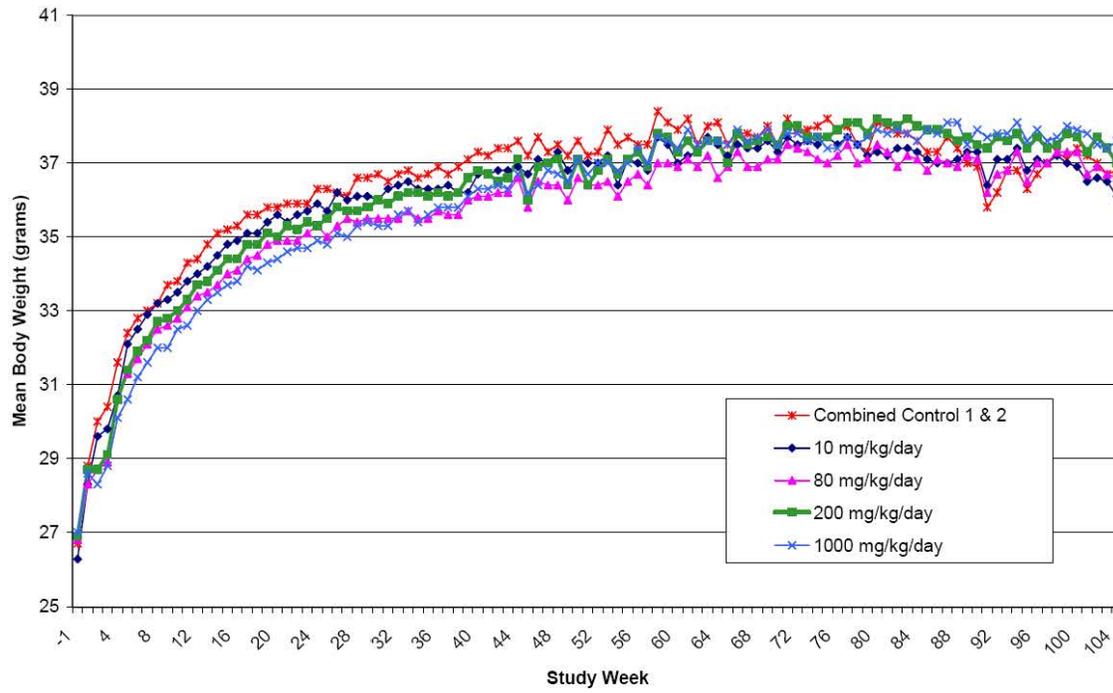
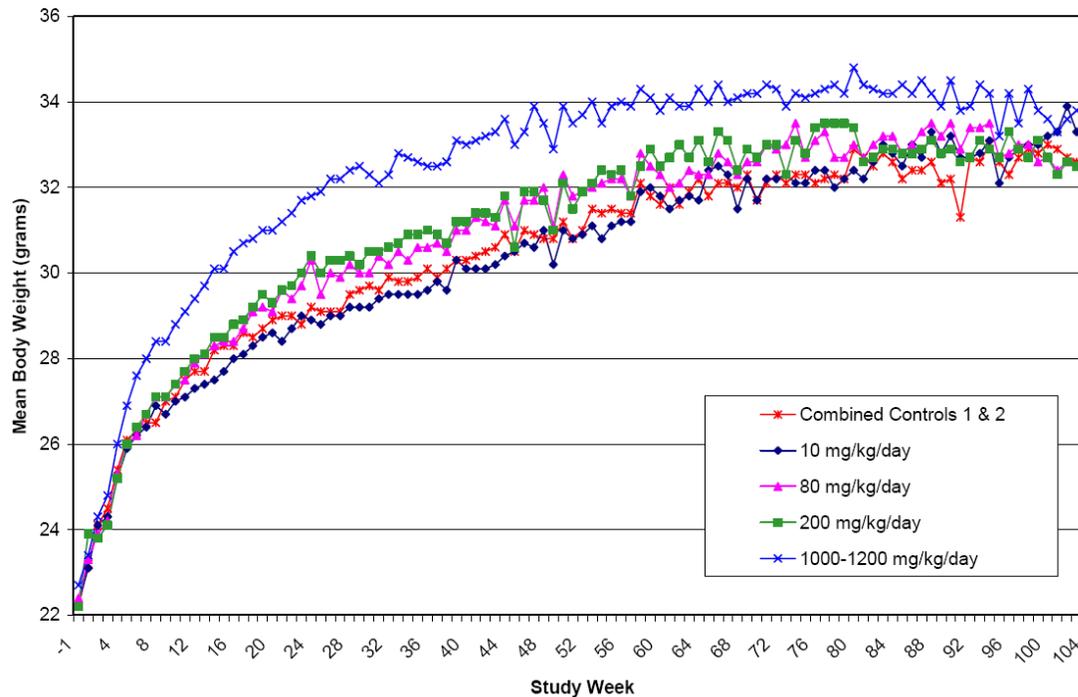


Figure 2: 98R003: Carcinogenicity Study in Mice on Flibanserin
Mean Body Weights, Combined Control Groups - Females



Body weight changes in both males and females were considered drug related. Mean male body weights were significantly decreased (3 to 6%) at the HD during study weeks 2 to 22, but were not decreased upon study termination. Mean body weights in the HD females were significantly increased over combined controls (3 to 11%) during weeks 5 to 82. There were no significant differences at study termination.

Food consumption: Greater than controls in HD males and females. Because of possible palatability issues, there was significant food spillage and food consumption may be overestimated.

Gross pathology: Only treatment related macroscopic change was increase in liver masses in HD males (15/70) versus controls (0/70, 9/70).

Histopathology:

Non-neoplastic: Increased incidence of hepatocellular (centrilobular and midzonal) hypertrophy in males treated with ≥ 80 mg/kg and HD females.

Neoplastic:

There was an increased incidence of tumors in the liver and mammary gland

Incidence and Percentage of Selected Proliferative Changes in the Mammary Gland of Female CD-1 Mice

Group description	Control 1	Control 2	Low	Mid 1	Mid 2	High
Dose Level (mg/kg/day) Flibanserin	0	0	10	80	200	1200
Number of Animals Examined	70	70	70	70	70	70
Adenocarcinoma	0 0%	1 1.4%	3 4.3%	3 4.3%	5 7.1%	5 7.1%
Metastasis (Lung)	0	0	1	1	4	5
Metastasis (Bronchial L. Nodes) ^a	0	0	0	0	2	3
Adenoacanthoma, Malignant ^b	0 0%	0 0%	0 0%	0 0%	1 1.4%	2 2.9%
Combined Carcinomas	0 0%	1 1.4%	3 4.3%	3 4.3%	6 8.6%	7 10%
Hyperplasia	2	0	0	2	0	2

^a Bronchial Lymph Nodes

^b Also known as Malignant Adenosquamous Carcinoma

**Table 3.2.3:6
Statistical Analysis of Mammary Gland Adenocarcinomas, Malignant Adenoacanthomas, and Adenocarcinomas and Malignant Adenoacanthomas Combined (Females)**

M-Adenocarcinomas (common)								
Dose mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1200	Trend
Examined	N	70	70	70	70	70	70	-
Incidence	I	0	1	3	3	5	5	-
p value versus	C1 + C2	-	-	0.0937	0.1013	0.0154	0.0134	0.0064
	C1	-	1.000	0.0827	0.0666	0.0246	0.0218	0.0185
	C2	-	-	0.3013	0.2695	0.1104	0.0951	0.0575
M-Adenoacanthomas (rare)								
Incidence	I	0	0	0	0	1	2	-
p value versus	C1 + C2	-	-	1.000	1.000	0.3544	0.1458	0.0194 ^d
	C1	-	1.000	1.000	1.000	0.5600	0.3466	0.0328
	C2	-	-	1.000	1.000	0.4912	0.2710	0.0287
M-Adenocarcinomas and M-Adenoacanthomas Combined (common)								
Incidence	I	0	1	3	3	6	7	-
p value versus	C1 + C2	-	-	0.0937	0.1013	0.0062 ^a	0.0024 ^a	0.0008 ^c
	C1	-	1.000	0.0827	0.0666	0.0138	0.0076 ^a	0.0033 ^c
	C2	-	-	0.3013	0.2695	0.0621	0.0323	0.0119

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.05, pairwise comparison, Peto test, rare tumor

c p < 0.005, trend, Peto test, common tumor

d p < 0.025, trend, Peto test, rare tumor

Table 1 Historical control data for mammary gland adenocarcinomas and malignant adenoacanthoma in CD-1 mice (from: (b) (4))

Study ID	Start [y]	Animals examined	Adenocarcinoma		Adenoacanthoma, malignant	
			with lesions	[%]	with lesions	[%]
35	1993	54	0	0.00	0	0.00
36	1993	64	0	0.00	0	0.00
37	1993	49	1	2.04	0	0.00
38	1993	62	2	3.23	0	0.00
39	1993	49	2	4.08	0	0.00
40	1994	50	2	4.00	0	0.00
41	1994	56	2	3.57	0	0.00
42	1994	57	1	1.75	0	0.00
43	1995	60	5	8.33	0	0.00
44	1995	38	2	5.26	0	0.00
45	1995	52	0	0.00	2	3.85
46	1995	68	2	2.94	0	0.00
47	1996	48	1	2.08	1	2.08
48	1996	46	0	0.00	0	0.00
49	1996	55	0	0.00	0	0.00
50	1996	53	0	0.00	0	0.00
51	1998	54	1	1.85	0	0.00
52	1999	65	2	3.08	0	0.00
53	1999	57	3	5.26	0	0.00
54	2000	55	1	1.82	0	0.00
all studies		1092	27	2.5	3	0.3
			range min	0.00		0.00
			range max	8.33		3.85

Incidence and Percentage Summary of Selected Proliferative and Non-Proliferative Findings in the Liver

Group description	Control 1		Control 2		Low		Mid 1		Mid 2		High	
	0		0		10		80		200		1000/1200	
Dose Level (mg/kg/day) Flibanserin												
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number of Animals Examined	70	70	70	70	70	70	70	70	70	70	70	70
PROLIFERATIVE FINDINGS												
Carcinoma, Hepatocellular	1 1.4%	0 0%	6 8.6%	0 0%	7 10.0%	0 0%	8 11.4%	5 7.1%	9 12.9%	1 1.4%	11 15.7%	4 5.7%
Adenoma, Hepatocellular	3 4.3%	1 1.4%	3 4.3%	0 0%	2 2.9%	0 0%	0 0%	1 1.4%	3 4.3%	0 0%	5 7.1%	2 2.9%
Combined Adenomas/Carcinomas, Hepatocellular	4 5.7%	1 1.4%	8 11.4%	0 0%	8 11.4%	0 0%	8 11.4%	5 7.1%	11 15.7%	1 1.4%	13 18.6%	6 8.6%
Foci, Acidophilic	0	0	1	0	0	0	0	0	3	0	6	1
Foci, Basophilic	0	0	1	0	0	0	2	1	2	0	8	3
Foci, Mixed	0	0	2	0	0	0	0	0	1	0	7	2
Foci, Vacuolated	0	0	0	0	0	0	0	0	0	1	3	0
Foci, Clear Cell	0	0	0	0	3	0	0	0	0	0	0	0
Foci, Combined	0	0	4	0	3	0	2	1	5	1	17	4
DRUG-RELATED NON- PROLIFERATIVE FINDINGS												
Hypertrophy Hepatocellular Centrilobular	0	0	0	0	0	0	2	0	6	0	13	3
Hypertrophy Hepatocellular Midzonal	0	1	0	0	3	1	6	0	6	0	25	6

Statistical Analysis of Combined Hepatocellular Adenomas and Carcinomas

Hepatocellular Adenomas and Carcinomas Combined (common)								
Dose Level mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1000 M 1200 F	Trend
No.Examined/ Sex	N	70	70	70	70	70	70	-
MALES								
Incidence	I	4	8	8	8	11	13	-
p value	C1+C2	-	-	0.3518	0.2859	0.1192	0.0239	0.0188
versus	C1	-	0.2452	0.2417	0.1987	0.0865	0.0113	0.0092
	C2	-	-	0.7020	0.6551	0.3475	0.1435	0.0851
FEMALES								
Incidence	I	1	0	0	5	1	6	-
p value	C1+C2	-	-	1.000	0.0599	0.5862	0.0046 ^a	0.0024 ^b
versus	C1	-	0.4314	1.000	0.3088	0.8114	0.0491	0.0153
	C2	-	-	1.000	0.0619	0.4912	0.0092 ^a	0.0025 ^b

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.005, trend, Peto test, common tumor

Table 3.2.3:3

Statistical Analysis of M-Hepatocellular Carcinomas in the Liver of Mice

M-Hepatocellular Carcinomas (common)								
Dose Level mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1000 M 1200 F	Trend
No.Examined/ Sex	N	70	70	70	70	70	70	-
MALES								
Incidence	I	1	6	7	8	9	11	-
p value	C1+C2	-	-	0.1661	0.0865	0.0764	0.0065 ^a	0.0062
versus	C1	-	0.1198	0.0367	0.0185	0.0162	0.0028 ^a	0.0035 ^b
	C2	-	-	0.5364	0.4189	0.3997	0.1111	0.0859
FEMALES								
Incidence	I	0	0	0	5	1	4	-
p value	C1+C2	-	-	1.0000	0.0152	0.3544	0.0045 ^a	0.0045 ^b
versus	C1	-	0.10000	1.0000	0.1066	0.5600	0.0248	0.0203
	C2	-	-	1.0000	0.0619	0.4912	0.0338	0.0180

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.005, trend, Peto test, common tumor

Table 2 Historical control data for hepatocellular carcinomas in CD-1 mice 1981 to 2000

Males					Females				
Study ID ^{1,2}	Start [y]	total examined	with lesions	[%]	Study ID ^{1,2}	Start [y]	total examined	with lesions	[%]
CQ	1985	50	14	28.00	CQ	1985	50	1	2.00
CR	1985	50	0	0.00	CR	1985	50	2	4.00
CP	1985	50	1	2.00	CP	1985	51	1	1.96
BX	1981	52	9	17.31	BX	1981	52	1	1.92
DN	1988	48	4	8.33	DN	1988	49	0	0.00
DX	NS	50	8	16.00	DX	NS	50	1	2.00
CX	1983	72	12	16.67	CX	1983	71	0	0.00
DU	1989	50	7	14.00	DU	1989	50	2	4.00
EG	1989	50	5	10.00	EG	1989	49	1	2.04
DZ	1990	49	8	16.33	DZ	1990	49	0	0.00
27	1989	50	3	6.00	30	1992	50	0	0.00
28	1992	49	5	10.20	31	1990	60	2	3.33
29	1990	60	4	6.67	32	1991	70	1	1.43
30	1991	67	10	14.93	33	1991	58	2	3.45
31	1991	60	2	3.33	34	1992	117	0	0.00
32	1993	59	8	13.56	35	1993	59	1	1.69
33	1993	70	4	5.71	36	1993	70	3	4.29
34	1993	50	3	6.00	37	1993	50	0	0.00
35	1993	65	1	1.54	38	1993	65	0	0.00
36	1993	50	8	16.00	39	1993	51	0	0.00
37	1994	50	2	4.00	40	1994	50	0	0.00
38	1994	65	0	0.00	41	1994	65	0	0.00
39	1994	65	5	7.69	42	1994	65	1	1.54
40	1995	60	0	0.00	43	1995	60	0	0.00
41	1995	60	2	3.33	44	1995	41	0	0.00
42	1995	60	4	6.67	45	1995	59	0	0.00
43	1995	70	6	8.57	46	1995	70	0	0.00
44	1996	50	4	8.00	47	1996	50	0	0.00
45	1996	50	5	10.00	48	1996	50	0	0.00
46	1996	90	5	5.56	49	1996	60	0	0.00
47	1996	60	2	3.33	50	1996	70	1	1.43
48	1996	60	7	11.67	51	1998	60	0	0.00
49	1998	65	6	9.23	52	1999	65	0	0.00
50	1999	70	7	10.00	53	1999	60	1	1.67
51	1999	60	9	15.00	54	2000	55	0	0.00
52	2000	55	2	3.64					
March 1995*¹		471	54	11.46			521	9	1.73
March 2005*²		1570	114	7.26			1530	12	0.78
1993 – 2000*		1284	90	7.00			1175	7	0.60
all studies*		2041	168	8.23			2051	21	1.02

Study ID with letters: Spontaneous Neoplastic Lesions in the CrI:CD-1[®] (ICR)BR Mouse. March 1995, (b)(4), information prepared by (b)(4) Appendix A.

Study ID with numbers: Spontaneous Neoplastic Lesions in the CrI:CD-1[®] (ICR)BR Mouse in Control Groups from 18 Month to 2 year Studies. March 2005, (b)(4), information prepared by (b)(4) Appendix B.

Study CQ excluded for males due to exceptionally high incidence(shaded grey)

**Table 3 Historical control data for hepatocellular carcinomas in CD-1 mice
December 1993 (92 - 104 weeks)**

Males					Females				
Study ID ¹	Start [y] ²	total examined	with lesions	[%]	Study ID ¹	Start [y] ²	total examined	with lesions	[%]
618	ns	50	8	16.00	618	ns	50	1	2.00
670	ns	49	8	16.33	670	ns	49	0	0.00
617	ns	50	5	10.00	617	ns	50	0	0.00
293	ns	50	7	14.00	293	ns	50	2	4.00
694	ns	50	0	0.00	694	ns	50	0	0.00
483	ns	50	5	10.00	483	ns	49	1	2.04
056	ns	50	2	4.00	056	ns	50	0	0.00
001	ns	50	5	10.00	001	ns	50	0	0.00
total		399	40	10.03			398	4	1.01
range			min	0.00				min	0.00
			max	16.33				max	4.00

Background tumour incidences from carcinogenicity studies in CRL:CD one Swiss mice, OA39/BTI(mice).1297, _____: Appendix C.
ns = not specified

b(4)

Historical control data were from the same strain and same vendor (_____ four different production sites). Studies were initiated between 1993 and 2000 for mammary tumors and 1981 to 2000 for hepatocellular tumors.

b(4)

Toxicokinetics:

Table 3.1.4:1 Mean Plasma Concentrations of Flibanserin in Mice

Group	Dose (mg/kg/day)	Gender	Flibanserin Plasma Concentration (ng/mL)		
			Week 24	Week 53	Week 78
G1	0 (Control)	Male	0	24.6 ± 55.0	2.32 ± 5.19
		Female	0	0	0
G2	0 (Control)	Male	0	0	0
		Female	0	0	0
G3	10	Male	60.4 ± 69.2	0 ^a	12.5 ± 14.4
		Female	6.62 ± 14.80	0 ^a	21.5 ± 12.6
G4	80	Male	124 ± 60	131 ± 79 ^a	142 ± 69
		Female	123 ± 48	47.4 ± 65.0 ^a	203 ± 181
G5	200	Male	211 ± 47	228 ± 67	286 ± 169
		Female	181 ± 120	204 ± 79	185 ± 89
G6	1000 (males)	Male	807 ± 635	1253 ± 301	1218 ± 407
	1200 (females) ^b	Female	571 ± 215	735 ± 482	571 ± 276

a NOTE: The LOQ of the assay for these samples was higher (100 ng/mL) than for samples in Weeks 24 and 78 (10 ng/mL). As a consequence, and because a concentration of zero ng/mL was used in place of BLQ for calculations of mean±SD, the mean concentrations for the 10 and 80 mg/kg/day dose group in Week 53 are biased to be lower than if an LOQ of 10 ng/mL had been possible. Therefore, comparisons to other groups may be inaccurate.

b The initial dose in Group G6, 1000 mg/kg/day, was escalated to 1200 mg/kg/day in Week 23 for females only.

No AUC data were available from the mouse carcinogenicity study. Direct exposure comparisons with humans cannot be made.

Serum drug and prolactin levels were determined in a 34 week study in the same strain of mice and at the same doses as the carcinogenicity study. For additional information on the prolactin measurements, see under special toxicology studies, below.

Summary Table 1 Mean toxicokinetic parameters of flibanserin after dietary administration to mice

Parameter	Week	Gender	10 mg/kg	80 mg/kg	200 mg/kg	1000 mg/kg	1200 mg/kg
C(max)	3	m	39.1	176	376	1430	-
[ng/mL]	3	f	47.6	192	313	-	1710
AUC(0-24h)	3	m	591	3150	6020	16300	-
[ng·h/mL]	3	f	671	2370	5680	-	20800
C(1000)	3	m	18.5	115	217	628	-
[ng/mL]	3	f	18.6	66.1	188	-	477
C(0800)^a	13	m	18.0	207	247	787	-
	13	f	16.2	101	197	-	558
	26	m	23.0	150	233	748	-
	26	f	16.5	88.6	311	-	764

^a actual collection between 0800 and 1050 hours

In humans taking 100 mg, drug AUC_{0-ss} was 2080 ng.h/ml. These TK data are used to compare mouse and human exposures for the carcinogenicity study.

Prolactin levels - means

Prolactin ng/ml	control	10 mg/kg	80 mg/kg	200 mg/kg	1000/1200 mg/kg
wk 14	129 ± 58*	146 [^] ± 107	134 ± 42	115 ± 55	177 ± 104
wk 34	106 ± 153	33 ± 31	146 ± 152	103 ± 66	104 ± 77

* ± SD

[^] In another table, the value was given as 128

Summary: Flibanserin was tested for carcinogenicity in a two year study in mice. Mortality was similar for males and females among all treated groups and controls.

Only sign of toxicity was distended abdomen in HD males. Changes in body weight occurred during the course of the study for both males (decrease) and females (increase) and were considered treatment related but body weights were no different than controls at study termination.

In the mammary gland of female mice, there was a clear increase in adenocarcinomas (incidence was 0, 1 in the two controls and 3, 3, 5, 5 in the treated gps). When combined with malignant adenoacanthomas (also called adenosquamous carcinoma; a variation of adenocarcinoma), the controls had 0, 1 and the treated gps had 3, 3, 6, 7 total malignant mammary tumors which is higher than the historical controls for any one group. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes.

In male mice, there was a statistically significant increase trend in hepatocellular carcinomas (1 and 6 in male controls, and 7, 8, 9, 11, in the treated gps) which was also significant in the pairwise test at the HD. For hepatocellular carcinomas in females, the results were 0 and 0 for controls and 0, 5, 1, 4 in the treated gps. There was a significant trend and pairwise difference at the 1200 mg/kg dose level when compared with pooled controls but not when compared with control 1 or control 2 separately. For combined adenomas and carcinomas, females showed a significant trend and pairwise difference at 1200 mg/kg when compared to pooled controls and control 2.

The maximum percent incidence in the historical controls for hepatic carcinoma controls was 17% for males and 4% for females (Sponsor excluded the first 1985 study because of the exceptionally high incidence). In this study, the percents were 1.4%, 8.6%, 10%, 11.4%, 12.9%, 15.7% for males and 0%, 0%, 0%, 7.1%, 1.4%, 5.7% for females.

There was a clear increase in combined foci of cellular alteration in livers of high dose males and possibly in females as well.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: **Study of fertility and early embryonic development to implantation in rats by oral administration, gavage.**

Key study findings: No effects on fertility

Study no.: 69S; U95-2214

Volume #, and page #: Electronic

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. (b)(4)
(b)(4) GmbH, Biberach an der Riss, Germany

Date of study initiation: January 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9403;

Methods

Doses: 20, 80, 200 mg/kg

Species/strain: Chbb: (b)(4) (SPF) rats

Number/sex/group: 24/sex/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg

Satellite groups used for toxicokinetics: none

Study design: Males were dosed 28 days before mating and females 14 days before mating. Dosing continued without interruption until GD 6 in females. Males were dosed continuously until successful mating. Dams were sacrificed on GD 14-16 and subjected to cesarean section with an in situ macroscopic inspection.

Results

Mortality: none

Clinical signs: Some sedation in HD males. Sedation, prone posture, reduction of spontaneous activity and timidity were seen in HD females at the beginning of treatment. They were normal from the 4th day on.

Body weight: Some slight decrease in MD and HD females on GD 3 and 6 but same as controls on GD 14.

Food consumption: No significant changes

Toxicokinetics: not done

Necropsy: Females were normal

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

The normal duration of the estrus cycle for this strain of rat is 4-5 days. The duration of the estrus cycle was changed to shorter than 4 days in 4.2% of MD animals and longer than 5 days in 8.3 and 29.2% of MD and HD animals. The mean number of corpora lutea was significantly increased in the HD group.

Parameter (mean)	Control	5 mg/kg	15 mg/kg	100 mg/kg	Historical range [#]
Corpora lutea	15.6	16.3	15.9	17.8*	13.5-17.5

* significant difference (P<0.05), # = from vehicle controls and unaffected dosages

There were no effects on fertility (copulation index, fertility index, gestation index)

Parameter [%]	Control	20 mg/kg	80 mg/kg	200 mg/kg
Copulation index [#]	95.8	100	95.8	100
Fertility index [#]	95.8	95.8	95.8	100
Gestation index	95.6	100	95.6	100

[#] dams pregnant without sperm found in vaginal smear included

There were no effects on litter parameters (implantations, resorptions, viable fetuses)

Embryofetal development

Study title: **Study for effects on embryo-fetal development in rats by oral administration, gavage**

Key study findings: Maternal toxicity in MD and HD. Developmental abnormalities in the HD. Several different malformations in treated groups with no dose response. NoAEL considered to be 80 mg/kg (~15 X human exposure).

Study no.: 56S; U95-2254

Volume #, and page #:

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. [REDACTED] ^{(b) (4)} GmbH, Biberach an der Riss, Germany

Date of study initiation: September, 1994

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9403;

Methods

Doses: 20, 80, 400 mg/kg

Species/strain: Chbb: [REDACTED] ^{(b) (4)} (SPF) rats

Number/sex/group: 24 pregnant females/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg

Satellite groups used for toxicokinetics: 6/gp

Study design: Pregnant females were dosed from GD 7-16. Animals were sacrificed on GD 22.

Results

Mortality (dams): None

Clinical signs (dams): The number of pregnant rats/group were 20, 22, 18, and 22 in the control to high dose, respectively. An additional dam in the high dose group had total embryo resorptions. The clinical signs of the low dose group were similar to the control group. Within 2-3 hrs, MD females showed somnolence, sedation and timidity after the first treatment until the 9th treatment. In the HD group, there were severe clinical sign including somnolence, reduction of spontaneous activity, sedation, prone position, timidity, catalepsy, and others. After the 7th treatment there was slight sedation, somnolence and timidity and after the end of treatment, the animals behaved normally.

Body weight (dams):

There was moderate decrease in BW gain in the HD group and to a lesser extent, the MD group. Total body weight gains were 121, 118, 102, 73g control to HD, respectively.

Dose (mg/kg)	Mean of Body Weight Gain (g) relative to GD 7			
	GD 8	GD 12	GD 16	GD 21
control	1.7	24.1	45.3	121.3
20	0.6	18.2*↓	41.7↓	118.0
80	-3.7*↓	13.5*↓	31.5*↓	101.9*↓
400	-23.5*↓	-7.5*↓	10.0*↓	73.0*↓

* significant difference (P<0.05), ↓ decreased, GD = gestation day

▨ = administration period

Food consumption (dams):

There was a slight decrease in food consumption in the MD group and a moderate decrease at the HD.

Toxicokinetics:

Geometric means of $C_{max,ss}$ and AUC_{0-24h} and medians of $t_{max,ss}$ on GD 12 were

Dose [mg/kg/day]	N	$t_{max,ss}$ [h]	$C_{max,ss}$ [ng/ml]	AUC_{0-24h} [(ng/ml)×h]
20	6	1	1108	7131
80	6	1	3785	31592
400	6	2	8447	86643

Human AUC_{0-t} is 2080 ng.h/ml

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

One HD animal had complete resorption.

Parameter (mean)	Control	20 mg/kg	80 mg/kg	400 mg/kg	Historical range#
Fetal body weight [g]	5.5	5.5	5.2*	4.7*	4.8 - 5.6 g

* significant difference (P<0.05), # = from vehicle controls and unaffected dosages

Offspring (malformations, variations, etc.):

There was one runt (fetuses weighing less than 65% of the weighted control mean values) in the MD and 10 in the HD groups. Skeletal and visceral abnormalities were seen in controls and treated animals with equal distribution. In the HD group, there were a greater number of fetuses with reduced ossification of fore limbs and a greater number with lumbar ribs.

Malformations were seen only in the treated groups. They included frequent malformations like cleft or fused vertebrae (2 at MD, 2 at HD) or a single malformation (agnathia) at the LD. In the HD, there were 2 fetuses with anophthalmia and one with hydrocephalus, all within one litter.

Fetal findings (%)

Findings	Control	Dose mg/kg			Historical Data %
	G 0	20 G 1	80 G 2	400 G 3	
Runts			1 (0.40)	10 (3.29)	0.19
Variations:					
retinal fold	24 (17.91)	31 (20.26)	16 (13.44)	21 (14.19)	0.13*
dilated renal pelvis	6 (4.48)	4 (2.61)	1 (0.84)	-	0.84*
distance between A.carotis communis sinistra and A.subclavia sinistra enlarged	1 (0.75)	-	-	-	-
cervical ribs	2 (1.38)	4 (2.53)	-	1 (0.64)	1.74*
lumbar ribs	1 (0.69)	1 (0.63)	4 (3.12)	9 (5.77)	0.35*
delayed ossification of fore limbs	1 (0.69)	-	-	11 (7.05)	2.56*
delayed ossification of sternbrae	1 (0.69)	1 (0.63)	1 (0.78)	1 (0.64)	2.68*
delayed ossification of vertebral body	1 (0.69)	1 (0.63)	1 (0.78)	1 (0.64)	0.43 (ossification delay in general)*
split or displaced sternbrae	3 (2.07)	1 (0.63)	3 (2.34)	1 (0.64)	0.34*
rudimentary sternbrae	-	-	-	1 (0.64)	-
hypoplastic 13th rib	-	-	1 (0.78)	-	0.43*
short ribs	-	-	-	1 (0.64)	-
Malformations:					
dilated ventricle of telencephalon (hydrocephalus)	-	-	-	1 (0.33) ⁺	0.09*
agnathia	-	1 (0.32) ⁺	-	-	-
anophthalmia	-	-	-	1 (0.33) ⁺ 1 (0.67)	-
cleft vertebrae	-	-	2 (1.56)	1 (0.64)	0.11
fused vertebra	-	-	-	1 (0.74)	0.02

+ calculated for all living fetuses

* These data origin from an internal historical data set which is filed in the Teratology Laboratory at Dr. JmbH, Biberach

b(4)

The complete resorption in one HD dam and the decreased fetal body weights may be attributable to the significant reduction in maternal body weight in the HD group. The two fetuses in the high dose group with anophthalmia one of them also with hydrocephalus, occurred in the one litter.

Study title: Study for effects on embryo-fetal development in rabbits by oral administration, gavage

Key study findings: No teratology findings. Increased fetal resorptions at HD.

Study no.: 68S; U95-2267

Volume #, and page #:

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. (b)(4) (b)(4) GmbH, Biberach an der Riss, Germany

Date of study initiation: January, 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9402-P;

Methods

Doses: 20, 40, 80 mg/kg

Species/strain: Chbb:HM (SPF) rabbits

Number/sex/group: 21 pregnant females/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 5 ml/kg

Satellite groups used for toxicokinetics: 6/gp

Study design: Pregnant females were dosed from GD 6-18. Animals were sacrificed on GD 29.

Results

Mortality (dams): One high dose dam died on GD 21, cause of death not stated.

Clinical signs (dams): The number of pregnant rabbits/group were 17, 17, 20 and 21 in control to high dose, respectively. Complete resorptions occurred in one MD dam and in 5 HD dams. There were abortions in one MD dam (2 fetuses) and one HD dam (5 fetuses).

Coprostasis (fecal impaction) was seen in 2/20 MD rabbits and in 14/21 HD rabbits.

I assume that the dams were culled to 17/group except for the HD which had only 15 pregnant dams remaining.

Body weight (dams):

Dose (mg/kg)	n	Mean of Body Weight Gain [g] relative to GD 6				
		GD 7	GD 8	GD 18	GD 21	GD 28
control	17	5.6	9.2	128.1	150.5	272.7
20	17	-5.1	-24.3	113.8	149.3	300.9
40	17	-27.7↓*	-55.7↓*	32.7↓*	84.2	238.5
80	15	-57.5↓*	-104.0↓*	-94.7↓*	-14.4↓*	180.1↓*

* significant difference (P<0.05), ↓ decreased

Food consumption (dams):

FC was decreased during treatment in the MD and HD group.

Toxicokinetics:

Geometric means of $C_{max,ss}$ and AUC_{0-24h} and medians of $t_{max,ss}$ on GD 13/14:

Dose [mg/kg/day]	N	$t_{max,ss}$ [h]	$C_{max,ss}$ [ng/ml]	AUC_{0-24h} [(ng×h/ml)]
20	3	2	1286	7535
40	3	2	1821	15720
80	3	2	4852	53930

Human AUC_{0-t} is 2080 ng.h/ml

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Gestation index was 100% in controls and LD, 85% in MD and 71% in HD due to aborted fetuses in one MD and one HD dam, complete abortion in one HD dam and to complete resorptions in one MD and 5 HD dams

Parameter (mean)	Control	20 mg/kg	40 mg/kg	80 mg/kg	Historical range#
Corpora lutea	6.8	7.3	8.1*	8.0	7.5-9.1
Late resorptions	0.1	0.1	0.1	0.8*	0-0.1
Resorption rate	5.8	6.6	2.4	17.0*	4.6-14.4

* significant difference (P<0.05) , # = from vehicle controls

Offspring (malformations, variations, etc.):

Five fetuses of the HD group were classified as runts (less than 65% of control value).

Parameter (mean)	Control	20 mg/kg	40 mg/kg	80 mg/kg	Historical range#
Fetal body weight [g]	39.0	38.5	36.2*	35.9*	34.6-39.6

* significant difference (P<0.05) , # = from vehicle controls

There were no variations or malformations in the treated groups that were considered treatment related. None occurred more than once or at a significantly greater frequency than concurrent controls and all were within the historical control range.

Findings	Control	Dose mg/kg			Historical Data* / %
		20	40	80	
Runts		-	-	5 (5.0%)	1.2
Variations:					
Flexure	2 (1.9%)	-	1 (0.8%)	-	0.9
Ventricular septal defect (VSD)	3 (2.8%)	3 (2.7%)	4 (3.1%)	2 (2.0%)	38.3
short 12th rib	-	1 (0.9%)	-	-	0.1
13th rib	-	1 (0.9%)	1 (0.8%)	-	0.4
Malformations:					
Hydrocephalus	-	-	1 (0.8%)	-	0.05
Synostosis of sternebrae	2 (1.9%)	-	-	1 (1.0%)	0.25

* These data origin from an internal historical data set which is filed in the Teratology Laboratory at Dr. ~~_____~~ GmbH, Biberach

b(4)

Prenatal and postnatal development

Study title: **Study for effects on pre- and postnatal development including maternal function in rats by oral administration, gavage**

Key study findings: Decreased number of offspring, some developmental effects.

Study no.: 96B026; U97-2294

Volume #, and page #:

Conducting laboratory and location:

Date of study initiation: March, 1996

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403-P

Methods

Doses: 20, 80, 200 mg/kg

Species/strain: Chbb: ~~_____~~ (SPF) rats

Number/sex/group: 24 females/group

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg

b(4)

Satellite groups used for toxicokinetics: none

Study design: Dosing began on GD 6 and continued until postpartum (pp) day 21

Results

F₀ in-life: Twenty control and low dose and 21 mid and high dose females became pregnant. One HD dam died after the first treatment. Slight treatment related sedation was seen in the LD on treatment day 1 and in the MD from treatment day 1 to 10. In the HD group there was slight to severe sedation and somnolence, reduction of spontaneous activity and occasional pilo-erection, chromorhinodacryorrhea and dyspnea. Gestation period was 22 days for the control group, 3 dams in the LD and MD had gestations of 23 days and 8/20 dams in the HD group had a gestation of 23 days.

Body weight gain of pregnant dams of all treated groups was dose dependently and significantly decreased during gestation. However, by lactation day (LD) 21, only the HD group had decreased body weight.

Dose [mg/kg]	Mean of body weight gain (relative to GD 6) during gestation [g]				
	GD 7	GD 10	GD 17	GD 20	GD 21
control	4.4	15.6	62.9	109.4	127.5
20	-0.3*↓	8.6*↓	47.7*↓	84.8*↓	100.0*↓
80	-1.0*↓	6.1*↓	38.5*↓	73.7*↓	88.4*↓
200	-11.0*↓	1.0*↓	25.4*↓	55.9*↓	69.6*↓

* significant difference (P<0.05), ↓ decreased, GD=gestation day

Dose [mg/kg]	Mean of body weight gain (relative to LD 1) during lactation [g]			
	LD 4	LD 7	LD 14	LD 21
control	10.6	16.2	39.1	42.9
20	5.8	14.0	36.2	47.6
80	5.3	11.1	30.5*↓	44.0
200	0.8*↓	4.2*↓	23.0*↓	25.7*↓

* significant difference (P<0.05), ↓ decreased, LD=lactation day

F₀ necropsy:

No macroscopic changes were seen in the dams at weaning. All litter parameters were comparable in control, LD and MD groups. The HD group had significantly reduced numbers of implantations and newborns, due primarily to postimplantation loss.

Parameter (mean; range)	Control	20 mg/kg	80 mg/kg	200 mg/kg	Historical range#
Implantations	15.8	14.3	14.6	12.5*↓	14.3; 13.5-15.0
Number of offspring	14.1	12.4	12.7	10.8*↓	12.8; 12.8-12.9
Postimplantation loss [%]	10.7	13.3	12.8	16.6	12.2; 10.0-14.0

* significant difference (P<0.05) , # = from vehicle controls

At birth anophthalmia was seen in two pups in the high dose group, exceeding the historical control range for the strain. No variations were seen in controls or treated groups.

In the 200 mg/kg dose group viability rate was decreased to 49.3% and weaning rate to 85.5%. In the 80 mg/kg dose group the effect on viability was 82%. All offspring survived after weaning.

At delivery the body weights of the F₁ pups from the 80 and 200 mg/kg groups were significantly lower than the control and below the mean historical control mean. Body weight gain of these groups remained significantly decreased during lactation.

F₁ physical development:

Dose [mg/kg]	Mean of body weight [g]	Mean of body weight gain (relative to LD 1) [g]			
	LD 1	LD 4	LD 7	LD 14	LD 21
Control	5.9	1.4	6.4	22.0	38.1
20	5.9	1.2	6.0	21.0	36.6
80	5.6*↓	0.7*↓	4.7*↓	18.0*↓	32.6*↓
200	5.3*↓	0.1*↓	2.6*↓	14.4*↓	27.7*↓

* significant difference (P<0.05) , ↓ decreased , LD=lactation day

Time points for incisor eruption, growth of fur, opening of the ear canals, opening of the eyes, correct running, descent of the testes and vaginal opening were similar for controls, LD and MD. In the HD, growth of fur, opening of the ear canals and vaginal opening were significantly delayed for one day in 10/72 (fur), 12/72 (ear) and 5/21 (vagina) pups or more than one day in 6/72, 3/72 and 3/21 pups.

Pupillary reflex, air-righting reflex and hearing were not affected by treatment.

F₁ behavioral evaluation:

Biel Water T-maze Test: Results of the learning approach in wk 6 and test of memory function in wk 7 were similar in all groups.

Test on motility (Actifram): Time dependent activity was similar between all groups. In controls, locomotor activity could be seen at 4 pm due to switch off of room light. The reaction

in the MD and HD groups was significantly decreased (less total activity). In the LD, only the females were affected.

F₁ reproduction:

No treatment related effects were seen. Copulation and fertility indices were not affected by treatment and there were no abortions, resorptions of entire litters or intercurrent deaths. Mean numbers of viable fetuses, resorption rate and preimplantation loss were comparable between treated and control groups.

2.6.6.7 Local tolerance

Not done

2.6.6.8 Special toxicology studies

Study title: **34-week oral (diet) toxicokinetic study in the mouse**

Key study findings: no increase in serum prolactin levels

Study no.: 06R074

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim, Pharm, Ridgefield, CT

Date of study initiation: Sept, 2006

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, lot 05071202M, purity not stated

Methods

Doses: 10, 80, 200, 1000 (males) 1200 (females) mg/kg

Species/strain: CD-1 mice

Number/sex/group or time point (main study): 35/sex/gp

Route, formulation, volume, and infusion rate: oral in diet

Satellite groups used for toxicokinetics or recovery: none

Results:

Mortality: none drug related

Clinical signs: none drug related

Body weights: At the end of the study, HD males weighted about 15% less than controls. There were non-significant decreases in BW in the other male groups as well. No effects on BW in females.

Toxicokinetics:

Summary Table 1 Mean toxicokinetic parameters of flibanserin after dietary administration to mice

Parameter	Week	Gender	10 mg/kg	80 mg/kg	200 mg/kg	1000 mg/kg	1200 mg/kg
C(max)	3	m	39.1	176	376	1430	-
[ng/mL]	3	f	47.6	192	313	-	1710
AUC(0-24h)	3	m	591	3150	6020	16300	-
[ng-h/mL]	3	f	671	2370	5680	-	20800
C(1000)	3	m	18.5	115	217	628	-
[ng/mL]	3	f	18.6	66.1	188	-	477
C(0800)^a	13	m	18.0	207	247	787	-
	13	f	16.2	101	197	-	558
[ng/mL]	26	m	23.0	150	233	748	-
	26	f	16.5	88.6	311	-	764

^a actual collection between 0800 and 1050 hours

In humans taking flibanserin 100 mg, the AUC_{0-ss} is 2080 ng.h/ml.

Prolactin levels - means

Prolactin ng/ml	control	10 mg/kg	80 mg/kg	200 mg/kg	1000/1200 mg/kg
wk 14	129 ± 58*	128 ± 107	134 ± 42	115 ± 55	177 ± 104
wk 34	106 ± 153	33 ± 31	146 ± 152	103 ± 66	104 ± 77

* ± SD

Prolactin levels (ng/ml), week 14, individual data, summary statistics and t-tests

Animal	Control	Low-Dose	Low-Mid-Dose	High-Mid-Dose	High-Dose
1	136.65	77.93	93.94	70.27	163.47
2	179.06	142.06	160.03	200.57	185.50
3	169.86	315.66	169.87	102.10	114.38
4	185.08	156.46	180.49	175.00	140.99
5	105.97	4.24	155.71	54.58	126.31
6	19.53	177.52	93.57	121.59	101.86
7	107.31	19.75	81.15	79.38	404.58
N	7	7	7	7	7
Mean	129.066	127.660	133.537	114.784	176.727
SD	58.257	106.628	42.087	54.851	104.468
Geom. Mean	107.951	69.869	127.407	104.120	158.790
Geom. SD	2.200	4.546	1.403	1.612	1.584
p value	-	0.3363	0.7124	0.9359	0.3930

Prolactin levels (ng/ml), week 34, individual data, summary statistics and t-tests

Animal	Control	Low-Dose	Low-Mid-Dose	High-Mid-Dose	High-Dose
1	51.47	21.94	18.84	80.63	238.68
2	3.85	20.19	161.12	141.14	111.34
3	51.75	11.25	147.24	199.12	50.52
4	444.08	12.50	17.50	16.00	155.58
5	100.53	48.02	186.28	128.04	33.89
6	87.98	97.23	39.61	53.91	115.12
7	5.36	19.75	451.20		20.97
N	7	7	7	6	7
Mean	106.431	32.983	145.970	103.140	103.729
SD	153.368	30.839	152.206	66.030	77.078
Geom. Mean	41.608	24.784	82.512	79.384	77.694
Geom. SD	5.357	2.148	3.510	2.479	2.404
p value	-	0.4077	0.2760	0.3225	0.3196

Analyzed by ANOVA followed by Dunnett's test. Sponsor also did statistical tests on the geometric means. There were no significant effects.

In female mice given flibanserin, there were no effects on prolactin levels at either 14 or 34 wks at doses up to 1200 mg/kg or approximately 10 times the human exposure.

As quoted by sponsor

Prolactin levels measured in Drug Weeks 14 and 34 did not show any evidence of drug-related differences as compared to vehicle Control animals, nor were any dose-related changes observed. In Drug Week 34, estrus stage as evaluated by vaginal smear showed no correlation to prolactin levels or flibanserin dose level.

Summary: TK study with flibanserin gave exposure multiples in female mice of approximately 0.3, 1, 3 and 10 times the human exposure in humans taking 100 mg. The doses had no effect on plasma prolactin levels and cannot explain the increase in mammary tumors seen in mice given flibanserin

Study title: Four week oral (gavage) immunotoxicity study in female rats

Key study findings:

Study no.: 04B105

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim Pharma, Biberach/Riss, Germany

Date of study initiation: May, 2004

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 99111078M, 99.4% pure

Formulation/vehicle: 0.5% methylcellulose

Methods

Doses: 0, 20, 100, 250 mg/kg/day

Study design: Flibanserin was administered daily for at least 28 days by oral gavage to groups of 10 female Chbb ^{(b)(4)} rats (gps 1-4). A fifth group of 10 females was gavaged with 25 mg/kg cyclosporin A. An additional 10 animals received either 0 or 250 mg/kg flibanserin for 28 days and were kept for a 4 week recovery period.

Group No.	Daily dose of BIMT 17 BS [mg/kg]	Females	
		Main study	Recovery
1	0 (vehicle)	151-160	161-170
2	20	251-260	-
3	100	351-360	-
4	Day 1: 400 Day 2- 28: 250	451-460	461-470
5	Cyclosporin A 25 mg/kg	551-560	-

High dose was reduced due to unexpected severe catalepsy in individual animals and inter-individual aggressiveness in all animals at 400 mg/kg, the HD was reduced from day 2 on to 250 mg/kg. Neither the catalepsy nor the aggressiveness had been seen in other studies at 400 mg/kg.

Apparently, the aggressiveness was due to a combination of flibanserin treatment and group housing. On day three, animals were housed in individual cages.

Results:

Semiquantitative comparison of cell markers in Cyclosporin-A treated animals versus control

Cell marker	CD 2	CD 3	CD 8	CD 45RA
Lymphocyte subset	T-subset	T-subset	T-subset	B-subset
Thymus				
Cortex	unchanged	unchanged	unchanged	unchanged
Medulla	reduced	reduced	reduced	reduced*
Spleen				
Follicle	unchanged	unchanged	unchanged	unchanged
PALS	reduced	reduced	reduced	reduced*
Marginal zone	unchanged	unchanged	unchanged	unchanged
Red pulp	unchanged	unchanged	unchanged	unchanged
Lymph node, mesenterial				
Follicle	unchanged	unchanged	unchanged	unchanged
Paracortex	reduced	reduced	reduced	reduced*
Sinusoidal lymphocytes	unchanged	unchanged	unchanged	unchanged

unchanged: Similar or equal to Control / No noteworthy difference when compared to Control
 reduced: Reduced volume of the respective anatomical zone as a consequence of the reduced number of positive staining lymphocytes. Note: the remaining lymphocytes may stain positive.
 reduced* Note: there are generally only a few or no B-lymphocytes within the respective anatomical zone.

Results of flow cytometry of peripheral blood cells (means)

Blood, spleen and thymus cells were placed into flow cytometry tubes containing combinations of antibodies to rat leukocyte cell surface proteins. These antibodies had been conjugated to different fluorochrome dyes to allow detection and discrimination of the different cell populations.

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.97	99.98	99.95	99.99*	99.96	100.00	100.00
T-lymphocytes (CD45 ⁺ CD3 ⁺ cells) (% of leukocytes)	44.15	43.75	42.64	42.32	28.36*	50.26	48.16
T-helper-lymphocytes (CD3 ⁺ CD4 ⁺ cells) (% of T-lymphocytes)	83.80	83.84	82.87	82.12	80.41	80.70	79.58
Double positive T-lymphocytes (CD3 ⁺ CD4 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	1.54	0.70*	1.00	1.01	0.66*	2.16	2.83
Cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	16.86	15.79	17.34	18.01	18.90	20.60	22.28
B-lymphocytes (CD45 ⁺ CD45RA ⁺ cells) (% of leukocytes)	45.60	45.93	47.30	46.47	58.73*	35.78	33.54
Monocytes (CD3 ⁻ CD4 ⁺ cells) (% of analysed cells)	3.59	3.15	2.81*	2.88*	6.14*	9.04	11.58
Total Natural Killer Cells (CD45 ⁺ CD161 ⁺ cells) (% of leukocytes)	4.80	4.11	3.97	4.16	5.36	5.51	6.47
Ratio of T-lymphocytes (CD3 ⁺) to B-lymphocytes (CD45RA ⁺) (calculated)	1.00	1.04	0.96	0.96	0.50*	1.46	1.51
Ratio of T-helper-lymphocytes (CD3 ⁺ CD4 ⁺) to cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺) (calculated)	5.22	5.58	5.15	4.72	4.53	4.08	3.63

* Statistically significant differences (p<0.05) versus Control

Cyclosporine A treatment tended to decrease T-cells and increase B-cells in peripheral blood. There was an isolated effect of flibanserin treatment to decrease monocytes in the MD and HD.

Results of flow cytometry of spleen cells (immune cell activity)

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.97	99.98	99.99*	99.99*	99.98*	100.00	100.00
T-lymphocytes (CD45 ⁺ CD3 ⁺ cells) (% of leukocytes)	44.67	44.01	45.11	44.73	37.95	45.47	45.58
T-helper-lymphocytes (CD3 ⁺ CD4 ⁺ cells) (% of T-lymphocytes)	67.05	67.66	66.83	68.68	60.48	67.77	73.15*
Double positive T-lymphocytes (CD3 ⁺ CD4 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	0.87	0.95	0.91	1.07	0.87	1.06	1.23
Cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	28.38	28.92	30.98	28.88	35.69*	29.52	24.31
B-lymphocytes (CD45 ⁺ CD45RA ⁺ cells) (% of leukocytes)	44.62	43.45	44.10	43.76	46.60	27.17	31.37*
Monocytes (CD3 ⁻ CD4 ⁺ cells) (% of analysed cells)	4.18	5.30	4.92	5.77	6.93*	12.81	12.26
Total Natural Killer Cells (CD45 ⁺ CD161 ⁺ cells) (% of leukocytes)	6.50	6.45	6.75	6.43	8.29	16.03	11.94*
Ratio of T-lymphocytes (CD3 ⁺) to B-lymphocytes (CD45RA ⁺) (calculated)	1.06	1.02	1.11	1.14	0.84	1.73	1.49
Ratio of T-helper-lymphocytes (CD3 ⁺ CD4 ⁺) to cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺) (calculated)	2.54	2.43	2.41	2.53	1.72*	2.38	3.14

* Statistically significant differences (p<0.05) versus Control

Cyclosporin administration resulted in a decrease of both the ratio of T to B lymphocytes and the ratio of T-helper to cytotoxic T-lymphocytes. There was a relative increase in both monocytes and natural killer cells in these animals.

Results of flow cytometry of thymus cells

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Number of thymus cells (x 10 ⁻⁷)	27.10	25.57	30.87	30.80	23.59	25.35	30.88
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.51	99.73	99.82	99.30	99.80	99.56	99.64
CD3 ⁺ thymus cells (% of thymus cells)	21.76	18.14	20.91	18.61	6.97*	14.39	20.13
CD4 ⁺ thymus cells (% of analysed cells)	95.81	95.29	94.69*	94.94	95.46	96.66	96.92
CD8 ⁺ thymus cells (% of analysed cells)	91.93	92.59	91.79	92.60	98.45*	95.83	97.06
CD4 ⁺ CD8 ⁻ thymus cells (% of analysed cells)	6.79	5.96	6.66	5.97	0.72*	3.34	2.48
CD4 ⁻ CD8 ⁺ thymus cells (% of analysed cells)	3.03	3.39	3.90*	3.77*	3.81	2.60	2.72
CD4 ⁺ CD8 ⁺ thymus cells (% of analysed cells)	88.90	89.20	87.90	88.82	94.64*	93.23	94.35
CD4 ⁻ CD8 ⁻ thymus cells (% of analysed cells)	1.28	1.45	1.55	1.44	0.83*	0.83	0.46*
CD3 ⁺ CD4 ⁺ CD8 ⁻ thymus cells (% of CD3 ⁺ cells)	26.50	28.59	28.93	27.52	3.23*	19.03	9.37
CD3 ⁺ CD4 ⁻ CD8 ⁺ thymus cells (% of CD3 ⁺ cells)	7.03	5.91	7.48	8.18	5.30	5.93	4.87
CD3 ⁺ CD4 ⁺ CD8 ⁺ thymus cells (% of CD3 ⁺ cells)	63.35	62.72	60.99	61.49	89.57*	73.44	85.08*
CD3 ⁺ CD4 ⁻ CD8 ⁻ thymus cells (% of CD3 ⁺ cells)	3.12	2.79	2.60	2.81	1.90*	1.61	0.69*
Ratio of CD4 ⁺ to CD8 ⁺ thymus cells (calculated)	1.04	1.03*	1.03	1.03	0.97*	1.01	1.00*

* Statistically significant differences (p<0.05) versus Control

Cyclosporine treatment reduced the number of isolated thymocytes and shifted the thymocyte subsets. There was a reduction in more mature CD3⁺ and CD4⁺CD8⁻ thymocytes whereas the relative number of immature double positive CD4⁺CD8⁺ was increased. As a consequence, the relative number of mature CD3⁺CD4⁺CD8⁻ cells was markedly reduced. There was a small reduction in mature CD3⁺CD4⁻CD8⁺ thymocytes and the relative number of CD3⁺CD4⁺CD8⁺ thymocytes increased.

Natural Killer Cell activity of spleen cells

Daily Dose [mg/kg]	Mean Specific Release [%] at various effector to target cell ratios					
	ratio 200:1	ratio 100:1	ratio 50:1	ratio 25:1	ratio 12:1	ratio 6:1
Main study 0 (Control)	9.2	6.5	4.9	3.1	1.6	1.2
Main study 20 BIMT 17 BS	11.6	8.3	6.1	3.7	2.0	1.3
Main study 100 BIMT 17 BS	14.3*	10.3*	7.1	4.5	2.4	1.5
Main study 400/250 BIMT 17 BS	14.6*	10.5*	7.4	4.2	2.0	1.2
Main study 25 Cyclosporine A	25.7*	18.9*	12.7*	7.6*	4.1	2.6
Recovery: 0 (Control)	27.3	22.2	15.7	9.7	5.5	3.0
Recovery: 400/250 BIMT 17 BS	36.7*	30.3	20.9	12.6	7.0	4.3

* Statistically significant differences (p<0.05) versus Control

In the main study, animals treated with flibanserin had an increased killing of YAC-1 target cells compared to controls at both 100 and 250 mg/kg. At the higher dose, this increase was still seen after the recovery period. Treatment with Cyclosporin-A resulted in a pronounced increase in spleen cell NKC activity along with an increased phenotypic presence of NKC's in the spleen. The sponsor believes that the increase activity with HD flibanserin was due to the abnormally low control activity level. Hard to say although the NKC activity of the control for the recovery group was considerably higher. However, if control activity was higher, then the Cyclosporine A activity would not have shown an increase.

I'm no expert on immunotoxicity studies but it seems clear that cyclosporin A had activity in altering the numbers of T and B cells in blood, spleen and thymus. In contrast, flibanserin treatment clearly had no effects on immune cell numbers or ratios. However, flibanserin treatment at both 100 and 250 mg/kg did increase natural killer cell activity. Whether this was due to abnormally low activity in controls is not clear.

Overall, it seems that the effect of flibanserin administration on immune activity is minimal at most.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Flibanserin is a 5-HT_{1A} agonist and 5-HT_{2A} antagonist that is intended to treat women with hypoactive sexual desire disorder (HSSD). In studies in mice, chronic flibanserin administration decreased serotonin levels in the median prefrontal cortex and the nucleus accumbens shell while increasing norepinephrine levels. Effects on dopamine appear mixed.

Flibanserin had a number of neurological effects, some severe at high doses but not unexpected for a centrally acting drug. In rats, flibanserin produced mild elevations of blood pressure and heart rate but had no effect on the ECG rhythm or waveform morphology. There were no respiratory effects in rats.

Flibanserin is metabolized extensively but is considered the active moiety since most of the metabolites are pharmacologically inactive and only two active metabolites cross the blood brain barrier, and neither are near the concentration of flibanserin.

In chronic toxicity studies in rats and dogs, flibanserin produced clear clinical signs of discomfort at high doses (exposures 28 times higher in rats and 100 times higher in dogs than human exposure). In dogs there were dose-related ophthalmologic and histopathologic changes of the eyes characterized by discrete, focal, smoky opacity or by focal thickening of the corneal epithelium. This effect was also seen in a shorter, 13 week study in dogs but was not seen in any study in rats.

Also in dogs, flibanserin produced fatty changes in the liver of males and females after 26 and 52 weeks. This effect seemed both dose and duration related with an increase in severity and numbers of dogs affected. Hepatocellular fatty changes were also seen in the 13 and 26 week rat toxicity studies.

There was an increase in fatty accumulation in the myocardium of dogs of both sexes without an increase in severity or number of dogs affected from 26 to 52 weeks.

This fat deposition occurred in both the MD and HD with the no effect level being 3 mg/kg or approximately 0.6 times the human exposure. The mechanism for the fat deposition is unknown but it did not produce measurable changes in cardiac electrical patterns measured throughout the study. Significantly, there was no increase in cardiac fat deposition in rats at any dose. There were fatty changes in the livers of both rats and dogs.

Because of the ophthalmologic findings in dogs, the Sponsor was asked to include ophthalmologic endpoints in a clinical trial. In A 24 week, randomized, double blind placebo controlled trial with an open-label extension, exams were performed at screening and end of treatment and included the best corrected distance visual acuity, tonometry (intraocular pressure measurement) and with pupils dilated, slit lamp evaluation of the anterior segment, including the cornea and lens. There were no increased findings or corneal abnormalities with flibanserin treatment.

Flibanserin was not mutagenic in vitro in bacteria or Chinese hamster ovary cells but was positive in the in vitro human lymphocyte assay. It was negative in the in vivo rat micronucleus assay and the Comet assay for DNA damage. Based on the weight of evidence, I consider flibanserin to be non-genotoxic.

When administered orally to mice and rats for two years, flibanserin increased the incidence of liver tumors. In male mice there was a significant increase in hepatocellular carcinomas. In females, there was an increase in the incidence of hepatocellular carcinomas and combined carcinomas and adenomas in the three higher dosed groups that was significant pairwise and for trend at the high dose group. In rats, there was a modest increase in the incidence of hepatocellular carcinomas in males but not in females (significant for trend against one control group only). Flibanserin induced centrilobular hepatocellular hypertrophy probably by inducing hepatic cytochrome P-450 enzymes. Sponsor believes that flibanserin is acting like phenobarbital in promoting liver tumors. Because flibanserin is an enzyme inducer in rodents and is essentially non-genotoxic, this seems like a reasonable explanation.

In the mammary gland of female mice, there was a clear dose-related increase in adenocarcinomas and malignant adenoacanthomas. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes. This effect was not seen in rats.

In reproduction studies in rats and rabbits, flibanserin administration did affect the duration of the estrus cycle in rats but had no effect on female fertility as measured by copulation index, fertility index or gestation index. When given during the period of organogenesis, flibanserin produced some visceral and skeletal variations in rats at the 400 mg/kg dose (approximately 42 times human exposure) and there were different malformations in pups from all treated groups without a discernable pattern. There were resorptions in rabbits treated with 80 mg/kg (26 times human exposure) but there was no increase in fetal variations or malformations. In a pre- and postnatal development study in rats, flibanserin produced maternal toxicity in all groups with a significant reduction in number of implantations and newborns from dams treated with the HD (200 mg/kg or roughly 30 times human exposure). Dams treated with the HD showed poor maternal care leading to failure to thrive and high mortality. There were some development delays in these pups but fertility and the F₂ generation seemed unaffected.

In general, flibanserin administration produced no clear signal for adverse effects on fetal development. Although almost all malformations occurred in fetuses from treated rats they affected different organ systems without an obvious common mechanism. In both the embryofetal and the prenatal and postnatal development study, flibanserin administration resulted in pups with anophthalmia. In the rat embryofetal development study, two pups from one litter from the HD had anophthalmia and in the postnatal study, there were two affected fetuses from the high dose group (drug blood levels not determined but probably around 30 times human exposure extrapolating roughly from the rat embryofetal development toxicokinetic data). There was one fetus with hydrocephalus in both treated rats (42 times human exposure) and rabbits (8 times human exposure). Considering the different types of malformations, lack of dose response and the fairly high drug exposure levels in which they occurred, the animal data do not indicate to me that the offspring are at significant risk if flibanserin is inadvertently taken by a pregnant woman.

In special toxicology studies, flibanserin increased natural killer cell activity but had no other effects on the rat immune system and did not seem to produce self-administration behavior in rats.

In general, flibanserin produced few significant toxicological effects in rats or dogs. In reproduction studies, flibanserin did not affect fertility and was not obviously teratogenic. In carcinogenicity studies, flibanserin produced malignant mammary tumors in mice but not rats.

Unresolved toxicology issues (if any): none

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/s/

ALEXANDER W JORDAN
07/16/2015



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22526
SERIAL NUMBER: 001
DATE RECEIVED BY CENTER: Resubmission March 29, 2013
PRODUCT: Trade Name (flibanserin)
INTENDED CLINICAL POPULATION: Women with hypoactive sexual desire disorder
SPONSOR: Sprout Pharmaceuticals Inc
DOCUMENTS REVIEWED: ECT
REVIEW DIVISION: Division of Bone, Reproductive and Urological Products
PHARM/TOX REVIEWER: Alex Jordan, PhD
PHARM/TOX SUPERVISOR: Lynnda Reid, PhD
DIVISION DIRECTOR: Hylton Joffe, MD
PROJECT MANAGER: Charlene Williamson

Date of review submission to DARRTS: August 28, 2013

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EXECUTIVE SUMMARY

I. Recommendations

Recommendation on approvability: I recommend approval of flibanserin (Trade Name) for women with hypoactive sexual desire disorder.

Recommendation for nonclinical studies: none

Recommendations on labeling

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C

In reproduction toxicity studies in rats given flibanserin during the period of organogenesis, there were sporadic malformations at exposures ≥ 3 times human exposure based on drug blood levels (AUC). In rabbit reproduction studies, there was an increase in resorptions at 26 times human exposure but there was no increase in fetal malformations. There are no adequate and well controlled studies of flibanserin in pregnant women. Trade Name should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

8.2 Nursing Mothers

It is not known if flibanserin is excreted in human milk. However, flibanserin is excreted in rat milk. Because many drugs are excreted in human milk, caution should be exercised when Trade Name is administered to a nursing mother.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility:

Carcinogenesis

Oral administration of flibanserin for 2 years produced a significant dose-related increase in the incidence of malignant mammary gland carcinomas in female mice at drug levels approximately 3 and 10 times the levels in women taking the recommended dose. In male and female mice, flibanserin administration increased the incidence of liver tumors at exposures approximately 10 times the exposure for women taking 100 mg. There was a non-significant increase in liver tumors in male rats.

Mutagenesis

Flibanserin was negative for mutagenesis in vitro in *Salmonella typhimurium* (Ames test) and in Chinese hamster ovary cells. It was positive for chromosomal aberrations in cultured human lymphocytes but was negative for chromosomal aberrations in vivo in the rat bone marrow micronucleus assay and for DNA damage in rat liver in the Comet assay.

Impairment of fertility

Flibanserin administration to female rats 2 weeks before mating until gestation day 6 resulted in slight changes in the duration of the estrus cycle, decreases in body weight gain and some neurological signs at high doses. Flibanserin administration had no effect on fertility or early embryonic development.

In my earlier NDA review, I had suggested restricting flibanserin to women with a history of breast cancer or at high risk for breast cancer due to the increased incidence of mammary tumors in female mice. I have eliminated that suggestion in this review because of the difficulty in prescribing and compliance coupled with the fact that the risk to of breast cancer to women from flibanserin is exceedingly small if at all. Although the increase in mammary tumors in mice was significant, it did not occur in female rats, flibanserin is not genotoxic and the incidence (percent) of mice with mammary cancer only slightly exceeded the historical control incidence.

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings: In rats, chronic administration of flibanserin resulted in an increase in weights of the thymus, adrenal and liver with a reduction in fat deposition in the liver of males and an increase in hepatocellular hypertrophy in females treated with 400 mg/kg daily (approximately 29 times human exposure). In dogs, chronic flibanserin administration produced corneal opacities, increased heart rate and an increase in the frequency and intensity of fatty liver and heart which seemed dose and duration dependent. The fatty accumulation in the heart occurred only in dogs at exposure levels in females of approximately 5 times the exposure in women taking 100 mg. In the adrenal, there was an increase in fat accumulation which was dose but not duration dependent. There was a degeneration of the mucous membranes of the trachea in dogs treated with 15 or 75 mg/kg/day (5 to 53 time human exposure). In mice, there was a dose-related statistically significant increase in malignant mammary tumors. This was not seen in rats. The weight of evidence indicates that flibanserin is not genotoxic. Flibanserin had no significant effects on fertility in male or female rats. When flibanserin was given to rats during embryogenesis, there was an increase in fetal variations at the high dose and there were sporadic non dose-related fetal malformations. There were no adverse fetal effects in rabbits other than an increase in resorptions at the high dose. In a pre- and postnatal development study in rats, flibanserin reduced the number of implantations and newborns from dams treated with the HD (200 mg/kg or roughly 30 times human exposure). Dams treated with the HD showed poor maternal care leading to failure to thrive and high mortality. There were some development delays in these pups but fertility and the F₂ generation seemed unaffected.
- B. Pharmacologic activity: Flibanserin acts on the serotonin receptor and functions as a 5-hydroxytryptamine (5-HT)_{1A} agonist 5-HT_{2A} antagonist that decreases extracellular serotonin and increases norepinephrine in rat prefrontal

cortex, medial preoptic area and nucleus accumbens. There are also regional, dose-related effects on dopamine. These effects are believed to be related to its effect on increased sexual desire in females.

- C. Nonclinical safety issues relevant to clinical use: The major issue is the increased incidence of mammary tumors in mice. Flibanserin is a new molecular entity with a neurochemical mechanism indicated for hypoactive sexual desire disorder in women. Mammary tumors are not particularly common in mice so there is some concern about an increased risk of breast cancer. However, flibanserin does not seem to be genotoxic and there was no increased incidence of mammary tumors in rats. The incidence of mammary tumors in mice while significantly elevated compared to concurrent controls, was only somewhat higher than the historical control maximum, thus the risk, if any, for breast cancer in women seems slight.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-526

Review number: 1

Sequence number/date/type of submission: EDR

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Sprout Pharmaceuticals

Manufacturer for drug substance: (b) (4)

Reviewer name: Alex Jordan, PhD

Division name: Reproductive and Urological Products

HFD #:

Review completion date:

Drug:

Trade name:

Generic name: flibanserin

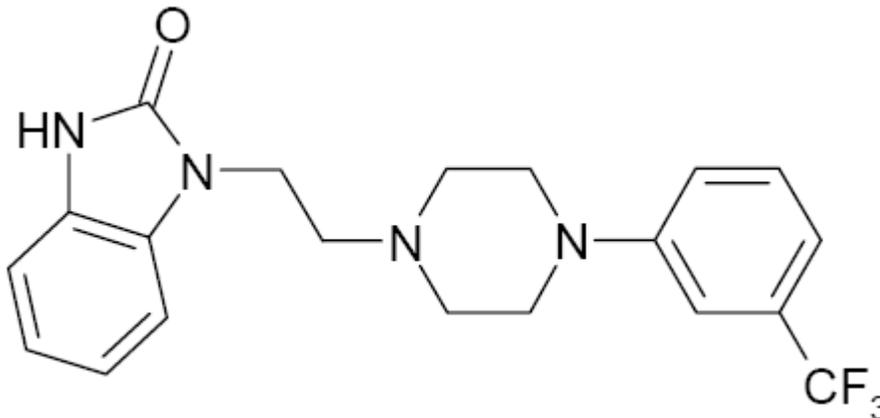
Code name: BIMT 17 BS

Chemical name: 2H-benzimidazol-2-one, 1,3-dihydro-1-[2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl].

CAS registry number:

Molecular formula/molecular weight: 390.4 g/mol

Structure:



Relevant INDs/NDAs/DMFs: IND (b) (4)

Drug class: Serotonin modulator

Intended clinical population: Women with hypoactive sexual desire disorder

Clinical formulation:

Table 1 Qualitative and quantitative composition of flibanserin film-coated tablets, 100 mg

Ingredient	mg per Tablet	Function	Reference to Standards
Flibanserin	100.000	Active ingredient	Company Standard
Lactose monohydrate (b) (4)	(b) (4)	(b) (4)	NF/Ph. Eur./JP
Microcrystalline cellulose (b) (4)			NF/Ph. Eur./JP
Hypromellose (b) (4)			USP/Ph. Eur./JP
Croscarmellose sodium			NF/Ph. Eur./JP
Magnesium stearate (b) (4)			NF/Ph. Eur./JP
(b) (4) Pink (b) (4)			USP/Ph. Eur./JP
			Company Standard
Total			347.0

Route of administration: oral

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Flibanserin acts preferentially as a post-synaptic 5-hydroxytryptamine (HT) [serotonin]_{1A} receptor agonist and a 5-HT_{2A} receptor antagonist. Flibanserin had high affinity for human 5-HT_{1A} recombinant receptors ($K_i = 6.59$ nM) and was a full agonist on 5-HT_{1A} receptors when receptors were expressed in Chinese hamster ovary (CHO) cells. Flibanserin had high affinity ($K_i = 15.3$ nM) and was a full antagonist for human 5-HT_{2A} receptors when expressed in CHO cells. Flibanserin also had moderate affinity for 5-HT_{2B}, 5-HT_{2C} and dopamine (DA) D₄ receptors. Flibanserin does not have physiologically significant affinity for human monoamine transporters, does not inhibit reuptake of these neurotransmitters into synaptosomes expressing rat monoamine transporters and does not inhibit monoamine oxidase type A or B in rat brain.

Flibanserin was originally developed to treat depression. In Phase 2 depression trials, flibanserin was not associated with sexual dysfunction, which is unusual for serotonergic antidepressants. A multi-dimensional measure of sexual dysfunction, the Arizona Sexual Experiences Scale (ASEX), was included in the four Phase 2b depression studies for comparison of flibanserin, standard antidepressants, and placebo. In one of these trials,

flibanserin was superior to both the positive comparator and placebo on the ASEX scale, mainly on the "sex drive" item in women.

Hypoactive sexual desire disorder (HSDD) is defined in the DSM-IV-TR as a deficiency or absence of sexual fantasies and desire for sexual activity that causes marked distress or interpersonal difficulty and is not due to the physiological effects of a substance or general medical condition. The biological causes of HSDD are only poorly understood and to date there are no approved pharmacological treatments in the US. Clinical development and off-label usage has focused on hormone products including a testosterone transdermal system that has been approved for surgically postmenopausal women in Europe.

The therapeutic mechanism of action of flibanserin in HSDD is unknown but there is evidence for the involvement of serotonin. Serotonin reuptake inhibitors (SSRIs) produce a number of sexual side effects with the most common being anorgasmia or delayed orgasm. There are also reports of spontaneous orgasm in conjunction with fluoxetine treatment and reports of hypersexual effects following treatment with fluoxetine or the serotonin releaser, fenfluramine suggesting that both inhibited and enhanced sexual responses to serotonergic agents may be produced. Flibanserin has been shown to stimulate 5-HT_{1A} receptors while blocking 5-HT_{2A} receptors and has moderate affinity to 5-HT_{2B,2C} receptors. These 5-HT receptor mediated mechanisms directly affect serotonergic neurotransmission but also have some indirect effects on dopaminergic and noradrenergic neurotransmitter signaling. Flibanserin decreases extracellular serotonin levels and increases the extracellular dopamine concentration in the medial prefrontal cortex in rats. This effect is completely abolished by the selective 5-HT_{1A} antagonist WAY100.635.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Flibanserin binding studies to various receptors in vitro revealed a high affinity for 5-HT_{1A} ($K_i = 6.59$ nM) and 5-HT_{2A} ($K_i = 15.3$ nM). Flibanserin has a moderate affinity for 5-HT_{2B} ($K_i = 89.3$ nM), 5-HT_{2C} ($K_i = 88.3$ nM), and dopamine D₄ receptors ($K_i = 167$ nM). Flibanserin does not have physiologically significant affinity for monoamine transporters 5-HT: ($K_i = 2.63$ uM), DA: ($K_i = 2.63$ uM), NA ($K_i = 2.63$ uM), and does not inhibit monoamine A and B oxidase.

In human post mortem studies, flibanserin significantly reduced the activity of forskolin-stimulated adenylyl cyclase postsynaptically (prefrontal cortex), and in the hippocampus, but had no effect in the raphe nuclei (presynaptic level).

Flibanserin acts as a full agonist on 5-HT_{1A} receptors in reducing forskolin-stimulated adenylyl cyclase activity. This was observed in all tissues in the human dorsal raphe (largest serotonergic nucleus where some antidepressants are believed to act) which contrasts with other 5-HT_{1A} receptor agonists, which are inactive in reducing forskolin-stimulated AC activity in the cortex.

Flibanserin 5-HT_{1A} agonist activities result in many of its pharmacological properties in vivo. Flibanserin decreases firing rates of 5-HT neurons in the rat dorsal raphe nucleus and alters firing rates in the hippocampus. Flibanserin also reduces neuronal cortical activity and these decreases can be inhibited by 5-HT_{1A} antagonists.

Drug activity related to proposed indication:

Effect of acute and chronic treatment with flibanserin 15 and 45 mg/kg po on extracellular serotonin, norepinephrine, dopamine, GABA and glutamate in the medial prefrontal cortex and in nucleus accumbens shell of freely moving female rats.

This study was conducted at (b) (4) under study key 191 (parts A and B). Rats (280-350 g) were from (b) (4). Drug was flibanserin batch 05071202M.

Female Wistar rats in the nonreceptive stage (not in estrous) were anesthetized and placed in a stereotaxic frame and I shaped probes were inserted in the medial prefrontal cortex (mPFC) and Nucleus Accumbens shell (NAcc).

The rats were orally dosed with 0, 15 or 45 mg/kg flibanserin two times a day for 21 days. On the day of the experiment, the probes were connected with flexible PEEK tubing to a microperfusion pump and perfused with artificial CSF at a flow rate of 1.5 ul/min. Microdialysis samples were collected at 30 min intervals. After the experiment the rats were sacrificed and the brains removed and the position of each probe was verified histologically.

Concentrations of 5-HT, NA, DA, GABA and Glu were determined by HPLC separation and electrochemical and fluorometric detection.

Effects of chronic treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle

	5-HT	NA	DA	GABA	Glu
mPFC (15 mg/kg)	Decrease +	Increase +	----	----	----
NAcc (15 mg/kg)	----	----	----	----	----
mPFC (45 mg/kg)	Decrease +	Increase +	Increase +	----	----
NAcc (45 mg/kg)	Decrease +	----	(Decrease +)	----	----

---- no statistical significance
 (significant only at a single time point)
 + statistically significant

Sponsor concludes: At an oral dose of 15 and 45 mg/kg, extracellular serotonin and norepinephrine were significantly changed in the medial prefrontal cortex compared to controls.

At an oral dose of 45 mg/kg extracellular dopamine in the medial prefrontal cortex and serotonin in the nucleus accumbens were significantly changed compared to vehicle.

Basal absolute values of the neurotransmitters vary between the doses given. This variation is an effect of the compound when given chronically and is dose dependent in the prefrontal cortex with respect to dopamine and norepi.

Effects of acute and chronic treatment with flibanserin on extracellular serotonin, norepinephrine, dopamine, GABA and Glutamate in the Medial Preoptic Area of freely moving female rats.

Nonreceptive female Wistar rats were anesthetized and placed in a stereotaxic frame and I shaped probes were inserted into the medial preoptic area (MPOA).

Rats were given flibanserin orally twice a day for 21 days. On the day of the experiment, the probes were connected to flexible PEEK tubing and perfused with artificial CSF. On the day of the experiment the animals were challenged with flibanserin at different doses depending on the pretreatment dose. Animals treated with vehicle were challenged with vehicle on the first day. On the second day they received an acute dose of 45 mg/kg flibanserin.

Effect of chronic treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle.

	5-HT	NA	DA	GABA	Glu
MPOA (15 mg/kg)	Decrease +	Increase +	Decrease (+)	----	----
MPOA (45 mg/kg)	Decrease +	Increase +	----	----	----

---- not statistically significant

+ statistically significant

(+) significant only at a single time point

Effect of acute treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle.

	5-HT	NA	DA	GABA	Glu
MPOA (45 mg/kg)	Decrease +	Increase +	Increase (+)	----	----

---- not statistically significant

+ statistically significant

Flibanserin reduced 5-HT release in the mPFC, mPOA and Nacc, but not in the hippocampus. Flibanserin increased extracellular DA in the mPFC and in the mPOA of the hypothalamus, but not in the Nacc or hippocampus. Repeated treatment of flibanserin induced selective effects. In rats treated twice daily for 15 or 45 mg/kg for 21 days, flibanserin administration decreased serotonin and increased NA in the mPFC and mPOA. DA levels were increased only in the mPFC (45 mg/kg) and not in the mPOA or Nacc. Serotonin was decreased in the Nacc (45 mg/kg) but no changes in NA or DA were observed. Repeated treatment increased basal levels of DA and NA in the mPFC but only NA was increased in the Nacc.

Animal model for female sexual behavior

The bilateral chamber test is used to examine sexual incentive motivation. The chamber consists of an upper and lower level which allows the female to escape the male and thus pace mating. Female Long-Evans rats (13 per group) were ovariectomized and rendered sexually receptive with injections of estradiol and progesterone. Flibanserin was administered orally at doses of 5, 15 and 45 mg/kg for 28 days. Sexual behavior was noted during 30 minute period tests on days 1, 8, 15, 22 and 29. Proceptivity was defined as gestures such as solicitations (headwise orientation toward the male followed by abrupt runaway terminated by the assumption of a crouching posture), hops and darts and ear wiggings. Receptivity or readiness to allow copulation is represented by lordosis.

Flibanserin induced a dose-dependent increase in solicitations on days 15 and 22 but not on the other days. There were no other effects of sexual motivation such as ear wiggles or hops and darts. Similarly, there was no effect on lordosis behavior.

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects:

In mice, flibanserin administration at an oral dose of 64 mg/kg resulted in a significant reduction in locomotor activity and hypothermia. Four of eight mice exhibited hindlimb abduction, one exhibited flaccid abdominal tone, two had reduced grip strength and one showed grasping in the traction test. After 128 mg/kg flibanserin, a complete inhibition of locomotor activity, an increase in hot plate reaction time and hypothermia were observed. All treated animals had marked hindlimb abduction and head weaving. One mouse showed straub-tail (erect tail often seen with opiate treatment), three had ptosis, six had reduced grip strength, six exhibited grasping in the traction test and two mice were cataleptic.

Cardiovascular effects:

Arterial blood pressure, heart rate and ECG (lead II) were measured in two male and two female Beagles which had previously been chronically implanted with telemetry transmitters. Following a predose recording for 30 minutes, the animals were orally dosed with 3, 10 or 30 mg/kg flibanserin or vehicle control.

Flibanserin treatment induced mild but statistically significant elevations in mean arterial blood pressure at the high dose. Time to peak effect was approximately 1-2 hrs after dosing and these effects had abated after about 5 hrs.

Mean heart rate was mildly elevated approximately 3 hrs after dosing at 30 mg/kg.

Flibanserin produced no adverse changes to the ECG rhythm or waveform morphology at any dose. There were treatment-related changes in the RR, PR and uncorrected QT interval which would be anticipated from mild elevation of heart rate at the high dose. There was no evidence of QT prolongation at doses (3, 10 and 30 mg/kg) that produced geometric overall mean plasma concentrations at one hour of 250, 549 and 1190 ng/ml, respectively.

Maximum changes in arterial pressure in comparison with mean pre-dose values and the approximate times of peak effect

Treatment & dose (mg/kg)	Maximum increase from pre-dose values at time measured, for:					
	Systolic (mmHg)		Diastolic (mmHg)		Mean (mmHg)	
	Pre*	Change	Pre*	Change	Pre*	Change
Vehicle	137	+14 (1 hr)	87	+15 (1 hr)	104	+15 (1 hr)
Flibanserin (3 mg/kg)	139	+17 (3.17 hr)	91	+14 (4.17 hr)	107	+14 (3.17 hr)
Flibanserin (10 mg/kg)	138	+23 (1 hr)	89	+18 (1 hr)	105	+20 (1 hr)
Flibanserin (30 mg/kg)	134	+27 (1.17 hr)	87	+24 (2 hr)	103	+26 (2 hr)

Flibanserin was shown to block hERG-mediated potassium current in HEK293 cells with an IC₅₀ of 1.18 μ M.

Fraction of hERG current (I/I_0) at four different concentrations of flibanserin

Concentration	individual experiment			mean	SD
0.1 μ M	0.90	0.87	0.93	0.90	0.03
0.3 μ M	0.61	0.73	0.64	0.66	0.06
1.0 μ M	0.46	0.44	0.58	0.49	0.08
3.0 μ M	0.37	0.31	0.27	0.32	0.05

Flibanserin has no effect on the action potential configuration in guinea pig papillary muscles at concentrations up to 10 uM. Increasing the concentration up to 30 uM produced a shortening of the action potential.

Pulmonary effects:

Flibanserin was investigated for effects on respiratory rate, tidal volume and minute volume following a single oral administration to rats at doses of 20, 100 and 250 mg/kg.

The low dose of 20 mg/kg had no effect on any parameter. At 100 mg/kg and above, a moderately delayed decrease in respiratory rate and moderate to marked delay of the normal decrease in tidal volume were observed.

Renal effects: not done

Gastrointestinal effects: not done

Abuse liability:

Effects of flibanserin and amphetamine in the self-administration paradigm in male Wistar rats

Flibanserin was compared to amphetamine and saline in the self-administration paradigm in male rats. Rats were trained to lever-press in the operant chambers with sucrose pellets as reinforcement, on a fixed ratio (FR)-one schedule of reinforcement.

Operant chambers were equipped with two levers – one designated active, the other inactive. Drug delivery was accomplished via a jugular vein catheter through an injector mounted on a swivel-arm apparatus, which was connected to a syringe pump delivering a 10 uL drug infusion upon active lever responding. Each drug delivery was followed by a 20 second time-out period in which lever pressing produced no consequence.

During the acquisition/maintenance phase, all animals were trained to lever press for infusions of their assigned drug (amphetamine, flibanserin or saline) on an FR-1 schedule of reinforcement to establish self-administration behavior.

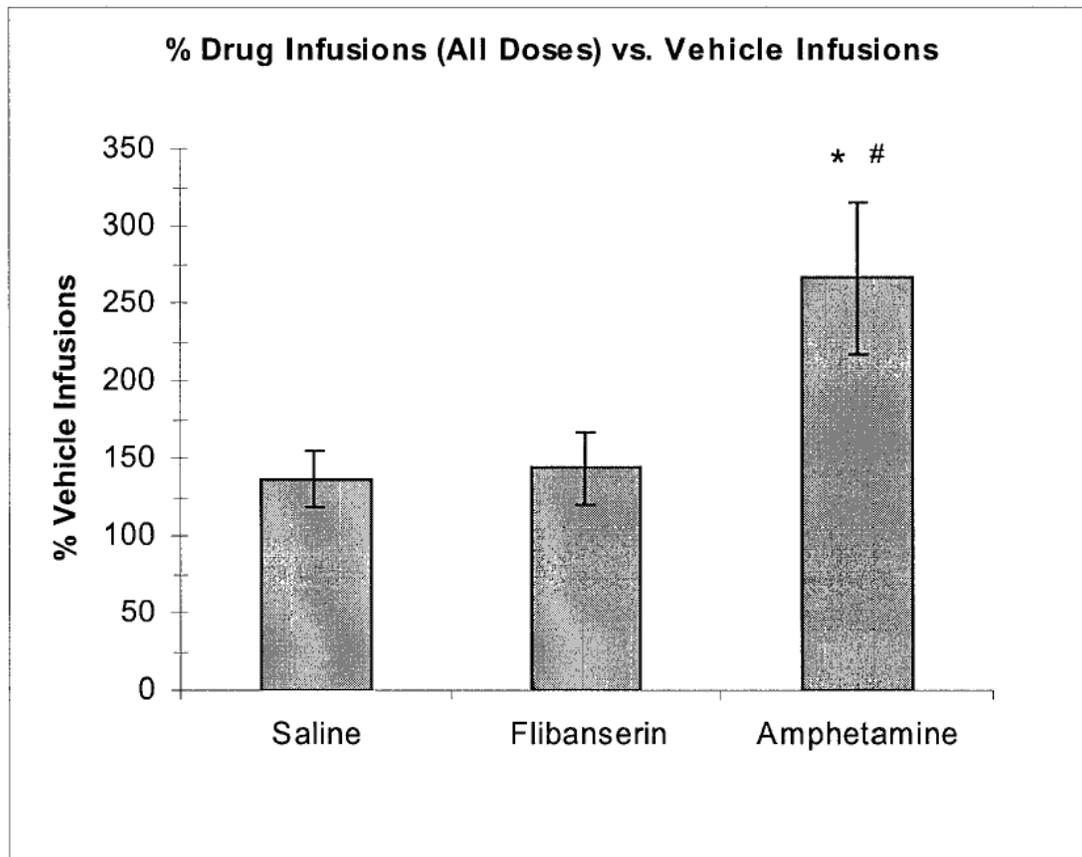
Flibanserin was dissolved in 25% polyethylene glycol and administered at doses of 0, 25, 50 and 100 ug/infusion. D-amphetamine was given at doses of 0, 5, 10 and 20 ug/infusion.

Dosing Schedule for Dose-Response Self-Administration Phase

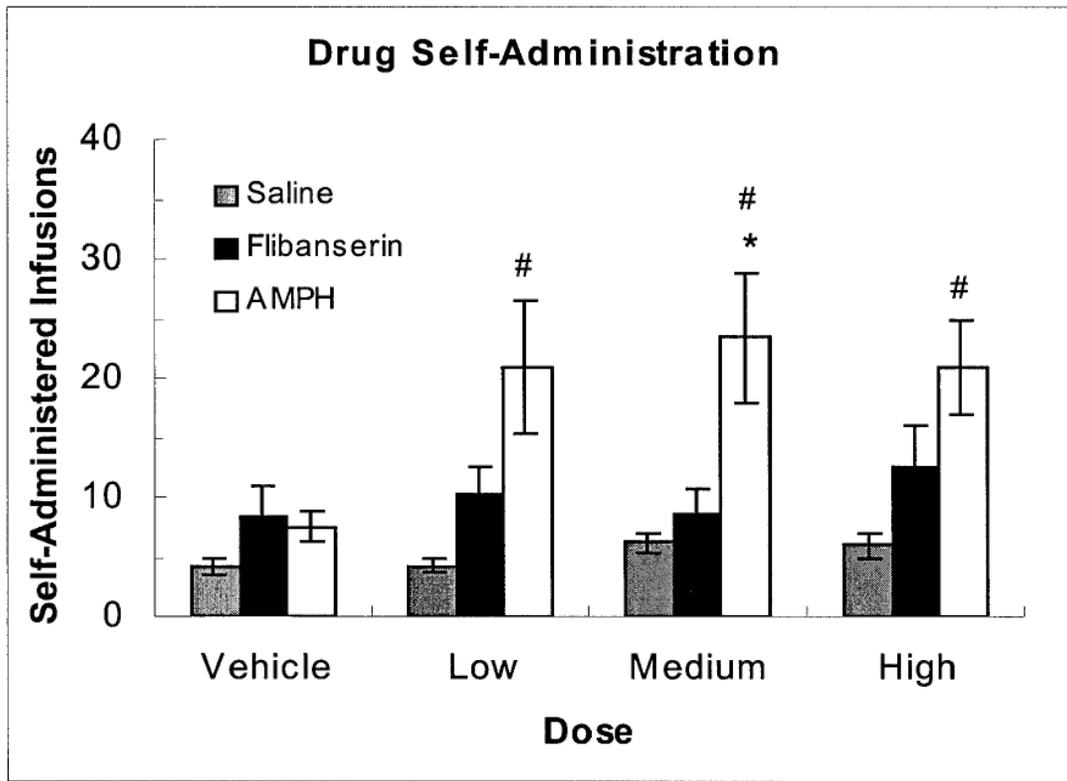
Dose-Response Test Days	Amphetamine Group	Flibanserin Group	Saline Group
Day 1-4	0.5 mg/mL (5 µg/infusion)	2.5 mg/mL (25 µg/infusion)	0.9% saline
Day 5-8	1 mg/mL (10 µg/infusion)	5.0 mg/mL (50 µg/infusion)	0.9% saline
Day 9-12	2 mg/mL (20 µg/infusion)	10 mg/mL (100 µg/infusion)	0.9% saline
Day 13-15	0 mg/mL (0 µg/infusion)	0 mg/mL (0 µg/infusion)	0.9% saline

- The total duration of the Dose-Response Phase was 15 days.
- Testing of the vehicle (0 µg/infusion) was limited to 3 days, rather than 4, to reduce the likelihood of extinction of self-administration behaviour.
- Animals responded on an FR-1 schedule of reinforcement.
- One priming infusion of the appropriate drug commenced all sessions
- Infusion volume for all reinforcers was 10 µL, with a 20 second time-out (during which lever pressing produced no consequence) following each infusion

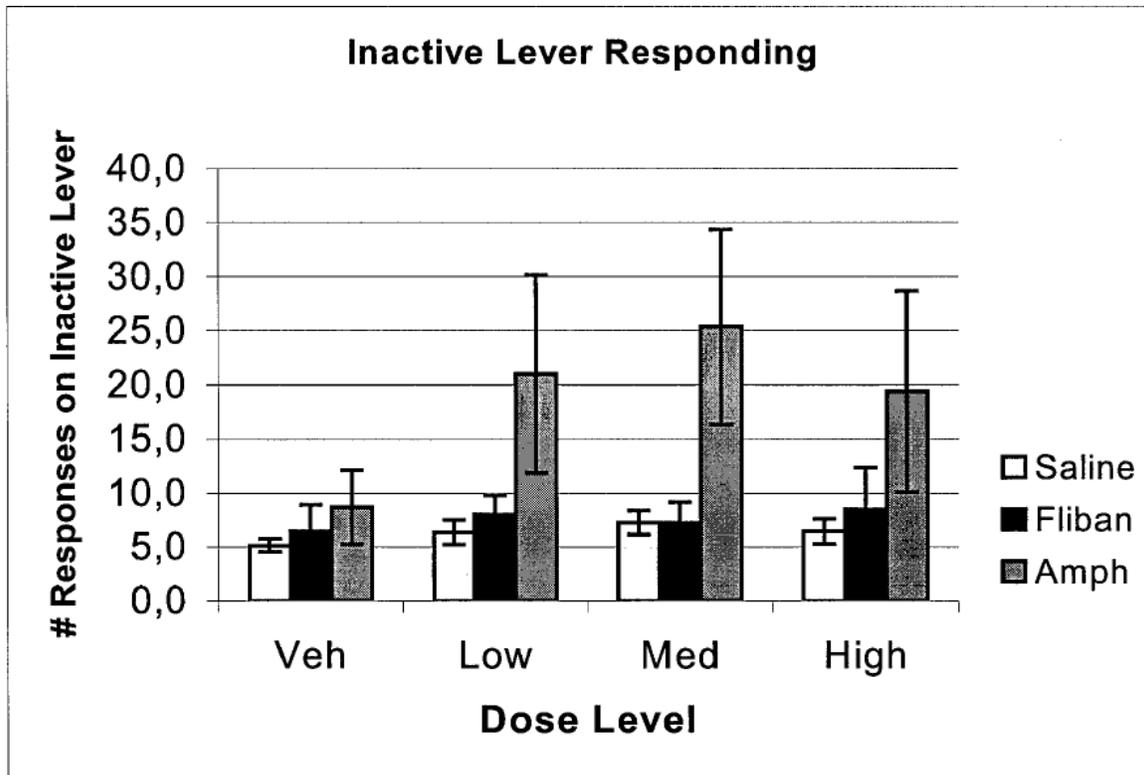
Sample sizes were amphetamine n=6, flibanserin n=5 and saline n=4.



Bars represent the mean (\pm SEM) number of infusions delivered for each dose group, expressed as a percentage of the mean number of vehicle administrations. Flibanserin did not differ significantly from saline.



Mean ± SEM of number of self-administered infusions of each test drug at each dose levels. None of the test doses of flibanserin differed significantly from saline.



Relative to vehicle, animals had a significantly higher number of self-administered infusions of amphetamine than flibanserin or saline in the first days of the experiment. However, amphetamine treated rats had a higher number of inactive lever pulls than controls, indicating that amphetamine increased overall activity such that the self administration of amphetamine may be a response to hyperactivity and not a signal of self-reward.

All data are highly variable and amphetamine showed an overall tendency to be self-administered, the results with flibanserin seem to indicate that this compound may not be self-administered, even if no definitive conclusions can be drawn due to the small sample size and the failure of the positive control to clearly elicit self-administration behavior.

2.6.2.5 Pharmacodynamic drug interactions

Flibanserin was active in several animal models sensitive to antidepressants and anxiolytics but it did not elicit depression- or anxiety-like behavior. Flibanserin had antinociceptive activity in two models of acute pain which was antagonized by naloxone and potentiated by morphine. Flibanserin has a low affinity for opioid receptors so the action may be indirect. Flibanserin did not impair learning and memory in a water maze test in rats.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

In male and female rats, absorption was relatively fast after oral administration of a suspension with a T_{max} of approximately 1 h. Absolute bioavailability (F) was 35% in males and 65% in females despite almost complete absorption suggesting a moderate first pass effect. After IV administration the clearance (CL) was 27 ml/min/kg in males and 18 ml/min/kg in females indicating a fast clearance of parent drug. Plasma t_{1/2} of parent was approximately 1-2 hrs at steady state. In contrast, the t_{1/2} for radioactivity in plasma was 66 h after oral dosing indicating the presence of long-lived metabolite(s). In humans, the t_{1/2} is 10-11 hrs for parent drug and 66 hrs for radioactivity.

In a pilot study in the cynomolgus monkey, drug bioavailability was only about 4% resulting in low blood levels and the decision not to use cynomolgus monkeys for toxicity studies. The AUC_{0-∞} for flibanserin in monkeys following oral administration of 3 mg/kg of flibanserin was 100 ng.h/ml compared to 2100 ng.h/ml in dogs following a similar dose.

Absorption in humans

Pharmacokinetics of flibanserin in healthy women following a single dose. Study no. 511.97 U07-1869

Table 11.5.2.2: 1 Comparison of geometric mean (gCV in %) key pharmacokinetic parameters of Flibanserin by treatment

Flibanserin		1 x 25 mg (F1_25) (N=22)	2 x 25 mg (F2_25) (N=22)	1 x 50 mg (F1_50) (N=22)	2 x 50 mg (F2_50) (N=22)	1 x 100 mg (F1_100) (N=20)
AUC _{0-∞}	[ng·h/mL]	515 (44.9)	1140 (38.6)	1140 (39.2)	2250 (34.2)	2320 (32.8)
AUC _{0-∞,norm}	[ng·h/mL/mg]	20.6 (44.9)	22.8 (38.6)	22.8 (39.2)	22.5 (34.2)	23.2 (32.8)
%AUC _{tz-∞}	[%]	5.74 (42.5)	3.36 (67.5)	3.58 (60.3)	2.78 (63.5)	2.48 (59.6)
AUC ₀₋₁₂	[ng·h/mL]	403 (39.4)	831 (32.9)	827 (31.5)	1610 (29.6)	1670 (33.7)
AUC _{0-12,norm}	[ng·h/mL/mg]	16.1 (39.4)	16.6 (32.9)	16.5 (31.5)	16.1 (29.6)	16.7 (33.7)
AUC ₀₋₂₄	[ng·h/mL]	472 (41.4)	999 (35.0)	1000 (34.6)	1960 (31.4)	2020 (32.9)
AUC _{0-24,norm}	[ng·h/mL/mg]	18.9 (41.4)	20.0 (35.0)	20.0 (34.6)	19.6 (31.4)	20.2 (32.9)
C _{max}	[ng/mL]	140 (34.8)	282 (35.8)	253 (27.2)	483 (38.6)	540 (36.5)
C _{max,norm}	[ng/mL/mg]	5.59 (34.8)	5.64 (35.8)	5.07 (27.2)	4.83 (38.6)	5.40 (36.5)
t _{max} ¹	[h]	0.750 (0.500-2.03)	0.750 (0.500-1.50)	0.750 (0.500-2.00)	0.750 (0.500-2.00)	0.750 (0.500-3.00)
t _½	[h]	8.22 (24.5)	9.95 (27.4)	9.77 (25.7)	10.1 (21.0)	10.5 (16.1)

¹ median and range

The AUC_{0-∞} for flibanserin in women taking a single dose of 100 mg was 2320 ng.h/ml, C_{max} was 540 ug/ml and the T_{1/2} was 10.5 hrs.

Human pharmacokinetics in patients (single dose). Study no. 511.105 U07-1871

Single dose	50 mg q.d. (N=30)		100 mg q.d. (N=28)		25 mg b.i.d. (N=33)		50 mg b.i.d. (N=32)	
	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]
AUC₀₋₁₂ [ng·h/mL]	685	36.4	1150	52.4	391	50.5	745	45.9
AUC_{0-12,norm} [ng·h/mL/mg]	13.7	36.4	11.5	52.4	15.7	50.5	14.9	45.9
AUC₀₋₂₄ [ng·h/mL]	817	39:4	1420	54.7	---	---	---	---
AUC_{0-24,norm} [ng·h/mL/mg]	16.3	39:4	14.2	54.7	---	---	---	---
AUC_{0-tz} [ng·h/mL]	816	39.3	1420	54.6	391	50.5	744	45.8
AUC_{0-∞} [ng·h/mL]	904	43.5	1630	54.6	474	53.2	919	52.0
AUC_{0-∞,norm} [ng·h/mL/mg]	18.1	43.5	16.3	54.6	19.0	53.2	18.4	52.0
%AUC_{tz-∞} [%]	8.34	56.5	11.0	59.8	16.4	36.8	17.3	42.5
C_{max} [ng/mL]	217	40.8	336	50.7	136	41.9	250	40.0
C_{max,norm} [ng/mL/mg]	4.33	40.8	3.36	50.7	5.45	41.9	4.99	40.0
t_{max}¹ [h]	0.875	0.500-3.00	1.00	0.500-3.00	0.750	0.500-3.00	1.00	0.500-3.00
t_{1/2} [h]	8.45 ²	23.3 ²	9.33	27.8	5.93 ³	24.8 ³	6.06	27.0
MRT_{po} [h]	8.46	29.2	10.0	35.4	6.47	24.6	6.79	30.2
CL/F [mL/min]	922	43.5	1020	54.6	878	53.2	907	52.0
V_z/F [L]	676	33.2	827	62.6	457	58.2	476	43.7

Source Data: Section 15, [Table 6.3: 1](#), [Table 6.2.1: 1](#) and [Table 6.2.1: 15](#)

¹ median and range

² N=31; ³ N=34

AUC_{0-∞} was 1630 ng.h/ml in patients following a single dose of 100 mg

Human pharmacokinetics in patients (steady state) Study no. 511.105 U07-1871

Steady state	50 mg q.d. (N=30)		100 mg q.d. (N=29)		25 mg b.i.d. (N=33)		50 mg b.i.d. (N=31)	
	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]
AUC_{τ,ss} [ng·h/mL]	991	47.7	2080	46.6	653	60.1	1400	46.9
AUC_{τ,ss,norm} [ng·h/mL/mg]	19.8	47.7	20.8	46.6	26.1	60.1	28.1	46.9
R_{A,AUC} []	1.20 ²	19.3 ²	1.44 ³	63.5 ³	1.70 ⁴	26.4 ⁴	1.84	21.4
C_{max,ss} [ng/mL]	234	41.2	469	42.7	168	50.9	346	34.4
C_{max,ss,norm} [ng/mL/mg]	4.68	41.2	4.69	42.7	6.72	50.9	6.91	34.4
R_{A,Cmax} []	1.09 ²	23.5 ²	1.36 ³	58.0 ³	1.28 ⁴	40.1 ⁴	1.37	31.4
t_{max,ss} ¹ [h]	1.00	0.417-4.00	1.00	0.500-3.00	1.00	0.500-3.00	0.750	0.500-3.00
t_{1/2,ss} [h]	10.1	23.3	11.4	24.3	11.9	25.5	11.6	21.4
MRT_{po,ss} [h]	9.44	34.5	11.3	27.8	11.1	30.2	12.1	29.5
CL/F_{ss} [mL/min]	841	47.7	803	46.6	638	60.1	593	46.9
V_Z/F_{ss} [L]	734	33.9	795	53.9	655	46.8	594	38.4

Source data: Section 15 [Table 6.3.1:1](#)

¹ median and range

² N=28; ³ N=27; ⁴ N=32

The steady state drug level (AUC_i) in patients taking 100 mg was 2080 ng.h/ml. This is the figure I have used for all cross-species comparisons of drug exposure.

Pharmacokinetic parameters for various species following a single dose

Route	Parameter	Unit	Mouse (m)	Rat (m/f)	Rabbit (f)	Dog (m&f)	Cynomolgus Monkey (m&f)	Man ^{a, d} (m)
	Dose	mg/kg	5	5 / 5	3	3.0 p.o. 3.3 i.v.	3	50 mg p.o. 20 mg i.v.
oral	C(max)	µg/mL	0.17	0.42 / 0.61	nd	0.64	0.057	0.25
	t(max) ^b	h	1.0	0.67 / 1.0	nd	0.5	1.0	0.5
	AUC(0-∞)	(µg/mL)·h	1.1	1.1 / 3.0	nd	2.1	0.1	0.75
	MRT	h	7.0	2.7 / 3.8	nd	3.3	1.7	5.9
	t(½)	h	5.5	nd / 2.5	nd	4.4	0.73	6.8
	MAT	h	6.1	1.2 / 1.0	nd	0.17	-0.1	nd
	F(a)	%	nd	119 ^c / 111	>72 ^c	105 ^c	nd	90
	F	%	84	35 / 65	nd	44	4.1	33
i.v.	AUC(0-∞)	(µg/mL)·h	1.3	3.1 / 4.7	3.5	5.1	2.4	0.91
	MRT	h	0.92	1.5 / 2.8	3.0	3.2	1.8	6.3
	t(½)	h	0.75	0.9 / 2.1	3.5	2.9	2.0	7.2
	CL	(mL/min)/kg	62	27 / 18	15	11	21	366 mL/min
	V(ss)	L/kg	3.4	2.3 / 3.0	2.6	2.0	2.3	134 L
<i>in vitro</i>	F(b)	%	98	97 / nd	98	98	98	98

^a body weight = 72 to 86 kg^b median^c from urinary data^d geometric means reported for human except of t(max) (median) and plasma protein binding (arithmetic mean)

2.6.4.4 Distribution

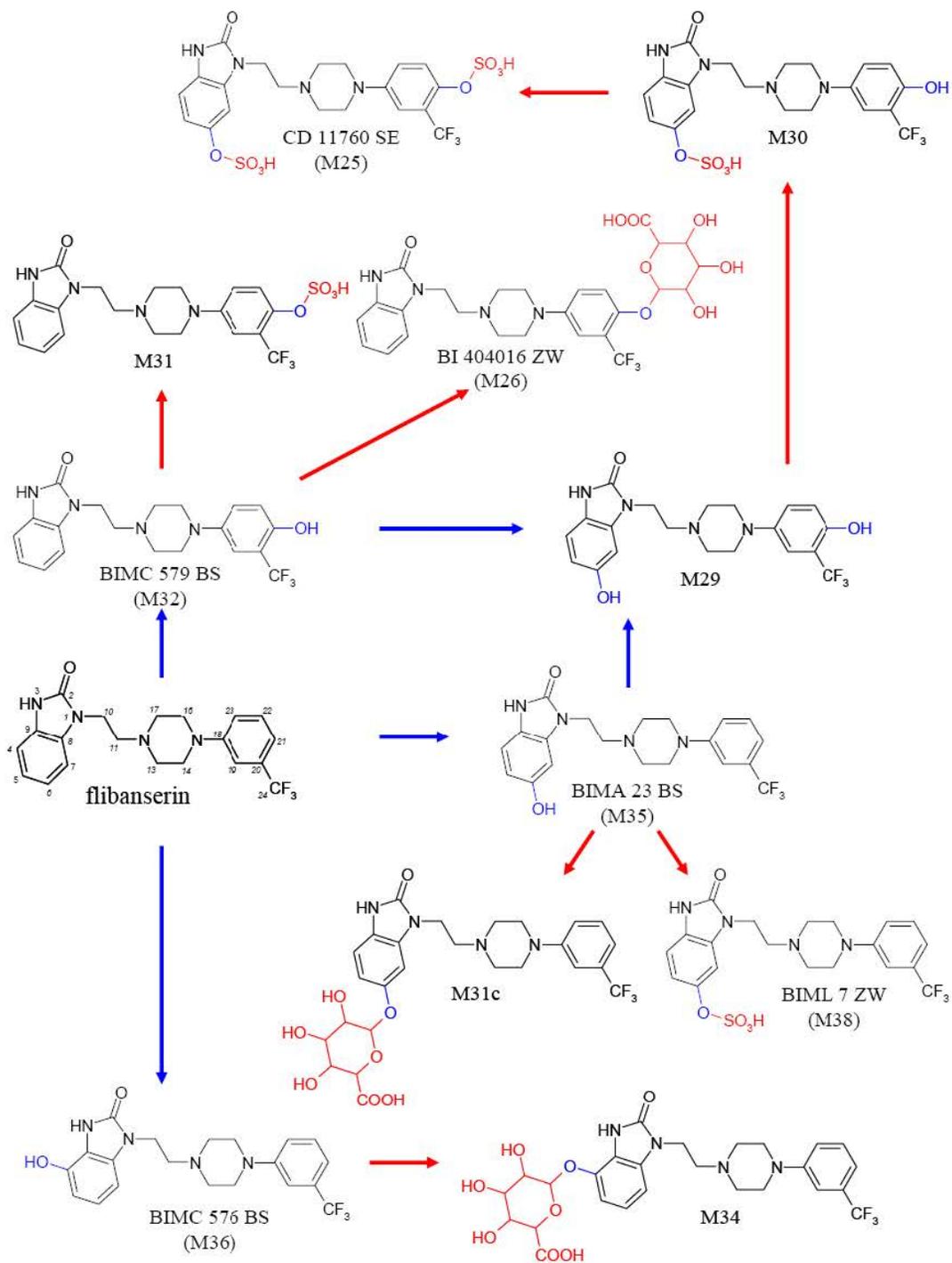
Flibanserin exhibited a high volume of distribution (approximately 2-3 L/kg) in all species examined. Thus, there is a species independent distribution of flibanserin from plasma into tissues.

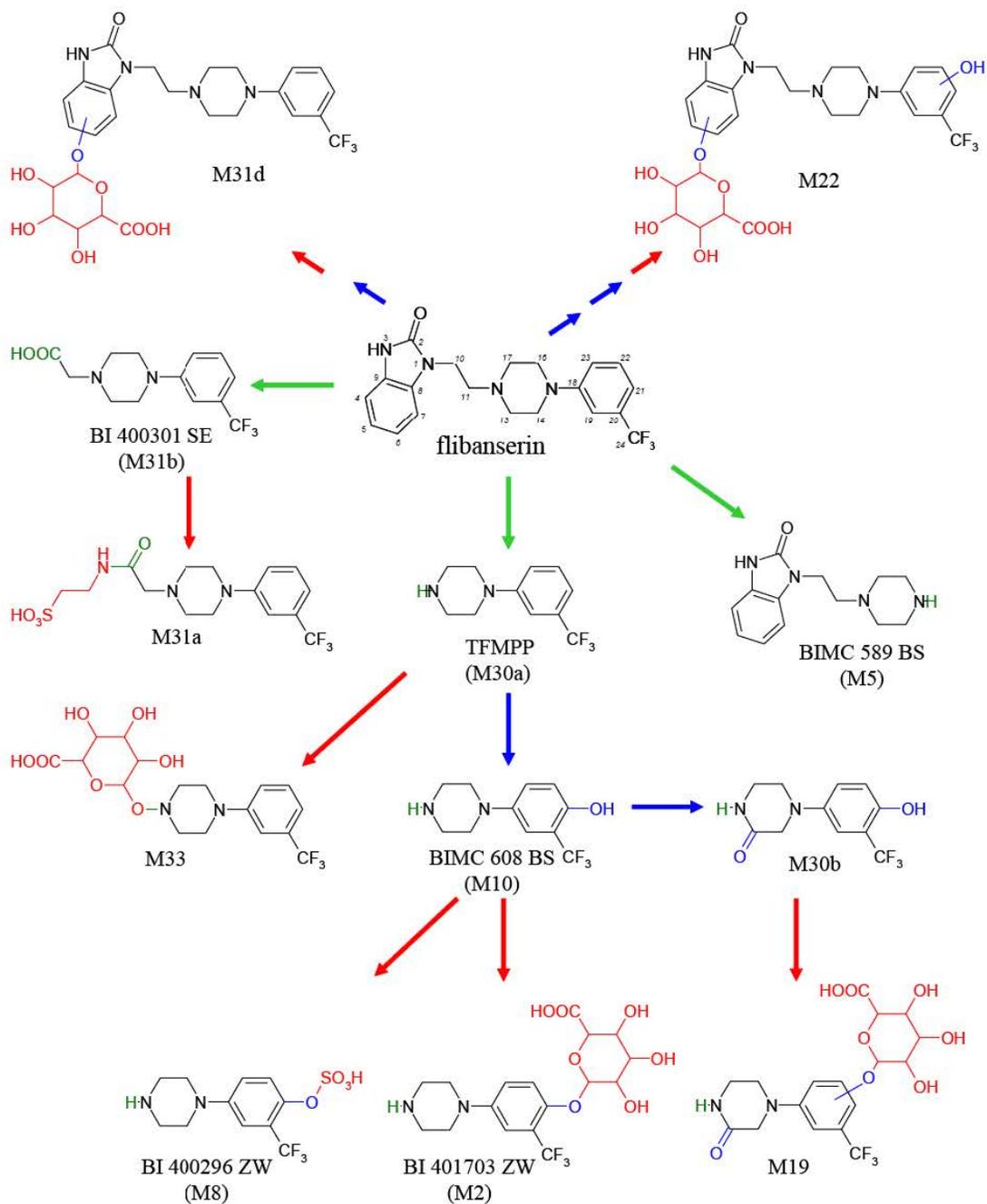
Tissue distribution was investigated in rats by whole body autoradiography. After repeated dosing, radioactivity was present in high concentrations in the liver, kidney, pancreas and brown fat. Pigmented rats showed higher concentrations of radioactivity in skin and eyes in comparison with non-pigmented ones, suggesting melanin binding of flibanserin or its metabolites. Flibanserin and its active minor metabolite M30a crossed the blood brain barrier to a large extent, whereas the active metabolites M35 and M38 did not.

2.6.4.5 Metabolism

After oral administration, flibanserin is almost completely metabolized in mice, rats, rabbits, dogs and humans to at least 35 metabolites. In humans, flibanserin is extensively metabolized by CYP3A4 and to a lesser extent by CYP2D6.

Flibanserin is mainly metabolized by aromatic hydroxylation to several mono- and di-alcohols of flibanserin. In addition, three cleavage pathways exist.





Metabolite Peak No.	BI-Code	mouse (m)	rat (m)	rabbit (f)	dog (m/f)			man (m)	
		5 mg/kg 2 h	5 mg/kg 2 h	3 mg/kg 2 h	3 mg/kg			50 mg	
					2 h (m)	2 h (f)	5.5 h (f)	1 h	4 h
M25/M26 ^a	BI 404016 ZW CD 11760 SE	55	72	826	235	219	682	940	139
M38 ^b	BIML 7 ZW	22	168	29	39	34	81	373	50
M39	flibanserin	237	161	274	1198	1318	230	308	166
M31b	BI 400301 SE	17 ^c	458 ^c	199 ^c	99 ^c	93 ^c	73 ^c	159	69
M8	BI 400296 ZW	nd	nd	nd	nd	nd	nd	138	89
M2	BI 401703 ZW	25	98	307	11	7	nd	23	132
M21		6	nd	nd	nd	nd	15	69	33
M1a		nd ^d	nd ^d	nd ^d	26	39	46	13	39
M30 ^e		6	20	25	nd	13	9	39	7
M1		52	128	110	124	139	66	13	39
M22		9	11	146	7	6	26	36	35
M41		78	43 ^f	144	377 ^f	750 ^f	571 ^f	13	31
M35	BIMA 23 BS	47	15	6	131	162	90	29	27
radioactivity		318	1447	2865	2791	3432	2333	2240	925

^a M25 and M26 could not be separated in plasma samples. Investigations by LC-MS showed that M25 dominated in animals, whereas M26 dominated in man

^b originally reported as M37 or M38, but shown to be only one metabolite (M38 = BIML 7 ZW)

^c in animal samples, M31 could not be separated from M31a, M31b, M31c, M31d

^d might be included in M1

^e in animal samples, M30 could not be separated from M30a (TFMPP) and M30b

^f M41/42

nd = not detected

Table 7.2.1: 3 Overview of geometric mean (gCV) key pharmacokinetic parameters of flibanserin and relevant metabolites after single oral administration of 100 mg flibanserin to healthy female subjects and HSDD patients (Datasets 1 + 3)

100 mg single dose	Flibanserin		BIMA 23 BS		BIML 7 ZW		TFMPP	
	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])
C_{max} (ng/mL)	100	399 (47.8)	51	30.6 (55.0)	51	452 (89.7)	50	5.47 (63.6)
AUC₀₋₁₂ (ng·h/mL)	81	1380 (44.8)	51	153 (45.7)	51	1000 (63.7)	50	36.7 (63.2)
AUC_{0-∞} (ng·h/mL)	100	2050 (47.6)	51	258 (49.3)	51	1460 (62.4)	50	58.6 (75.3)
RAUC_{0-∞,Met} ()	NC	NC	51	0.137 (34.7)	51	0.647 (80.0)	50	0.0530 (74.4)

NC = not calculated

Table 7.2.1: 4 Overview of geometric mean (gCV) key pharmacokinetic parameters of flibanserin and relevant metabolites after multiple oral administration of 50 mg flibanserin twice daily to healthy female subjects and HSDD patients (Datasets 1 + 3)

50 mg b.i.d.	Flibanserin		BIMA 23 BS		BIML 7 ZW		TFMPP	
	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])
$C_{max,ss}$ (ng/mL)	79	298 (41.7)	79	31.1 (37.9)	79	268 (48.4)	79	5.19 (75.8)
$AUC_{\tau,ss}$ (ng·h/mL)	79	1320 (46.6)	79	206 (45.8)	79	897 (34.2)	79	36.7 (82.9)
$C_{pre,ss}$ (ng/mL)	292	48.4 (81.0)	292	10.2 (75.7)	292	26.8 (54.5)	277	1.82 (93.2)
$RAUC_{\tau,ss,Met}$ ()	NC	NC	79	0.150 (42.7)	79	0.544 (53.5)	79	0.0470 (84.0)

NC = not calculated

Based on pharmacological tests including a receptor screen, three metabolites were considered potentially active, M35, M38 and M30_a (TFMPP). Considering the human plasma exposure at therapeutic doses and the brain penetration in rat in addition to the receptor binding data of flibanserin and its metabolites, sponsor concludes that flibanserin in the CNS active substance at therapeutic doses.

The metabolite M8 (BI 400296 ZW) was detected in humans but not in the species used in the toxicology studies. The toxicity of the metabolite was investigated in a 2 week IV dose range finding study, a 4 week GLP study and in in vitro and in vivo genotoxicity assays.

Flibanserin was administered IV to 10 male and 10 female Chbb: (b) (4) rats at daily doses of 0, 0.4, 4.0 and 40 mg/kg for four weeks.

There was no drug related mortality or clinical signs at any dose.

There were no clear drug-related effects on body weight or food consumption, ophthalmology, clinical chemistry and urinalysis. There was a dose related decrease in WBC (mainly lymphocytes) in males only but it was mild and not considered biologically significant (no effect in females).

There were no drug related organ weight changes or test item related microscopic changes.

The NoAEL was considered to be 40 mg/kg corresponding to a mean C_{max} of 64.2 ug/ml in males and 58.9 ug/ml in females. Corresponding mean AUC (0-24h) values were 48.9 and 44.5 ug.h/ml, respectively.

Based on a receptor screen, M8 was considered inactive. M8 was negative in the Ames test at doses up to 5000 ug/plate and the rat micronucleus assay in vivo when given at 40 mg/kg IV for 4 weeks. Plasma protein binding of the metabolite was fairly low (~50%) in both rats and humans.

Human exposure, plasma protein binding, receptor binding and brain/plasma ratios of flibanserin and metabolites

Code	Human C(max)ss at 100 mg q.d. [nM]	PPB man [%]	lowest K _i [nM]	C(max)/lowest K _i	Brain/plasma ^a ratio
Flibanserin	1200	98%	6.59	182	3.71
BIML 7 ZW	1320	97%	207	6.38	0.02
CD 11760 SE ^c	1050 ^b	nd	>10000	<0.105	nd
BI 401703 ZW	282	nd	>10000	<0.028	nd
BI 400296 ZW	281	52%	1760	0.160	nd
BI 400301 SE	159 ^c	nd	>10000	<0.016	nd
BIMA 23 BS	105	95%	7.65	13.7	0.08
BI 404016 ZW	36.6	nd	>10000	<0.004	nd
TFMPP	27.8	73%	32	0.869	2.80

PPB plasma protein binding in man

nd not determined

a based on total radioactivity data in tissues at the end of a 4h i.v. infusion of the respective radiolabelled compound to rats

b C(max) after single oral administration of 100 mg flibanserin to man [U09-1167, Module 5.3.5.3]

c based on C(max) after single oral dosing of 50 mg [¹⁴C]-flibanserin to man determined from pooled 1 h and pooled 4 h plasma samples [U99-1776]

In the above table, it can be seen that the drug concentration (Cmax) divided by the potency (K_i) gives essentially an activity ratio with a higher number denoting greater relative activity. Take into consideration the ability to penetrate the blood brain barrier and it can be seen that flibanserin is basically the only active substance.

Plasma protein binding of flibanserin at a concentration of 0.1 ug/ml was 97% in rat and 98% in mouse, rabbit, dog, monkey and human. Most of the binding was to serum albumin with some binding to α_1 -acid glycoprotein.

2.6.4.6 Excretion

Flibanserin excretion is primarily via feces in mice, rats and dogs with most of the rest through the urine. It is 50/40 feces/urine in humans.

Parameter	Units	Mouse p.o.	Rat p.o.	Rat i.v.	Rabbit p.o.	Dog p.o.	Dog i.v.	Man ^a p.o.	Man ^a i.v.
N/Gender		5m/5f	4m/4f	4m/4f	3f	2m/2f	2m/2f	6m	6m
Dose	mg/kg	5	5	5	3	3	3	50 mg	20 mg
Bile	% of dose	nd	nd/25.3	57.0/39.9	nd	nd	nd	nd	nd
Urine	% of dose	21.6/21.4	19.1/32.5	16.0/18.6	71.9	15.1	15.0	44.1	40.7
Feces	% of dose	70.8/50.0	73.3/70.0	79.1/78.3	19.9	82.4	80.9	50.9	56.0
Recovery	% of dose	92.5/71.4	92.5/105.4	95.2/98.4	92.4	98.7	97.6	95.5	97.0

^a geometric mean nd = not determined

Bile sampling period: male rat (0-5h), female rat (0-6h)

Urine sampling period: mouse (0-48h); male rat (0-96h); female rat, rabbit, dog (0-168h);
man (p.o. 0-264h, i.v. 0-192h)

Feces sampling period: mouse (0-48h); male rat (0-96h); female rat, rabbit, dog (0-168h);
man (p.o. 0-384h, i.v. 0-288h)

2.6.4.7 Pharmacokinetic drug interactions

See biopharmaceutics review

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

See final discussion

2.6.4.10 Tables and figures to include comparative TK summary

See above

02.6.5 PHARMACOKINETICS TABULATED SUMMARY

See above

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

See final discussion

Genetic toxicology:

See final discussion

Carcinogenicity:

See final discussion

Reproductive toxicology:

See final discussion

Special toxicology:

See final discussion

2.6.6.2 Single-dose toxicity

Single dose studies were conducted in mice and rats. Oral flibanserin was given at doses up to 4000 mg/kg in mice and rats. When given IV, the high doses were 80 mg/kg in mice and 90 mg/kg in rats. Additionally, an acute oral toxicity study was conducted in dogs at 50 and 100 mg/kg.

Oral doses resulted in significant mortality at 2000 mg/kg in mice and greater than 4000 mg/kg in rats. After IV administration, significant mortality was seen at 50 mg/kg for mice and 70 mg/kg for rats. Dogs had significant clinical signs after both 50 and 100 mg/kg.

2.6.6.3 Repeat-dose toxicity

Sponsor performed a number of toxicity tests in rats, mice and dogs. In a 13 week toxicity test in mice with a single dose of 1000 mg/kg/day, flibanserin treatment resulted in an increase in liver weight and hepatic lipidosis. Mean plasma flibanserin AUC_{0-24h} values for the mice were 18 ug.h/ml for males and 12 ug.h/ml for females. In a 13 week oral dose ranging study with doses of 20, 100 and 400 mg/kg (raised to 1200 and 2400 mg/kg in drug weeks 6 and 11 due to lack of toxicity), oral administration of flibanserin to CD-1 mice produced hepatocellular hypertrophy in MD and HD animals of both sexes with fatty accumulation.

In a 13 week toxicity study, rats were dosed with 20, 100 and 400 mg/kg which resulted in significant neurological signs at the HD with a reduction in body weight gain at all doses in males only. There was slight but significant decrease in RBC's with an increase in MCH in males. There was an increase in liver and ovary weights of females with reversible periacinar hypertrophy in the liver of the MD (males) and HD (males and females) at week 13. Mild reversible fatty changes were seen in livers of HD females.

In the 13 week rat study, serum prolactin levels were measured

PROLACTIN (ng/ml)				
	Dose mg/kg	MEDIAN	Interquartile Range	NI
CONTROL		52.45	27.05 - 67.41	10
BIMT17 BS	20	65.37	40.76 - 79.52	10
BIMT 17 BS	100	98.27 *	83.42 - 137.43	10
BIMT17 BS	400	174.00 **	142.27 - 275.93	10

* P < 0.05

** P < 0.01

In a 6 month toxicity study in rats at oral doses of 20, 100 and 400 mg/kg flibanserin administration produced hepatic hypertrophy. This change occurred in males at the two higher doses and in females at the highest dose only. In females at the highest dose, there was a mild increase in hepatic lipid content. No other histopathological changes were noted.

Tab. A: Geometric mean C_{max} - and AUC-values and median t_{max} -values (data of both sexes are combined).

Day	1			9			last		
	t_{max} mg/kg h	C_{max} ng/ml	$AUC_{0-\infty}$ ng/ml*h	$t_{max,ss}$ h	$C_{max,ss}$ ng/ml	AUC_{ss} ng/ml*h	$t_{max,ss}$ h	$C_{max,ss}$ ng/ml	AUC_{ss} ng/ml*h
20	1.5	552	2663	1	520	3321	1	521	3334
100	2	2791	50523	2	1617	17464	2	2321	20662
400	8	7315	- *	2	2755	31712	2	3069	41386

* not calculable due to the late C_{max}

This study showed very little toxicity in rats at exposures up to approximately 20 times the human exposure (2080 ng.h/ml) in women taking 100 mg.

In a 13 week study in 3 male and 3 female beagles per group, flibanserin given orally at doses of 3, 15 and 100 mg/kg/day produced significant clinical signs and lower body weight in males and females of the highest dose group. There was an increase in intraocular tension in the eyes of 3 of 6 dogs treated with 100 mg/kg. No other significant findings were noted.

Study title: 6-month oral toxicity study in the rat

Key study findings: Minimal toxicity

Study no.: Internal study no. I48

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim KG, Ingelheim, Germany.

Date of study initiation: Nov. 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: FIMT 17 BS, batch 9403-P, purity not stated

Methods

Doses: 15, 80, 400 mg/kg/day

Species/strain: Chbb: ^{(b)(4)} strain rats

Number/sex/group or time point (main study): 20/sex/gp

Route, formulation, volume, and infusion rate: oral gavage, flibanserin suspended in 0.5% Methocel-solution, 10 ml/kg.

Satellite groups used for toxicokinetics or recovery: none

Age: 57 days

Weight: 174-258 gg

Sampling times:

Unique study design or methodology (if any): None

Results

Mortality:

Group Sex	1 (control)		2		3		4	
	m	f	m	f	m	f	m	f
Died	1	1	0	1	1	0	2	0
Sacrificed	0	0	0	0	0	1	1	0
Total	1	1	0	1	1	1	3	0
%	5	5	0	5	5	5	15	0

The eight animals that died or were sacrificed all had congestion of the parenchymal organs, pulmonary edema and emphysema, intraorbital hemorrhages (one animal) and indications of gavage error (one animal). The cause of death was not determined.

Clinical signs:

Group Sex	1 (control)		2		3		4	
	m	f	m	f	m	f	m	f
Observation								
Ataxia	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20
Convulsions	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20
Dyspnoea/altered respiration	0/20	0/20	0/20	0/20	1/20	0/20	1/20	0/20
Lateral recumbency	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20
Sternal recumbency	0/20	0/20	0/20	0/20	0/20	0/20	2/20	9/20
Phobia	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Resistance to administration	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Sedation	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Poor condition/ruffled coat	0/20	0/20	0/20	0/20	1/20	0/20	2/20	2/20

Body weights:

Males

Week	Group			
	1	2	3	4
13	163	167 (102)	149 (92)	122 (75)
26	223	215 (97)	193 (86)	145 (65)

Females

Week	Group			
	1	2	3	4
13	68	72 (105)	77 (113)	84 (124)
26	89	94 (105)	100 (113)	105 (118)

() = percent of control body weight

For males, the decrease in body weight gain was statistically significant for the mid and high dose. In females, flibanserine treatment resulted in a significant increase in body weight in mid dose animals at weeks 10 and 11 and a significant increase in body weight in high dose rats from week 5 onward.

Food consumption: Animals of the two higher dosed groups demonstrated a statistically significant tendency to consume more food than the controls.

Ophthalmoscopy: No drug induced lesions could be determined with slit-lamp exams.

Hematology: From week 6 onwards high dose males and females showed slightly decreased RBC counts and hemoglobin values.

Clinical chemistry:

GPT: Females in the mid and high dose had a slight but significant increase in GPT (ALT) values during the entire treatment phase. There was no dose dependency.

AP: There was a slight reduction in enzyme activity in mid and high dose males during the entire treatment.

Triglycerides: Moderate to marked dose dependent decrease in serum triglycerides was seen in the mid and high dose males and females during the entire treatment.

Cholesterol: Slight elevations of serum levels were seen in high dose females during the entire treatment and in mid dose females during weeks 6 and 26 and, as a tendency, in low dose females at the end of the study.

Bilirubin: Minimal reductions in serum values in the high dose animals were within the normal range.

Glucose: Slight decreases in mid and high dose animals of both sexes.

Inorganic phosphate: Mid and high dose females showed a slight, dose dependent increase in serum levels. There was also a small increase in low dose females at the end of the study.

Prolactin: Levels were measured in all animals 2 hours after drug administration in weeks 8 and 23 of the study. In week 15 prolactin was determined 24 hours after dosing. There was a dose dependent increase of serum prolactin 2 hours after dosing in both males and females of the mid and high dose groups in week 8 and week 23. No change was seen after 24 hours in the low and mid dose groups and a slight decrease was seen in the high dose group.

Dose mg/kg	Male Rats	Week 8 2 h p. Appl.	Week 15 24 h p. Appl.	Week 23 2 h p. Appl.
Control	Median NI	65.43 20	66.32 20	93.09 19
15	Median NI	91.01 20	60.86 20	97.50 20
80	Median NI	**134.82 20	39.32 20	**173.98 20
400	Median NI	**198.99 20	**9.94 19	**249.14 17

Dose mg/kg	Female Rats	Week 8 2 h p. Appl.	Week 15 24 h p. Appl.	Week 23 2 h p. Appl.
Control	Median NI	103.86 19	15.24 19	48.63 18
15	Median NI	59.31 20	10.30 20	89.09 19
80	Median NI	132.67 19	10.60 19	**202.08 18
400	Median NI	290.11 20	<4.40 20	**335.22 18

** = significantly different from the control group (p < 0.01)

Urinalysis: There were no biologically relevant differences between control and treated animals.

Gross pathology: No biologically significant findings.

Organ weights (specify organs weighed if not in histopath table):

In males there was an increase in absolute thymus weights in all dosed groups and a significant increase in relative thymus weights in the low and high dose groups only. Group 2, 3 and 4 mean absolute values were 20, 18, and 30% higher than control values.

There was an increase in adrenal weights in the mid and high dose groups of males only. Group 2, 3 and 4 mean absolute values were 2, 14 and 19% higher than control.

In males, there was an increase in the relative liver weights in the high dose group. No change in absolute weight.

In females there was an increase in both absolute and relative liver weights in the mid and high dose groups. Group 2, 3 and 4 mean absolute weights were 9, 17 and 44% higher than control values.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (x), no ()

Only changes were seen in the liver of the high dose group. There was a reduction of the regularly occurring fat deposition with the lobuloperipheral hepatocytes in males which may explain the decrease in relative liver weights in males. In contrast, high dose females demonstrated a slight to mild hypertrophy of the lobulocentrally located hepatocytes which probably correlates with the increased liver weight.

Liver changes	G1		G2		G3		G4	
	0 mg/kg		15 mg/kg		80 mg/kg		400 mg/kg	
	M	F	M	F	M	F	M	F
Fatty change	18/20	20/20	20/20	8/20	19/20	14/20	4/20	17/20
Hepatocellular hypertrophy	0/20	0/20	0/20	0/20	0/20	0/20	0/20	20/20

There was no increase in myocardial fat deposition in rats at any dose.

Toxicokinetics

Geometric mean; males and females combined; n = 7-10.

Week	2			26		
Dose mg/kg	T _{max} h	C _{max} ng/mL	AUC _{ss} ng/mL*h	T _{max} h	C _{max} ng/mL	AUC _{ss} ng/mL*h
15	1.0	722	3820	1.1	791	3840
80	1.4	2020	17700	1.1	2690	22300
400	2.8	6040	61800	1.3	7100	59800

Exposures are 10 fold and 29 fold the human exposure (2080 ng.h/ml at 100 mg) at the mid and high doses, respectively.

Study title: 52-week oral (gavage) toxicity study in beagle dogs with interim autopsy after 26 weeks.

Key study findings: Severe neurological signs in HD with clear animal suffering, mild toxicities in LD and MD dogs

Study no.: U99-1576; internal study no. I49

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim KG, Ingelheim, Germany.

Date of study initiation: Dec. 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403-P & 9605-P, purity not stated.

Methods

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

Species/strain: Beagles

Number/sex/group or time point (main study): 3/sex/gp in the 6 month part of the study and 4/sex/gp in the 12 month part.

Route, formulation, volume, and infusion rate: oral gavage, flibanserin suspended in 0.5% Methocel-solution.

Satellite groups used for toxicokinetics or recovery: none

Age: 6-18 months

Weight: 8.9 – 14.5 kg

Unique study design or methodology (if any): None

Results

Mortality: One HD group 4 dog, sacrificed moribund on week 7.

Clinical signs: Salivation and ataxia in group 3 (15 mg/kg) on isolated occasions. In group 4 dogs salivation occurred after dose administration, the animals became recumbent, vocalized and were ataxic or convulsive from about 1 hour after drug administration until beyond 6 hours after administration. Dogs also exhibited dry eyes and various behavioral traits such as biting the metal drinking bowls or feeding racks, standing with head forward, and forcing head or limbs between the bars of the door or the enclosure. Symptoms were seen from 2 hours after administration onwards beyond 6 hours. In the course of the study the number of tremor and vocalization episodes decreased and the duration of vocalization became shorter.

Extreme aggression was seen in one high dose dog that also suffered from salivation, vocalization, sedation, ataxia, convulsions, refusal to eat, no defecation, and poor general condition. The dog was sacrificed on humane grounds.

Isolated cases of reduced locomotion, absence of feces or abnormal feces and miosis were also seen in the high dose group.

There was an increase in neurological signs (compulsive gnawing) with dose.

The continued treatment of the high dose dogs seems to me to be clear animal abuse. I'm not sure why the animal welfare monitor did not demand lowering the dose for these animals.

Body weights: Significant decrease BW gain in group 2 and 3 males but not in females. Group 4 males and females also gained less weight than control dogs but the difference for females was not significant.

Food consumption: No drug related effects.

Ophthalmoscopy: One MD dog and 7 HD dogs demonstrated dose-related ophthalmological and/or histopathological changes of the eyes. They were characterized by discrete, focal, smoky opacity or by focal facet (focal thickening of the cornea epithelium) in the central or peripheral region of the cornea. There was no increase in intensity with time and two of the opacities disappeared with time and did not reappear. Similar ocular findings were seen in the 13 week dog toxicity studies where 2/12 dogs at 100 mg/kg and 4/6 dogs at 100/75 mg/kg were affected.

EKG: There was an increase in heart rate in both male and female dogs of all treated groups at different time points during the study (mostly 3 hours after drug administration). From 18-58% in the low dose group to a mean maximum increase of 72 beats/minute (120%) in the male HD group. In general, males seemed more affected than females.

PQ-, QRS-, QT-intervals were within the normal historical range but there were increases in blood pressure in males and females of groups 2 and 3, 3 and 24 hours after administration in week 26 and 24 hours after administration in weeks 39 and 52 and in females of group 4, 24 hrs after administration in week 52. There was no dose response and the relationship to treatment is questionable.

Hematology: Individually reduced RBC counts and hemoglobin values in treated dogs without a dose response. Platelet counts were increased slightly, biologically and statistically significantly in group 4 dogs at all weeks tested. Thrombin and partial thrombin times were slightly reduced in these animals.

There were no differences in bone marrow smears from group 4 and control animals.

Clinical chemistry: Alkaline phosphatase values were slightly but significantly increased in group 4 dogs during the entire treatment phase.

Prolactin levels for groups 3 and 4 were elevated both for males and females. The increase was slight and not consistent, being less pronounced in males but not in females during later stages of the study.

There was a slight increase in cortisol values in both sexes of group 4 during the course of the study.

There was a slight increase in the α_2 -globulin fraction in group 4 animals which was accompanied by a corresponding change in the albumin-globulin ratio.

Urinalysis: There were no biologically relevant differences between control and treated animals.

Gross pathology: there was an increase in findings in treated groups but no particular lesion seemed significant. 2/14 high dose dogs had pale livers, two had some kidney changes (pale areas, abnormal brown yellowish color) compared to no liver or kidney effects in control dogs.

Organ weights (specify organs weighed if not in histopath table):

There were no significant changes in organ weights in treated animals compared to controls.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (x), no ()

Liver, fatty change in males after 26 and 52 weeks

Group:	Incidence	Males 26WK	Incidence	Males 52WK
Dose (mg/kg/d)	26WK	Animal No./Fatty Changes	52WK	Animal No./Fatty Changes
<i>controls:</i>	2/3	102, mild	4/4	104, mild
0		103, mild		105, very mild
				106, mild
				107, very mild
<i>low dose:</i>	0/3	none	4/4	204, very mild
3				205, moderate*
				206, mild to moderate
				207, very mild
<i>mid dose:</i>	3/3	301, mild	4/4	304, mild to moderate
15		302, moderate		305, moderate*
		303, moderate to marked*		306, mild to moderate
				307, mild
<i>high dose:</i>	2/2	401, moderate to marked*	4/4	403, moderate to marked*
75		402, moderate		404, mild
		(405 premature decedent)		406, mild to moderate*
				407, mild to moderate

Liver, fatty change in females after 26 and 52 weeks

Group:	Incidence	Females 26WK	Incidence	Females 52WK
Dose (mg/kg/d)	26WK	Animal No./Fatty Changes	52WK	Animal No./Fatty Changes
<i>controls:</i>	1/3	152, mild	1/4	157, very mild
0				
<i>low dose:</i>	1/3	253, moderate*	1/4	254, mild
3				
<i>mid dose:</i>	3/3	351, mild to moderate	2/4	355, mild
15		352, moderate*		356, mild to moderate*
		353, moderate		
<i>high dose:</i>	2/3	451, moderate	4/4	454, mild
75		452, mild		455, very mild
				456, very mild
				457, very mild

* Macroscopical findings consisted in some kind of discoloration of the liver.

Frequency and intensity of fatty liver seemed dose and possibly duration related. It was characterized as mild to marked with an increase in intensity in the mid and high dose groups. The fat droplets were present in the periportal and midzonal areas of the liver and did not coalesce into larger droplets.

Hepatocellular fatty change has also been observed in a 13 week toxicity study in rats which may have been an adaptive change perhaps due to the induction of metabolizing enzymes. Flibanserin is not an enzyme inducer in dogs.

Heart, fatty changes in males and females after 26 and 52 weeks

Dose Group	Weeks	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
control	26	0/3	no	0/3	no
control	52	0/4	no	0/4	no
low	26	0/3	no	0/3	no
low	52	0/4	no	0/4	no
mid	26	1/3	303, very mild	0/3	no
mid	52	1/4	306, mild	2/4	355, mild 356, moderate
high	26	1/3	402, mild	0/3	no
high	52	3/4	403, mild to moderate 404, very mild 407, mild to moderate	3/4	454, moderate 456, moderate to marked 457, moderate

There was a fine, focal or multifocal lipid droplet accumulation in the myocardium of the left ventricle. The frequency and severity seemed dose and duration dependent. Sponsor attributed the fatty disposition to a relative hypoxia due to the increased heart rate and/or the reduced physical condition (I assume due to the CNS toxicity). The no effect dose for fat deposition in the heart is 3 mg/kg or approximately 0.6 times the exposure of women taking 100 mg.

Trachea, degeneration of mucous membrane in males and females after 26 and 52 weeks

Study Group (mg/kg/d)	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
controls	0/4	no	0/4	no
3	0/4	no	0/4	no
15	3/4	304, moderate 305, mild 307, moderate	1/4	355, mild
75	4/4	403, mild 404, moderate 406, moderate 407, mild	3/4	454, moderate 455, moderate 456, moderate

The tracheal degeneration consisted of decreased size and number of goblet cells, mild and diffuse granulocytic infiltration in the superficial mucosal layers and in the lamina submucosa, loss of cilia and regenerative changes of the respiratory epithelium. The changes were drug and dose dependent without gender differences. The mechanism and relevance for humans for this effect is unknown.

Thymus, accelerated involution in males and females after 52 weeks.

Study Group (mg/kg/d)	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
controls	0/4	no	0/3	no
3	1/4	207, moderate	2/3	256, mild 257, moderate
15	3/4	304, moderate to marked 305, moderate 307, moderate to marked	1/4	357, moderate
75	3/4	403, mild 406, moderate to marked 407, moderate to marked	2/4	454, mild 455, moderate

There was thymic involution in animals of all groups including the controls. It was accelerated in animals of the dosed groups and considered by the sponsor to be a nonspecific stress response.

Histology conclusion: Administration of flibanserin for one year to dogs resulted in a dose and time dependent, mild increase of hepatocellular fatty change in all dose groups.

A dose-related focal or multi-focal, very mild to marked myocardial fatty change in the left ventricle in the mid and high dose groups.

A dose-related degeneration of the mucous membrane of the trachea in the mid and high dose groups.

A mild to marked, accelerated thymus involution in all dosed groups.

A dose-related, but not time dependent, focal thickening of the corneal epithelium.

Toxicokinetics:

Geometric C_{max}; AUC_{0-24h} and median T_{max} values in the male dogs (n = 3-7)

Gender	Week	Dose	[mg/kg]	3	15	75
male	1	T _{max}	[h]	1	1	6
		C _{max}	[µg/mL]	0.5	2.9	9.0
		AUC	[µg/mL·h]	1.3	12.1	114.0
	14	T _{max}	[h]	1	1	2
		C _{max}	[µg/mL]	0.4	3.2	10.2
		AUC	[µg/mL·h]	1.0	11.9	77.3
	25	T _{max}	[h]	1	1	3
		C _{max}	[µg/mL]	0.5	3.5	13.2
		AUC	[µg/mL·h]	1.5	14.9	135.0
	51	T _{max}	[h]	1	1	2.5
		C _{max}	[µg/mL]	0.6	3.3	13.2
		AUC	[µg/mL·h]	1.2	15.9	108.0
female	1	T _{max}	[h]	1	2	10
		C _{max}	[µg/mL]	0.3	0.8	5.9
		AUC	[µg/mL·h]	0.8	4.8	72.0
	14	T _{max}	[h]	1	2	3
		C _{max}	[µg/mL]	0.4	1.6	9.7
		AUC	[µg/mL·h]	1.1	6.6	73.9
	25	T _{max}	[h]	1	1	3
		C _{max}	[µg/mL]	0.5	2.8	14.1
		AUC	[µg/mL·h]	1.3	13.6	140.0
	51	T _{max}	[h]	1	2	2
		C _{max}	[µg/mL]	0.6	2.9	15.1
		AUC	[µg/mL·h]	1.9	13.7	161.0

Plasma AUC is nonlinear for dose and seems to be more pronounced at weeks 25 and 51. Human AUC_{0-24h} for women taking 100 mg is 2080 ng.h/ml.

Aside from the moderate increase in heart rate, the NoAEL for this study was 3 mg/kg.

2.6.6.4 Genetic toxicology

Study title: **Study of the capacity of the test article to induce gene mutations in strains of Salmonella typhimurium**

Key findings: Study was negative

Study no.: (b) (4) no. 910474; U93-0573

Volume #, and page #:

Conducting laboratory and location: (b) (4)

Date of study initiation: Nov. 1991

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 CL, lot and purity not stated

Methods

Strains/species/cell line: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100

Doses used in definitive study: 5, 15, 50, 150, 500 ug/plate

Basis of dose selection: Initial study used doses of 1, 10, 100, 1000 and 5000 ug/plate. Doses above 100 ug/plate were toxic

Negative controls: DMSO 0.1 ml/plate

Positive controls: Hydrazine sulfate 500 ug/plate, 9-aminoacridine 40 ug/plate, 2-nitrofluorene 2.5 ug/plate, doxorubicine 4 ug/plate, 2-aminofluorene 5 ug/plate

Incubation and sampling times: 72 h incubation

Results

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

Plates run in triplicate in two experiments. If the number of colonies reverted was at least double the number of spontaneously reverted colonies the test was considered positive.

Study outcome:

Toxicity was apparent from 100-300 ug/plate without S9 and 500-800 ug/plate with S9.

No mutagenic effect at concentrations up to 500 ug/plate with or without S9

Positive controls were active in all experiments

Study title: **Mutagenicity study in the V79 (HPRT) forward mutation assay**

Key findings: Study was negative

Study no.: U95-2191

Volume #, and page #:

Conducting laboratory and location: Dept Exp Path Tox, Dr. (b) (4) Gmbh,
Boehringer Ingelheim

Date of study initiation: March, 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9402-P, purity not stated

Methods

Strains/species/cell line: V79 Chinese hamster cell line

Doses used in definitive study: 10, 20, 30, 40, 50 ug/ml

Basis of dose selection: Dose range finding study from 5-500 ug/ml without metabolic activation. Concentrations above 50 ug/ml had no cells surviving. In the activation system, cellular toxicity was evident (59.6% survival) at a dose concentration of 40 ug/ml.

Negative controls: DMSO

Positive controls: ethylmethane sulfonate (EMS); methyl-N-nitro-nitrosoguanidine (MNNG); 7,12-dimethylbenz(a)anthracene (DMBA)

Incubation and sampling times: Cells were treated with the control and test article for 3 hrs. Experiments consisted of one negative control (DMSO), one positive control (EMS, MNNG or DMBA) and four compound-treated cultures. In the activation system the S9 was added during the incubation procedure.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Positive response is defined as a reproducible and concentration dependent increase in mutant frequency for a minimum of two successive concentrations.

COMPOUND ($\mu\text{g/ml}$)	% SURVIVORS		HPRT-MUTANTS/ 1 MIO. SURVIVORS	
	EXP. 1	EXP. 2	EXP. 1	EXP. 2
CONTROLS NEGATIVE DMSO	100.0	100.0	7.9	1.0
POSITIVE EMS 500 MNNG 0.2	99.9 -	- 43.8	161.6* -	- 155.0*
BIMT 17 BS				
10	96.4	96.0	6.9	0.4
20	86.4	94.6	7.1	0.6
30	53.4	57.8	7.5	5.0
35	-	0.6	-	-
40	0	-	-	-

COMPOUND ($\mu\text{g/ml}$)	% SURVIVORS		HPRT-MUTANTS/ 1 MIO. SURVIVORS	
	EXP. 1	EXP. 2	EXP. 1	EXP. 2
CONTROLS NEGATIVE DMSO	100.0	100.0	2.5	4.0
POSITIVE DMBA 5	32.8	84.1	117.1*	170.9*
BIMT 17 BS				
10	91.6	88.0	5.1	8.8
20	104.0	83.5	0	5.6
30	87.3	-	3.6	-
40	59.6	69.8	1.7	12.4*
50	-	14.1	-	0

P: PRECIPITATION

HISTORICAL DATA FOR MUTANT FREQUENCY: MEAN 6.0/1 MIO. (RANGE 0-35.2)

* SIGNIFICANT INCREASE ($P \leq 5\%$)

Study outcome: Study was negative. The positive at 40 $\mu\text{g/ml}$ in the activated experiment was considered not treatment related mainly because it fell within the historical control range. Sponsor also stated that there was no consistent, dose-related or reproducible increase in the mutant frequency compared to vehicle control. True, but since the next highest dose was toxic with very low % survival, it would be hard to detect a dose-response. Nevertheless, the dose is in the toxic range (% survivors are decreased)

and the result is within historical control range so I would agree with sponsor on their interpretation.

Study title: Mutagenicity study for chromosomal aberrations in human lymphocytes in vitro

Key findings: Study was positive

Study no.:

Volume #, and page #:

Conducting laboratory and location: Dr. (b) (4), Boehringer Ingelheim, Biberach an der Riss, Germany

Date of study initiation: January, 1997

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9507-P, purity not stated

Methods

Strains/species/cell line: Human peripheral blood lymphocytes

Doses used in definitive study: Nonactivation: 10, 20, 40, 50 ug/ml

Activation trial 1: 10, 100, 150 (175) ug/ml

Activation trial 2: 10, 100, 150 ug/ml

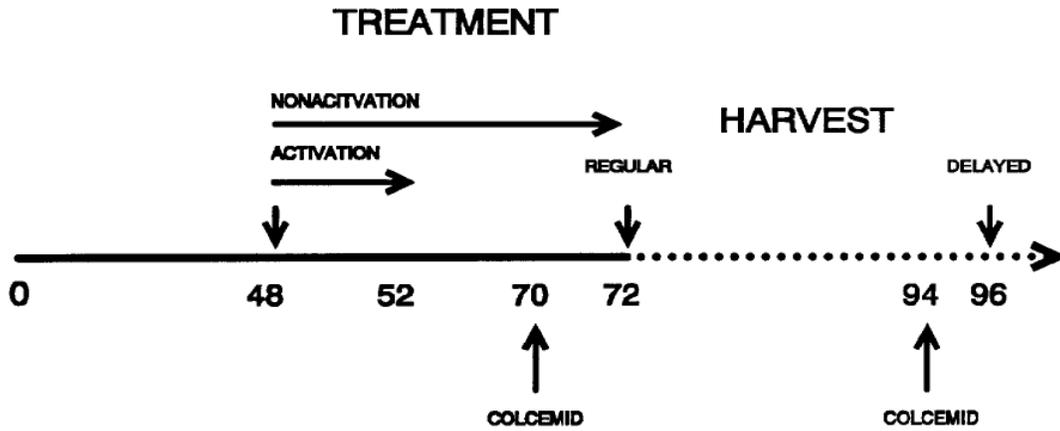
Basis of dose selection: Dose range finding study showed a marked toxicity at a dose concentration range of 50-500 ug/ml (without S9) and 100-500 ug/ml (with S9). Based on this the doses between 10 and 200 ug/ml were selected.

Negative controls: DMSO

Positive controls: Cyclophosphamide (CP), Adriamycin (ADR)

Incubation and sampling times:

Test	Culture Initiation	Treatment	Colcemid	Harvesting	
				Regular	Delayed
- S9	0	48 - 72	70 (94)	72	96
+ S9	0	48 - 52	70 (94)	72	96



Results

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

Positive study is defined as a reproducible and concentration dependent increase in aberration frequency in the exposed cultures.

Structural chromosome aberrations (% aberrant cells excluding gaps)

Metabolic activation: without

COMPOUND ($\mu\text{g/ml}$)	EXPERIMENT 1	EXPERIMENT 2	
	72. hr	72 hr	96 hr
CONTROLS			
NEGATIVE DMSO	1.0 NT	1.0 NT	1.0 NT
POSITIVE ADR 0.05	23.0 * LT	8.0 * NT	16.0 * NT
BIMT 17 BS			
10	0 NT	2.0 NT	ND
20	3.0 LT	1.0 LT	ND
40	0 ¹ HT	(0 ²) HT	ND
50	0 ¹ HT	(0 ²) HT	1.0 NT

ND: not done

1: <100 metaphases scored (toxic)

2: < 50 metaphases scored (toxic)

NT: no toxicity (MI 75 \geq 100 %)

LT: low toxicity (MI 50-74 %)

HT: high toxicity (MI \leq 49 %)*: Significantly different from the vehicle control ($P\leq 5\%$)

Historical negative control values (% aberrant cells excl. gaps): 0.5 (range 0-4)

Structural chromosome aberrations (% aberrant cells excluding gaps)

Metabolic activation: rat liver S9

COMPOUND ($\mu\text{g/ml}$)	EXPERIMENT 1	EXPERIMENT 2	
	72. hr	72 hr	96 hr
CONTROLS			
NEGATIVE DMSO	3.0 NT	1.0 NT	1.0 NT
POSITIVE CP 7	27.0 * NT	27.0 * HT	18.0 * LT
BIMT 17 BS			
10	1.0 NT	0 NT	ND
100	7.0 LT	5.0 NT	ND
150	8.5 ¹ LT	3.8 ¹ LT	4.0 LT
175	N.E. HT	ND	ND

¹: < 100 metaphases scored (toxic)

N.E.: Not evaluable

N.D.: Not done

NT: no toxicity (MI ≥ 100 %)

LT: low toxicity (MI 50-74 %)

HT: high toxicity (MI ≤ 49 %)

*: Significantly different from the vehicle control ($P \leq 5\%$)

Historical negative control values (% aberrant cells excl. gaps): 0.6 (range 0-3.0)

Study outcome: Study was negative without metabolic activation. Slightly increased aberration frequencies were observed in the activation system without dose dependency. Although the difference between treated and negative controls did not reach statistical significance, individual values were outside the historical control range (0-3%). The effects were reproducible and partly associated with cell toxicity as indicated by mitotic inhibition or poor metaphase quality. Because of the cellular toxicity, the sponsor suggests that the effect may be due to an indirect mechanism and not the result of a direct DNA mutation. This is total speculation and the study met the sponsor's criteria for a positive test.

Study title: **Rat bone marrow micronucleus test after oral administration**

Key findings: Result was negative

Study no.: MUT 265; U96-2432

Volume #, and page #:

Conducting laboratory and location: Dept Exp Path Tox, Boehringer Ingelheim, Ingelheim, Germany

Date of study initiation: Sept, 1994

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403A, purity 99.9%

Methods

Strains/species/cell line: Chbb: (b)(4) rats

Doses used in definitive study: 600, 1200, 1800 mg/kg

Basis of dose selection: Maximum tolerated dose using same dosing schedule

Negative controls: 0.5% methocel

Positive controls: Cyclophosphamide

Incubation and sampling times:

Doses were administered orally by gavage. Sampling was performed at 24 and 48 hrs after dosing.

Dose	Sampling time	Sex	Animal number
0.5% Methocel solution	24 h	m/f	001-005/051-055
BIMT 17 BS, 1800 mg/kg	24 h	m/f	101-105/151-155
BIMT 17 BS, 1800 mg/kg	48 h	m/f	201-205/251-255
BIMT 17 BS, 1800 mg/kg	--	m/f	301-302/351-352*
BIMT 17 BS, 1200 mg/kg	24 h	m	401-405
BIMT 17 BS, 600 mg/kg	24 h	m	501-505
Cyclophosphamide, 20 mg/kg	24 h	m/f	601-605/651-655

* Additional animals, to substitute animals in the test substance groups, in case of death.

Results

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

Study was valid

Study outcome:

Dose ^a	Sampling time	Sex	Number of rats	Micronucleated PE (‰) Mean ± standard deviation	p-value
Vehicle control	24 h	male	5	1.4 ± 0.9	
		female	5	1.1 ± 0.5	
BIMT 17 BS 1800 mg/kg	24 h	male	5	1.2 ± 0.3	0.758
		female	5	0.5 ± 0	1.000
BIMT 17 BS 1800 mg/kg	48 h	male	5	1.8 ± 0.4	0.262
		female	5	1.3 ± 1.3	0.433
BIMT 17 BS 1200 mg/kg	24 h	male	5	1.2 ± 0.8	0.714
BIMT 17 BS 600 mg/kg	24 h	male	5	1.0 ± 0.6	0.841
Cyclophosphamide 20 mg/kg	24 h	male	5	22.8 ± 7.0*	0.004
		female	5	21.4 ± 3.0*	0.004

^a Vehicle control, 20 ml 0.5% Methocel solution per kg.

* Significantly different from the vehicle control (p<0.05).

PE = polychromatic erythrocytes.

NE = normochromatic erythrocytes.

Study was negative

Study title: **In vivo comet assay for measurement of DNA damage in the liver of rats after repeat oral administration (gavage)**

Key findings: Study was negative

Study no.: 08B162

Volume #, and page #:

Conducting laboratory and location: Non-clinical drug safety, Boehringer Ingelheim, Biberach an der Riss, Germany

Date of study initiation: October, 2008

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 04040593M, 99.9% pure

Methods

Strains/species/cell line: Crl:WI(Han) male rats

Doses used in definitive study: 5 male rats/group were given either 400 or 1500 mg/kg flibanserin 28 and 4 hrs prior to necropsy. Vehicle controls received 0.5% hydroxyethylcellulose and the positive controls were given 200 mg/kg ethyl methanesulfonate, concurrently.

Basis of dose selection: Dose response study, 1800 mg/kg was significantly toxic.

Negative controls: 0.5% hydroxyethylcellulose

Positive controls: Ethyl methanesulfonate/EMS

Incubation and sampling times:

Results

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

Sponsors summary:

At necropsy samples from the target organ liver (right medial lobe) were immersed in chilled tissue buffer (Hank's balanced salt solution containing 20 mmol/L ethylenediaminetetraacetic acid disodium salt (EDTA) and 10 % dimethylsulfoxide (DMSO), pH 7.5).

(b) (4)

(b) (4)

The slides were coded in a random manner to allow an objective evaluation. The coverslipped slides were examined at 200 x magnification using fluorescent microscopy immediately after staining with ethidium bromide. A total of 150 cells (75 cells/slide) was analyzed. Images were visualized by a CCD camera and measured using the Kinetic Imaging software system "Komet 6 GLP". The head and tail areas of the image were identified and the light intensity of each was quantified and expressed as Olive Tail Moment (OTM) which is calculated automatically and saved to file. $OTM = (Tail.mean - Head.mean) \times Tail\%DNA/100$. Among different parameters including %DNA in tail used for comparison purposes, the OTM is regarded as the most relevant parameter to measure comet induction. Extensively damaged cells or cell clusters, so called "hedgehogs" were excluded from analysis. However, as a sign for toxicity, these "hedgehogs" (necrotic and apoptotic nuclei) were estimated in parallel. Pieces from the livers were taken adjacent to the sites used for comet analysis and fixed in 4 % neutral buffered formaldehyde solution. Since the comet result was negative, these samples were not processed and evaluated.

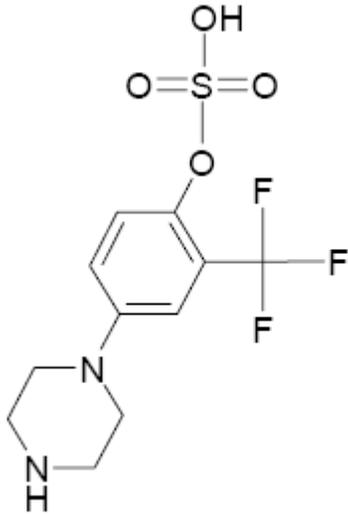
Study outcome:

	Flibanserin (mg/kg)			Pos. Control EMS (mg/kg)
	Control	400	1500	200
OTM:				
	0.18 (0.16-0.21)	0.20 (0.16-0.23)	0.22 (0.17-0.26)	0.41
%DNA in tail:				
	2.36 (2.21-2.65)	2.69 (2.02-2.99)	2.91 (2.50-3.32)	4.11
% Hedgehogs:				
	~2.4	~2.4	~2.4	~4.5

Flibanserin did not induce single strand breaks and alkaline-labile sites in the liver in this assay.

Flibanserin was negative in the in vitro Ames and HPRT assays but induced a non-significant increase in chromosomal aberrations in human peripheral erythrocytes in the presence of a metabolic activation system. However, flibanserin was negative in in vivo assays for clastogenicity (rat bone marrow micronucleus) and for DNA reactivity (Comet) when tested up to toxic doses. Based on the weight of evidence, I consider flibanserin negative for genotoxicity.

Metabolite BI 400296 ZW (M8)



The metabolite was tested for mutagenicity in the Ames assay against TA 1535, TA 1537, TA 98, TA 100 and TA102 with and without metabolic activation (study no. 06B221)

It was negative for all strains at concentrations up to 5000 ug/plate.

Mutagenic activation of M8 in *S. typhimurium* without metabolic activation**Experiment 1 (Plate test)**

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	10	50	58	131	434
BI 400296 ZW					
100	12	42	63	133	434
300	6	43	75	132	436
1000	7	47	66	128	392
3000	9	45	65	129	422
5000	9	42	63	139	434
Positive Controls					
NaN ₃ 5	<u>1054</u>	-	-	<u>1145</u>	-
9-AA 50	-	<u>558</u>	-	-	-
2-NF 10	-	-	<u>642</u>	-	-
MMC 0.5	-	-	-	-	<u>1160</u>

Experiment 2 (Preincubation)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	7	16	42	88	397
BI 400296 ZW					
100	7	18	44	79	376
300	7	14	45	81	403
1000	6	17	45	84	367
3000	7	13	39	88	381
5000	6	13	43	79	343
Positive Controls					
NaN ₃ 5	<u>761</u>	-	-	<u>1172</u>	-
9-AA 50	-	<u>340</u>	-	-	-
2-NF 10	-	-	<u>526</u>	-	-
MMC 0.5	-	-	-	-	<u>1667</u>

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Historical Range	6 - 22	3 - 30	16 - 68	49 - 150	249 - 458
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Mutagenic activation of M8 in *S. typhimurium* with metabolic activation

Experiment 1 (Plate test)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	12	55	59	139	528
BI 400296 ZW					
100	12	42	53	146	524
300	16	42	60	145	539
1000	12	44	55	156	510
3000	13	46	56	163	533
5000	15	49	61	170	536
Positive Controls					
2-AA 4	<u>133</u>	<u>158</u>	<u>986</u>	<u>1067</u>	-
2-AA 10	-	-	-	-	<u>1148</u>

Experiment 2 (Preincubation)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	10	16	39	107	486
BI 400296 ZW					
100	8	14	36	111	479
300	12	19	34	111	495
1000	9	16	39	108	472
3000	11	18	36	120	505
5000	11	14	37	112	502
Positive Controls					
2-AA 4	<u>162</u>	<u>376</u>	<u>1616</u>	<u>1411</u>	-
2-AA 10	-	-	-	-	<u>668</u>

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Historical Range	12 - 22	3 - 39	20 - 61	74 - 164	279 - 531
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2.6.6.5 Carcinogenicity

Study title: **Carcinogenicity study in rats by oral administration (dietary admixture) over a period of 2 years**

Key study findings: borderline positive for liver tumors in males

Adequacy of the carcinogenicity study and appropriateness of the test model:

Acceptable, doses and methods approved by exec CAC in fax dated 23 December 1997.

Evaluation of tumor findings:

Study no.: 97B090

Volume #, and page #: Electronic submission

Conducting laboratory and location: Dept. non-clinical drug safety, Boehringer Ingelheim Pharm, Biberach, Germany

Date of study initiation: March, 1998

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS (flibanserin), batch 720618, 99.44% pure

CAC concurrence: yes

Methods

Doses: 10, 30, 100 mg/kg

Basis of dose selection (MTD, MFD, AUC etc.):

Species/strain: rat/ Wistar Chbb: (b)(4)

Number/sex/group (main study): 50/sex/gp

Route, formulation, volume: oral in food, admixed in diet

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: 5/sex/gp

Age: 38-42 days

Animal housing: Up to 5/cage

Restriction paradigm for dietary restriction studies: no

Drug stability/homogeneity: stable

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: none

Results

Mortality:

Mortality	Daily dose of BIMT 17 BS [mg/kg]									
	0 (control 1)		0 (control 2)		10		30		100	
	M	F	M	F	M	F	M	F	M	F
Sacrificed	6	14	12	12	6	9	9	10	7	13
Found dead	3	0	3	1	0	3	0	2	4	2
Total	9	14	15	13	6	12	9	12	11	15
% mortality	18	28	30	26	12	24	18	24	22	30

M, F: males, females

Survival rate was equal or greater than 70% for all gps.

Clinical signs:

In parentheses: time period (first to final observation day during study)

Group	control 1		low dose		mid dose		high dose		control 2	
BIMT 17 BS [mg/kg]	0		10		30		100		0	
	M	F	M	F	M	F	M	F	M	F
No. of animals/group	50	50	50	50	50	50	50	50	50	50
No changes observed	18	19	20	19	28	24	25	18	12	23
Eye, milky, opaque	5 (482-722)	4 (279-722)	5 (510-722)	1 (475-722)	2 (412-722)	2 (356-722)	3 (489-723)	0	5 (615-723)	2 (601-723)
Hair loss	1 (700-722)	5 (398-722)	1 (608-671)	9 (447-722)	0	3 (622-722)	1 (447-630)	4 (398-723)	1 (629-723)	3 (398-723)
Pale appearance	0	7 (419-681)	0	6 (608-722)	0	6 (420-712)	0	5 (125-699)	1 (678-684)	2 (548-615)
Rough coat	2 (576-581)	9 (412-728)	0	7 (623-722)	4 (615-666)	10 (293-722)	4 (475-688)	8 (545-708)	4 (454-707)	4 (411-615)
Testes, increased size	1 (664-722)	---	7 (384-722)	---	5 (545-722)	---	6 (307-723)	---	12 (447-723)	---
Thickening, limbs	5 (412-643)	2 (580-663)	4 (405-663)	1 (195-722)	4 (426-722)	0	0	0	3 (384-650)	0
Vaginal discharge, red	---	4 (419-722)	---	7 (384-722)	---	6 (489-722)	---	4 (552-723)	---	1 (538-723)

M, F: males, females

No treatment related clinical signs.

Body weights:

Time	Group Dose [mg/kg]	control 1	low dose	mid dose	high dose	control 2
		0	10	30	100	0
Week -1	Males	168.80	171.29 (1.5)	170.93 (1.3)	169.36 (0.3)	171.93 (1.9)
	Females	137.35	140.49 (2.3) ↑	137.45 (0.1)	140.80 (2.5) ↑	138.91 (1.1)
Week 12	Males	428.34	424.97 (-0.8)	418.07 (-2.4)	403.03 (-5.9) ↓	437.71 (2.2)
	Females	267.93	265.09 (-1.1)	254.11 (-5.2) ↓	241.09 (-10.0) ↓	263.52 (-1.6)
Week 52	Males	584.20	566.21 (-3.1)	563.29 (-3.6) ↓	521.94 (-10.7) ↓	575.38 (-1.5)
	Females	313.64	309.81 (-1.2)	300.72 (-4.1) ↓	276.97 (-11.7) ↓	308.72 (-1.6)
Week 80	Males	612.26	597.89 (-2.4)	585.34 (-4.4) ↓	525.63 (-14.2) ↓	612.19 (0)
	Females	317.53	313.99 (-1.1)	300.71 (-5.3) ↓	283.49 (-10.7) ↓	318.93 (0.4)
Week 104	Males	640.72	612.93 (-4.3)	607.78 (-5.1) ↓	525.87 (-17.9) ↓	616.29 (-3.8)
	Females	323.85	321.84 (-0.6)	306.94 (-5.2) ↓	285.44 (-11.9) ↓	327.56 (1.1)

↑, ↓: significantly increased, decreased compared with control 1; p< = 0.05, many-to-one t-test, two-sided

There was a dose related decrease in BW gain that gradually increased over time. For males, the difference in absolute weight in the LD and MD were small, 4-5% when compared to control 1 (heaviest). The HD gp was reduced by ~18%

For females, the decrease in BW was negligible for the LD, 5% for MD and 12% for HD.

Food consumption:

Time	Group Dose [mg/kg]	control 1	low dose	mid dose	high dose	control 2
		0	10	30	100	0
Week -1	Males	150.74	149.51 (-0.8)	150.83 (0.1)	151.57 (0.6)	152.63 (1.2)
	Females	124.17	122.43 (-1.4)	120.57 (-2.9) ↓	119.74 (-3.6) ↓	119.89 (-3.4) ↓
Week 12	Males	204.75	200.84 (-1.9)	203.64 (-0.5)	199.07 (-2.8)	213.34 (4.2)
	Females	152.48	149.22 (-2.1)	141.40 (-7.3) ↓	130.09 (-14.7) ↓	156.50 (2.6)
Week 52	Males	182.77	178.86 (-2.1)	180.17 (-1.4)	177.43 (-2.9)	179.34 (-1.9)
	Females	123.33	123.06 (-0.2)	121.20 (-1.7)	113.94 (-7.6) ↓	132.12 (7.1) ↑
Week 80	Males	165.08	162.20 (-1.7)	164.04 (-0.6)	162.73 (-1.4)	166.78 (1.0)
	Females	120.03	116.49 (-3.0)	111.39 (-7.2) ↓	110.01 (-8.4) ↓	119.46 (-0.5)
Week 104	Males	174.87	164.82 (-5.8)	167.21 (-4.4)	152.98 (-12.5) ↓	173.44 (-0.8)
	Females	125.11	122.61 (-2.0)	117.43 (-6.1) ↓	113.04 (-9.7) ↓	129.73 (3.7)

↑, ↓: significantly increased, decreased compared with control 1; p< = 0.05, many-to-one t-test, two-sided

Non-consistent decrease in FC for both MD and HD males and females.

Hematology: No treatment related changes in hematology.

Organ Weight:

Daily dose of BIMT 17 BS [mg/kg]	0		10		30		100	
Gender (M = male, F = female)	M	F	M	F	M	F	M	F
Number of organs weighed	76	73	44	38	41	38	38	35
Body weight [g]	608.0	307.3	582.8	300.9	577.4	286.7	502.7	265.9
Liver								
Absolute weight [g]	16.0	9.9	16.2	10.2	17.5 [↑]	9.9	15.7	9.7
Liver weight to body weight ratio [%]	2.64	3.23	2.78 [↑]	3.39 [↑]	3.05 [↑]	3.46 [↑]	3.16 [↑]	3.64 [↑]
Liver weight to brain weight ratio [%]	672	460	691	480 [↑]	747 [↑]	465	673	465

[↑], [↓]: significantly increased, decreased compared with control 1; p < 0.05, many-to-one t-test, two-sided

Drug related slight increase in relative liver wt in all treated animals. The increase correlates with the histopath findings of centrilobular hepatocellular hypertrophy.

No other organ wt changes were considered toxicologically significant.

Gross pathology:

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
Gender (M = male, F = female)	M	F	M	F	M	F	M	F	M	F
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver:										
Nodule	1	2	1	3	5	2	5	0	1	0
Uterine cervix:										
Enlargement	-	5	-	7	-	13	-	10	-	5
Hard consistency	-	3	-	8	-	14	-	15	-	5

In the liver, there was an increase in nodules in MD and HD males but not in females. In the uterus, the enlargement and hard consistency may be due to fibrosis (see under non-neoplastic histopath)

Histopathology:Non-neoplastic:

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)	10	30	100	0 (control 2)
Number of animals examined	50	50	50	50	50
Uterine cervix:					
Fibrosis/fibroplasia moderate to severe, with/without necropsy correlate	34 (2.4) 14 3/11	36 (2.7) 23 5/18	37 (2.5) 17 8/9	39 (2.4) 17 13/4	35 (2.3) 13 4/9
Hyperplasia squamous cell	7 (2.6)	8 (2.0)	7 (2.6)	7 (2.6)	2 (1.5)

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
	M	F	M	F	M	F	M	F	M	F
Gender (M = male, F = female)										
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver										
Hypertrophy hepatoc. centrilobular / slight to moderate	12	1	13	0	24	0	32	4	14	0
Vacuolation centrilobular hepatoc. / moderate to severe	1	0	2	0	6	0	18	1	4	0
	14	2	18	0	26	0	26	0	12	0
	2	0	3	0	9	0	10	0	2	0
Kidneys										
Mineralisation tubular	0	8	0	4	0	1	0	30	1	1
Dilatation of tubules	0	4	1	1	2	3	0	9	1	1
Nephrosis, chronic-progressive	28	5	23	0	22	4	16	1	24	2
Rectum										
Edema	7	5	5	6	8	6	13	3	1	4
Infiltration, inflammatory	9	2	5	2	6	4	13	2	2	0
Spleen										
Hemosiderosis	16	26	10	29	16	30	14	43	15	29
Adrenal gland										
Peliosis / angiectasis	14	43	23	41	25	39	23	38	13	35

Neoplastic:

Group	control 1		low dose		mid dose		high dose		control 2	
	0		10		30		100		0	
Daily dose of BIMT 17 BS [mg/kg]	M	F	M	F	M	F	M	F	M	F
Gender (M = male, F = female)										
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver										
Carcinoma, hepatocellular	0	0	1	1	4	1	5	0	2	1
Adenoma, hepatocellular	2	2	1	0	1	2	1	0	1	0
Combined adenoma/carcinoma hepatocellular	2	2	2	1	5	3	6	0	3	1
Focus eosinophilic	2	2	2	1	1	0	3	4	3	1
Focus basophilic tigroid	18	10	18	10	11	16	6	2	16	21
Slight or more	10	4	9	3	4	8	0	1	5	10
Focus basophilic diffuse	2	2	4	3	2	3	4	1	4	4
Focus basophilic NOS	14	4	19	5	13	5	9	2	15	4
Focus clear cell	39	13	39	6	43	7	40	11	38	14
Adrenal gland										
Adenoma, cortical	6	1	3	5	3	3	0	4	2	3
Hyperplasia, cortical	32	29	32	28	26	19	20	19	30	28
Moderate to severe	16	8	11	9	5	7	5	7	11	14
Ovary										
Tumor sex cord stromal mixed [B]	-	13	-	7	-	3	-	2	-	8
Hyperplasia sex cord stromal	-	29	-	24	-	23	-	14	-	28
- severe	-	6	-	6	-	7	-	0	-	8

NOS: not otherwise specified

In the liver, the incidence of hepatocellular carcinomas was slightly higher in HD males. Two HD males died due to intra-abdominal hemorrhage from liver tumors in weeks 90 and 86. The carcinogenic finding was not significant when compared to pooled controls. There were no effects in females.

Statistical evaluation of hepatocellular carcinomas (Sponsors statistics) in males

Dose BIMT 17 BS	0 mg/kg (control 1)	0 mg/kg (control 2)	10 mg/kg	30 mg/kg	100 mg/kg	Trend
Examined (N)	50	50	50	50	50	
Incidences	0	2	1	4	5	
p	-	-	0.7460	0.1088	0.0430	0.0116
p1	-	0.2033	0.5116	0.0551	0.0253	0.0044 ^b
p2	-	-	0.9172	0.4162	0.2541	0.0788

p p value including pooled controls
p1 p value versus control 1
p2 p value versus control 2
^b significance level $p < 0.005$ for trend

Statistical evaluation of hepatocellular carcinomas and adenomas (combined tumors) in males

Dose BIMT 17 BS	0 mg/kg (control 1)	0 mg/kg (control 2)	10 mg/kg	30 mg/kg	100 mg/kg	Trend
Examined (N)	50	50	50	50	50	
Incidences	2	3	2	5	6	
p	-	-	0.7967	0.2330	0.1166	0.0408
p1	-	0.6543	0.7094	0.2493	0.1165	0.0308
p2	-	-	0.8834	0.4075	0.2895	0.0860

p p value including pooled controls
p1 p value versus control 1
p2 p value versus control 2

Other organs/tissues with significant numbers of tumors

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
	M	F	M	F	M	F	M	F	M	F
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Adrenal gland Tumor medullary [B]	4	2	4	2	6	8	2	2	5	1
Mammary gland Fibroadenoma	0	2	0	4	0	3	0	9	0	7
Pancreas Infiltration, inflammatory	4	0	6	0	5	4	10	0	2	2
Skin Fibromas ^a	0	1	1	2	0	0	0	3	1	0
Combined fibromas ^a and fibrosarcomas	1	1	1	3	0	0	1	3	2	1
Combined fibromatous tumors ^b	5	1	3	3	3	1	3	5	3	3
Thyroid gland Adenoma, follicular cell [B]	0	0	0	0	0	2	0	1	0	0

^a Including fibroma [B] and fibroma fibromatosis type [B]

^b Including fibroma [B], fibroma fibromatosis type [B], fibrosarcoma [M], fibrous histiocytoma [B], fibrous histiocytoma [M]

Other than possibly the liver, there were no treatment related tumor increases.

Toxicokinetics:

Group	low dose		mid dose		high dose	
	10 mg/kg		30 mg/kg		100 mg/kg	
Time	Males	Females	Males	Females	Males	Females
Week 13						
AUC(0-24h)	1960	1670	4610	5910	9670	18700
C(6:00 h)	70.6	108	235	322	584	1080
C(16:00 h)	89.2	28.9	145	147	196	400
Week 26						
AUC(0-24h)	1270	2400	4600	5440	8930	22200
C(6:00 h)	60.8	130	276	335	444	1170
C(16:00 h)	43.2	67.7	103	111	294	623
Week 52						
AUC(0-24h)	1330	1800	4300	4440	7520	16500
C(6:00 h)	48.5	91.9	217	202	389	803
C(16:00 h)	59.9	53.1	141	165	231	558
Week 104						
AUC(0-24h)	1780	1760	5960	7190	10000	17100
C(6:00 h)	96.7	108	311	353	516	1050
C(16:00 h)	50.8	36.9	180	245	314	278

	AUC of BIMT 17 BS (ng·h/mL)					
	10 mg/kg		30 mg/kg		100 mg/kg	
Rats (week 104)	M	F	M	F	M	F
AUC(0-24h)	1780	1760	5960	7190	10000	17100
Human (steady state)	2 mg/kg (50 mg BID)		4 mg/kg (100 mg BID)			
Geometric mean AUC _{ss}	2170 (2 x 1085)		5188 (2 x 2594)			
Safety margin	10 mg/kg		30 mg/kg		100 mg/kg	
Ratio rat/human (LD)	0.8	0.8	2.7	3.3	4.6	7.9
Ratio rat/human (HD)	0.34	0.34	1.1	1.4	1.9	3.3

Note: Human daily dose: 50 mg BID (LD, low dose), 100 mg BID (HD, high dose); individuals assumed with 50 kg bodyweight; U97-2256

Using 2080 ng·h/ml as the human exposure at a dose of 100 mg, the safety margins of exposure at the two higher doses for females are roughly 3 and 8, and for males 3 and 5, respectively.

Summary of rat study:

This was a 2 year carcinogenicity study in Wistar rats. The doses were 10, 30 and 100 mg/kg which gave multiples of human exposure to female rats of approximately 1, 3 and 8 for women taking 100 mg. These doses produced reductions in final BW of 18% for HD males and 12% for HD females. Decreased food consumption probably accounts for some of the weight decrease.

The doses were agreed to by the exec CAC in a meeting of Dec. 23, 1997.

The only tumorigenic effect was an increase in the incidence of hepatocellular carcinomas in males. Incidence was 0 and 2 in the controls, 1 in LD, 4 in MD and 5 in

HD. In a carcinogenicity study in mice, there was an increase in hepatocellular carcinomas in males and malignant mammary tumors in females. In the present study, the incidence of malignant mammary carcinomas was 1 and 2 in controls and 1 in LD, 0 in MD and 1 in HD and the incidence of mammary fibroadenomas was 2 and 7 in controls and 4, 3 and 9 in LD, MD and HD, respectively.

Historical controls in Wistar rats (hepatocellular carcinomas)

Study No.	Group No.	Start [m/y]	Duration [month]	Strain	Breeder	Males			Females		
						Animals exam.	with lesion	%	Animals exam.	with lesion	%
2	1	11/1984	24	WIST	A	50	2	4.0	50	0	0.0
13	1	05/1984	31	WIST	A	100	1	1.0	100	0	0.0
20	1	09/1986	24	WIST	A	100	4	4.0	100	0	0.0
21	1	06/1986	24	WIST	A	20	0	0.0	20	1	5.0
22	1	06/1986	25	WIST	A	50	3	6.0	50	0	0.0
28	1	02/1989	26	WIST	A	99	5	5.1	100	1	1.0
30	4	02/1989	26	WIST	A	100	2	2.0	100	0	0.0
49	4	05/1989	25	WIST	A	50	2	4.0	49	0	0.0
51	1	02/1990	25	WIST	A	50	0	0.0	50	0	0.0
65	6	02/1990	25	WIST	A	50	3	6.0	50	0	0.0
83	1	04/1993	25	WIST	A	20	2	10.0	20	0	0.0
89	1	09/1992	25	WIST	A	50	2	4.0			
93	1	09/1992	25	WIST	A				50	2	4.0
95	1	02/1989	26	WIST	A	100	8	8.0			
103	1	08/1994	25	WIST	A	50	2	4.0	50	0	0.0
117	1	08/1995	25	WIST	A	50	4	8.0	50	1	2.0
135	1	08/1995	24	WIST	A	20	1	5.0	20	0	0.0
139	1	05/1991	25	WIST	A	50	4	8.0	50	1	2.0
144	1	05/1991	25	WIST	A	20	0	0.0	20	0	0.0
156	1	10/1998	24	WIST	A	50	5	10.0	50	2	4.0
All 20 studies:						1079	50	4.6	979	8	0.8
Range MIN:								0.0			0.0
Range MAX:								10.0			5.0

m/y: month/year

The maximum percent was 10% in the historical controls and 10% for the HD in this study.

This study was borderline for liver tumors in males. Flibanserin increases the incidence and severity of centrilobular hepatocellular hypertrophy probably by inducing hepatic cytochrome P-450 enzymes. Sponsor believes that flibanserin acts similar to Phenobarbital in promoting liver tumors. It's a reasonable explanation since flibanserin was essentially negative in the available genotox studies and is probably not an initiator.

Combined with the data in mice (see below), flibanserin can be considered positive for liver tumors in male rodents.

Study title: **Twenty-four month oral (diet) carcinogenicity study in the mouse.**

Key study findings: mammary and hepatocellular carcinomas

Adequacy of the carcinogenicity study and appropriateness of the test model:

Deemed adequate by exec CAC

Evaluation of tumor findings:

Study no.: 98R003

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim Pharm, Ridgefield, CT

Date of study initiation: 3/1998

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 (flibanserin), lot 720618, purity not stated

CAC concurrence: yes

Methods

Doses: 10, 80, 200, 1000 (M), 1000/1200 (F). Doses for HD females increased from 1000 to 1200 mg/kg in drug week 23.

Basis of dose selection (MTD, MFD, AUC etc.): Doses were recommended by the Exec CAC in a July 14, 1998 meeting with the Division of Neuropharmacology. In a letter to the Sponsor they recommended that the high dose in females should be 1200 mg/kg based on MTD but apparently the sponsor decided initially on 1000 mg/kg and then increased the dose to 1200 mg/kg when little toxicity was apparent.

Species/strain: (b) (4) Crl:CD-1 (ICBR) mice

Number/sex/group (main study): 70/sex/gp

Route, formulation, volume: oral in feed

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: no

Age: 5-6 wks

Animal housing: individual cages

Restriction paradigm for dietary restriction studies: none

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: TK non GLP

Results

Mortality: Survivability similar between all gps including controls. 38-42 males survived and 22-41 females with no dose response.

Group	Flibanserin mg/kg/day	Males	Females
G1 Control I	0	40	22
G2 Control II	0	38	29
G3 Low	10	46	29
G4 Mid	80	42	41
G5 High-mid	200	44	28
G6 High	M: 1000 F: 1000/1200	40 -	- 32

- a Study termination started in Drug Week 105; numbers take into account all early deaths including animals found dead and sacrificed moribund, as well as dead due to accidental trauma.

Clinical signs: Noted mainly in 1000 mg/kg gp males and included distended/discolored abdomens, dermal blanching and hair staining. Hair staining was also seen in 200 mg/kg males. Distended abdomens was observed at wk 85 and increased to 70% at study termination compared to combined controls which had 39%. The abdominal finding was only seen in males at 1000 mg/kg dose level and not in females at any dose or lower dose males.

Body weights: No difference between treated and control males at study end. Mean body wts for 1000/1200 mg/kg females were consistently increased over combined controls from wk 5 to 82 and considered treatment related. From wk 82 on, wts between treated and control females were similar.

BW week	Control 1+2		10 mg/kg		80 mg/kg		200 mg/kg		1000/1200	
	M	F	M	F	M	F	M	F	M	F
104	36.4	31.9	36.0	33.6	36.8	32.7	37.6	32.0	37.3	32.5

Weekly body weight means

Figure 1: 98R003: Carcinogenicity Study in Mice on Flibanserin
Mean Body Weights, Combined Control Groups - Males

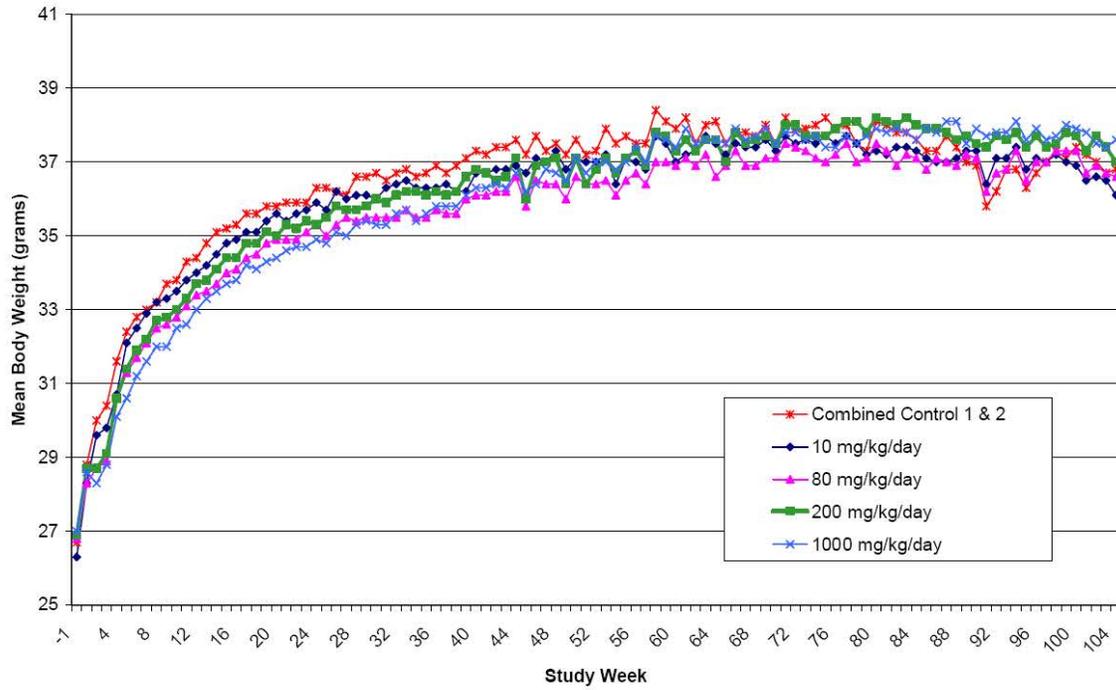
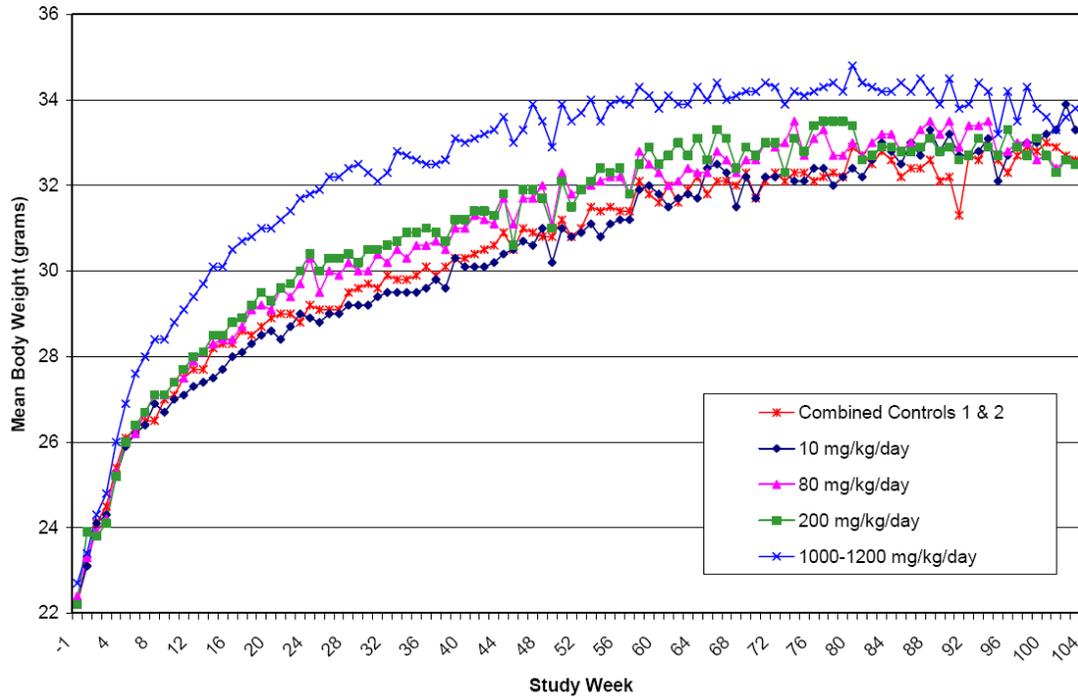


Figure 2: 98R003: Carcinogenicity Study in Mice on Flibanserin
Mean Body Weights, Combined Control Groups - Females



Body weight changes in both males and females were considered drug related. Mean male body weights were significantly decreased (3 to 6%) at the HD during study weeks 2 to 22, but were not decreased upon study termination. Mean body weights in the HD females were significantly increased over combined controls (3 to 11%) during weeks 5 to 82. There were no significant differences at study termination.

Food consumption: Greater than controls in HD males and females. Because of possible palatability issues, there was significant food spillage and food consumption may be overestimated.

Gross pathology: Only treatment related macroscopic change was increase in liver masses in HD males (15/70) versus controls (0/70, 9/70).

Histopathology:

Non-neoplastic: Increased incidence of hepatocellular (centrilobular and midzonal) hypertrophy in males treated with ≥ 80 mg/kg and HD females.

Neoplastic:

There was an increased incidence of tumors in the liver and mammary gland

Incidence and Percentage of Selected Proliferative Changes in the Mammary Gland of Female CD-1 Mice

Group description	Control 1	Control 2	Low	Mid 1	Mid 2	High
Dose Level (mg/kg/day) Flibanserin	0	0	10	80	200	1200
Number of Animals Examined	70	70	70	70	70	70
Adenocarcinoma	0 0%	1 1.4%	3 4.3%	3 4.3%	5 7.1%	5 7.1%
Metastasis (Lung)	0	0	1	1	4	5
Metastasis (Bronchial L. Nodes) ^a	0	0	0	0	2	3
Adenoacanthoma, Malignant ^b	0 0%	0 0%	0 0%	0 0%	1 1.4%	2 2.9%
Combined Carcinomas	0 0%	1 1.4%	3 4.3%	3 4.3%	6 8.6%	7 10%
Hyperplasia	2	0	0	2	0	2

^a Bronchial Lymph Nodes

^b Also known as Malignant Adenosquamous Carcinoma

Table 3.2.3:6

Statistical Analysis of Mammary Gland Adenocarcinomas, Malignant Adenoacanthomas, and Adenocarcinomas and Malignant Adenoacanthomas Combined (Females)

M-Adenocarcinomas (common)								
Dose mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1200	Trend
Examined	N	70	70	70	70	70	70	-
Incidence	I	0	1	3	3	5	5	-
p value versus	C1 + C2	-	-	0.0937	0.1013	0.0154	0.0134	0.0064
	C1	-	1.000	0.0827	0.0666	0.0246	0.0218	0.0185
	C2	-	-	0.3013	0.2695	0.1104	0.0951	0.0575
M-Adenoacanthomas (rare)								
Incidence	I	0	0	0	0	1	2	-
p value versus	C1 + C2	-	-	1.000	1.000	0.3544	0.1458	0.0194 ^d
	C1	-	1.000	1.000	1.000	0.5600	0.3466	0.0328
	C2	-	-	1.000	1.000	0.4912	0.2710	0.0287
M-Adenocarcinomas and M-Adenoacanthomas Combined (common)								
Incidence	I	0	1	3	3	6	7	-
p value versus	C1 + C2	-	-	0.0937	0.1013	0.0062 ^a	0.0024 ^a	0.0008 ^c
	C1	-	1.000	0.0827	0.0666	0.0138	0.0076 ^a	0.0033 ^c
	C2	-	-	0.3013	0.2695	0.0621	0.0323	0.0119

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.05, pairwise comparison, Peto test, rare tumor

c p < 0.005, trend, Peto test, common tumor

d p < 0.025, trend, Peto test, rare tumor

Table 1 Historical control data for mammary gland adenocarcinomas and malignant adenoacanthoma in CD-1 mice (from: (b) (4))

Study ID	Start [y]	Animals examined	Adenocarcinoma		Adenoacanthoma, malignant	
			with lesions	[%]	with lesions	[%]
35	1993	54	0	0.00	0	0.00
36	1993	64	0	0.00	0	0.00
37	1993	49	1	2.04	0	0.00
38	1993	62	2	3.23	0	0.00
39	1993	49	2	4.08	0	0.00
40	1994	50	2	4.00	0	0.00
41	1994	56	2	3.57	0	0.00
42	1994	57	1	1.75	0	0.00
43	1995	60	5	8.33	0	0.00
44	1995	38	2	5.26	0	0.00
45	1995	52	0	0.00	2	3.85
46	1995	68	2	2.94	0	0.00
47	1996	48	1	2.08	1	2.08
48	1996	46	0	0.00	0	0.00
49	1996	55	0	0.00	0	0.00
50	1996	53	0	0.00	0	0.00
51	1998	54	1	1.85	0	0.00
52	1999	65	2	3.08	0	0.00
53	1999	57	3	5.26	0	0.00
54	2000	55	1	1.82	0	0.00
all studies			27	2.5	3	0.3
			range min	0.00		0.00
			range max	8.33		3.85

Incidence and Percentage Summary of Selected Proliferative and Non-Proliferative Findings in the Liver

Group description	Control 1		Control 2		Low		Mid 1		Mid 2		High	
Dose Level (mg/kg/day)	0		0		10		80		200		1000/1200	
Flibanserin												
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number of Animals Examined	70	70	70	70	70	70	70	70	70	70	70	70
PROLIFERATIVE FINDINGS												
Carcinoma, Hepatocellular	1 1.4%	0 0%	6 8.6%	0 0%	7 10.0%	0 0%	8 11.4%	5 7.1%	9 12.9%	1 1.4%	11 15.7%	4 5.7%
Adenoma, Hepatocellular	3 4.3%	1 1.4%	3 4.3%	0 0%	2 2.9%	0 0%	0 0%	1 1.4%	3 4.3%	0 0%	5 7.1%	2 2.9%
Combined Adenomas/Carcinomas, Hepatocellular	4 5.7%	1 1.4%	8 11.4%	0 0%	8 11.4%	0 0%	8 11.4%	5 7.1%	11 15.7%	1 1.4%	13 18.6%	6 8.6%
Foci, Acidophilic	0	0	1	0	0	0	0	0	3	0	6	1
Foci, Basophilic	0	0	1	0	0	0	2	1	2	0	8	3
Foci, Mixed	0	0	2	0	0	0	0	0	1	0	7	2
Foci, Vacuolated	0	0	0	0	0	0	0	0	0	1	3	0
Foci, Clear Cell	0	0	0	0	3	0	0	0	0	0	0	0
Foci, Combined	0	0	4	0	3	0	2	1	5	1	17	4
DRUG-RELATED NON- PROLIFERATIVE FINDINGS												
Hypertrophy Hepatocellular Centrilobular	0	0	0	0	0	0	2	0	6	0	13	3
Hypertrophy Hepatocellular Midzonal	0	1	0	0	3	1	6	0	6	0	25	6

Statistical Analysis of Combined Hepatocellular Adenomas and Carcinomas

Hepatocellular Adenomas and Carcinomas Combined (common)								
Dose Level mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1000 M 1200 F	Trend
No.Examined/ Sex	N	70	70	70	70	70	70	-
MALES								
Incidence	I	4	8	8	8	11	13	-
p value	C1+C2	-	-	0.3518	0.2859	0.1192	0.0239	0.0188
versus	C1	-	0.2452	0.2417	0.1987	0.0865	0.0113	0.0092
	C2	-	-	0.7020	0.6551	0.3475	0.1435	0.0851
FEMALES								
Incidence	I	1	0	0	5	1	6	-
p value	C1+C2	-	-	1.000	0.0599	0.5862	0.0046 ^a	0.0024 ^b
versus	C1	-	0.4314	1.000	0.3088	0.8114	0.0491	0.0153
	C2	-	-	1.000	0.0619	0.4912	0.0092 ^a	0.0025 ^b

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.005, trend, Peto test, common tumor

Table 3.2.3:3

Statistical Analysis of M-Hepatocellular Carcinomas in the Liver of Mice

M-Hepatocellular Carcinomas (common)								
Dose Level mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1000 M 1200 F	Trend
No.Examined/ Sex	N	70	70	70	70	70	70	-
MALES								
Incidence	I	1	6	7	8	9	11	-
p value	C1+C2	-	-	0.1661	0.0865	0.0764	0.0065 ^a	0.0062
versus	C1	-	0.1198	0.0367	0.0185	0.0162	0.0028 ^a	0.0035 ^b
	C2	-	-	0.5364	0.4189	0.3997	0.1111	0.0859
FEMALES								
Incidence	I	0	0	0	5	1	4	-
p value	C1+C2	-	-	1.0000	0.0152	0.3544	0.0045 ^a	0.0045 ^b
versus	C1	-	0.10000	1.0000	0.1066	0.5600	0.0248	0.0203
	C2	-	-	1.0000	0.0619	0.4912	0.0338	0.0180

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.005, trend, Peto test, common tumor

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Table 2 Historical control data for hepatocellular carcinomas in CD-1 mice 1981 to 2000

Males					Females				
Study ID ^{1,2}	Start [y]	total examined	with lesions	[%]	Study ID ^{1,2}	Start [y]	total examined	with lesions	[%]
CQ	1985	50	14	28.00	CQ	1985	50	1	2.00
CR	1985	50	0	0.00	CR	1985	50	2	4.00
CP	1985	50	1	2.00	CP	1985	51	1	1.96
BX	1981	52	9	17.31	BX	1981	52	1	1.92
DN	1988	48	4	8.33	DN	1988	49	0	0.00
DX	NS	50	8	16.00	DX	NS	50	1	2.00
CX	1983	72	12	16.67	CX	1983	71	0	0.00
DU	1989	50	7	14.00	DU	1989	50	2	4.00
EG	1989	50	5	10.00	EG	1989	49	1	2.04
DZ	1990	49	8	16.33	DZ	1990	49	0	0.00
27	1989	50	3	6.00	30	1992	50	0	0.00
28	1992	49	5	10.20	31	1990	60	2	3.33
29	1990	60	4	6.67	32	1991	70	1	1.43
30	1991	67	10	14.93	33	1991	58	2	3.45
31	1991	60	2	3.33	34	1992	117	0	0.00
32	1993	59	8	13.56	35	1993	59	1	1.69
33	1993	70	4	5.71	36	1993	70	3	4.29
34	1993	50	3	6.00	37	1993	50	0	0.00
35	1993	65	1	1.54	38	1993	65	0	0.00
36	1993	50	8	16.00	39	1993	51	0	0.00
37	1994	50	2	4.00	40	1994	50	0	0.00
38	1994	65	0	0.00	41	1994	65	0	0.00
39	1994	65	5	7.69	42	1994	65	1	1.54
40	1995	60	0	0.00	43	1995	60	0	0.00
41	1995	60	2	3.33	44	1995	41	0	0.00
42	1995	60	4	6.67	45	1995	59	0	0.00
43	1995	70	6	8.57	46	1995	70	0	0.00
44	1996	50	4	8.00	47	1996	50	0	0.00
45	1996	50	5	10.00	48	1996	50	0	0.00
46	1996	90	5	5.56	49	1996	60	0	0.00
47	1996	60	2	3.33	50	1996	70	1	1.43
48	1996	60	7	11.67	51	1998	60	0	0.00
49	1998	65	6	9.23	52	1999	65	0	0.00
50	1999	70	7	10.00	53	1999	60	1	1.67
51	1999	60	9	15.00	54	2000	55	0	0.00
52	2000	55	2	3.64					
March 1995*¹		471	54	11.46			521	9	1.73
March 2005*²		1570	114	7.26			1530	12	0.78
1993 – 2000*		1284	90	7.00			1175	7	0.60
all studies*		2041	168	8.23			2051	21	1.02

Study ID with letters: Spontaneous Neoplastic Lesions in the CrI:CD-1[®] (ICR)BR Mouse. March 1995, (b)(4), information prepared by (b)(4). Appendix A.

Study ID with numbers: Spontaneous Neoplastic Lesions in the CrI:CD-1[®] (ICR)BR Mouse in Control Groups from 18 Month to 2 year Studies. March 2005, (b)(4), information prepared by (b)(4) (b)(4) Appendix B.

Study CQ excluded for males due to exceptionally high incidence(shaded grey)

**Table 3 Historical control data for hepatocellular carcinomas in CD-1 mice
December 1993 (92 - 104 weeks)**

Males					Females				
Study ID ¹	Start [y] ²	total examined	with lesions	[%]	Study ID ¹	Start [y] ²	total examined	with lesions	[%]
618	ns	50	8	16.00	618	ns	50	1	2.00
670	ns	49	8	16.33	670	ns	49	0	0.00
617	ns	50	5	10.00	617	ns	50	0	0.00
293	ns	50	7	14.00	293	ns	50	2	4.00
694	ns	50	0	0.00	694	ns	50	0	0.00
483	ns	50	5	10.00	483	ns	49	1	2.04
056	ns	50	2	4.00	056	ns	50	0	0.00
001	ns	50	5	10.00	001	ns	50	0	0.00
total		399	40	10.03			398	4	1.01
range			min	0.00				min	0.00
			max	16.33				max	4.00

- Background tumour incidences from carcinogenicity studies in CRL:CD one Swiss mice, OA39/BTI(mice).1297, (b) (4) Appendix C.
- ns = not specified

Historical control data were from the same strain and same vendor (b) (4) four different production sites). Studies were initiated between 1993 and 2000 for mammary tumors and 1981 to 2000 for hepatocellular tumors.

Toxicokinetics:

Table 3.1.4:1 Mean Plasma Concentrations of Flibanserin in Mice

Group	Dose (mg/kg/day)	Gender	Flibanserin Plasma Concentration (ng/mL)		
			Week 24	Week 53	Week 78
G1	0 (Control)	Male	0	24.6 ± 55.0	2.32 ± 5.19
		Female	0	0	0
G2	0 (Control)	Male	0	0	0
		Female	0	0	0
G3	10	Male	60.4 ± 69.2	0 ^a	12.5 ± 14.4
		Female	6.62 ± 14.80	0 ^a	21.5 ± 12.6
G4	80	Male	124 ± 60	131 ± 79 ^a	142 ± 69
		Female	123 ± 48	47.4 ± 65.0 ^a	203 ± 181
G5	200	Male	211 ± 47	228 ± 67	286 ± 169
		Female	181 ± 120	204 ± 79	185 ± 89
G6	1000 (males)	Male	807 ± 635	1253 ± 301	1218 ± 407
	1200 (females) ^b	Female	571 ± 215	735 ± 482	571 ± 276

a NOTE: The LOQ of the assay for these samples was higher (100 ng/mL) than for samples in Weeks 24 and 78 (10 ng/mL). As a consequence, and because a concentration of zero ng/mL was used in place of BLQ for calculations of mean±SD, the mean concentrations for the 10 and 80 mg/kg/day dose group in Week 53 are biased to be lower than if an LOQ of 10 ng/mL had been possible. Therefore, comparisons to other groups may be inaccurate.

b The initial dose in Group G6, 1000 mg/kg/day, was escalated to 1200 mg/kg/day in Week 23 for females only.

No AUC data were available from the mouse carcinogenicity study. Direct exposure comparisons with humans cannot be made.

Serum drug and prolactin levels were determined in a 34 week study in the same strain of mice and at the same doses as the carcinogenicity study. For additional information on the prolactin measurements, see under special toxicology studies, below.

Summary Table 1 Mean toxicokinetic parameters of flibanserin after dietary administration to mice

Parameter	Week	Gender	10 mg/kg	80 mg/kg	200 mg/kg	1000 mg/kg	1200 mg/kg
C(max)	3	m	39.1	176	376	1430	-
[ng/mL]	3	f	47.6	192	313	-	1710
AUC(0-24h)	3	m	591	3150	6020	16300	-
[ng·h/mL]	3	f	671	2370	5680	-	20800
C(1000)	3	m	18.5	115	217	628	-
[ng/mL]	3	f	18.6	66.1	188	-	477
C(0800)^a	13	m	18.0	207	247	787	-
	13	f	16.2	101	197	-	558
[ng/mL]	26	m	23.0	150	233	748	-
	26	f	16.5	88.6	311	-	764

^a actual collection between 0800 and 1050 hours

In humans taking 100 mg, drug AUC_{0-ss} was 2080 ng·h/ml. These TK data are used to compare mouse and human exposures for the carcinogenicity study.

Prolactin levels - means

Prolactin ng/ml	control	10 mg/kg	80 mg/kg	200 mg/kg	1000/1200 mg/kg
wk 14	129 ± 58*	146 [^] ± 107	134 ± 42	115 ± 55	177 ± 104
wk 34	106 ± 153	33 ± 31	146 ± 152	103 ± 66	104 ± 77

* ± SD

[^] In another table, the value was given as 128

Summary: Flibanserin was tested for carcinogenicity in a two year study in mice. Mortality was similar for males and females among all treated groups and controls.

Only sign of toxicity was distended abdomen in HD males. Changes in body weight occurred during the course of the study for both males (decrease) and females (increase) and were considered treatment related but body weights were no different than controls at study termination.

In the mammary gland of female mice, there was a clear increase in adenocarcinomas (incidence was 0, 1 in the two controls and 3, 3, 5, 5 in the treated gps). When combined with malignant adenoacanthomas (also called adenosquamous carcinoma; a variation of adenocarcinoma), the controls had 0, 1 and the treated gps had 3, 3, 6, 7 total malignant mammary tumors which is higher than the historical controls for any one group. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes.

In male mice, there was a statistically significant increase trend in hepatocellular carcinomas (1 and 6 in male controls, and 7, 8, 9, 11, in the treated gps) which was also significant in the pairwise test at the HD. For hepatocellular carcinomas in females, the results were 0 and 0 for controls and 0, 5, 1, 4 in the treated gps. There was a significant trend and pairwise difference at the 1200 mg/kg dose level when compared with pooled controls but not when compared with control 1 or control 2 separately. For combined adenomas and carcinomas, females showed a significant trend and pairwise difference at 1200 mg/kg when compared to pooled controls and control 2.

The maximum percent incidence in the historical controls for hepatic carcinoma controls was 17% for males and 4% for females (Sponsor excluded the first 1985 study because of the exceptionally high incidence). In this study, the percents were 1.4%, 8.6%, 10%, 11.4%, 12.9%, 15.7% for males and 0%, 0%, 0%, 7.1%, 1.4%, 5.7% for females.

There was a clear increase in combined foci of cellular alteration in livers of high dose males and possibly in females as well.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: **Study of fertility and early embryonic development to implantation in rats by oral administration, gavage.**

Key study findings: No effects on fertility

Study no.: 69S; U95-2214

Volume #, and page #: Electronic

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. (b) (4) GmbH, Biberach an der Riss, Germany

Date of study initiation: January 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9403;

Methods

Doses: 20, 80, 200 mg/kg

Species/strain: Chbb: (b)(4) (SPF) rats
 Number/sex/group: 24/sex/gp
 Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg
 Satellite groups used for toxicokinetics: none
 Study design: Males were dosed 28 days before mating and females 14 days before mating. Dosing continued without interruption until GD 6 in females. Males were dosed continuously until successful mating. Dams were sacrificed on GD 14-16 and subjected to cesarean section with an in situ macroscopic inspection.

Results

Mortality: none

Clinical signs: Some sedation in HD males. Sedation, prone posture, reduction of spontaneous activity and timidity were seen in HD females at the beginning of treatment. They were normal from the 4th day on.

Body weight: Some slight decrease in MD and HD females on GD 3 and 6 but same as controls on GD 14.

Food consumption: No significant changes

Toxicokinetics: not done

Necropsy: Females were normal

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): The normal duration of the estrus cycle for this strain of rat is 4-5 days. The duration of the estrus cycle was changed to shorter than 4 days in 4.2% of MD animals and longer than 5 days in 8.3 and 29.2% of MD and HD animals. The mean number of corpora lutea was significantly increased in the HD group.

Parameter (mean)	Control	5 mg/kg	15 mg/kg	100 mg/kg	Historical range [#]
Corpora lutea	15.6	16.3	15.9	17.8*	13.5-17.5

* significant difference (P<0.05), # = from vehicle controls and unaffected dosages

There were no effects on fertility (copulation index, fertility index, gestation index)

Parameter [%]	Control	20 mg/kg	80 mg/kg	200 mg/kg
Copulation index [#]	95.8	100	95.8	100
Fertility index [#]	95.8	95.8	95.8	100
Gestation index	95.6	100	95.6	100

[#] dams pregnant without sperm found in vaginal smear included

There were no effects on litter parameters (implantations, resorptions, viable fetuses)

Embryofetal development

Study title: Study for effects on embryo-fetal development in rats by oral administration, gavage

Key study findings: Maternal toxicity in MD and HD. Developmental abnormalities in the HD. Several different malformations in treated groups with no dose response. NoAEL considered to be 80 mg/kg (~15 X human exposure).

Study no.: 56S; U95-2254

Volume #, and page #:

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. (b) (4) GmbH, Biberach an der Riss, Germany

Date of study initiation: September, 1994

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9403;

Methods

Doses: 20, 80, 400 mg/kg

Species/strain: Chbb: (b) (4) (SPF) rats

Number/sex/group: 24 pregnant females/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg

Satellite groups used for toxicokinetics: 6/gp

Study design: Pregnant females were dosed from GD 7-16. Animals were sacrificed on GD 22.

Results

Mortality (dams): None

Clinical signs (dams): The number of pregnant rats/group were 20, 22, 18, and 22 in the control to high dose, respectively. An additional dam in the high dose group had total embryo resorptions. The clinical signs of the low dose group were similar to the control group. Within 2-3 hrs, MD females showed somnolence, sedation and timidity after the first treatment until the 9th treatment. In the HD group, there were severe clinical sign including somnolence, reduction of spontaneous activity, sedation, prone position, timidity, catalepsy, and others. After the 7th treatment there was slight sedation, somnolence and timidity and after the end of treatment, the animals behaved normally.

Body weight (dams):

There was moderate decrease in BW gain in the HD group and to a lesser extent, the MD group. Total body weight gains were 121, 118, 102, 73g control to HD, respectively.

Dose (mg/kg)	Mean of Body Weight Gain (g) relative to GD 7			
	GD 8	GD 12	GD 16	GD 21
control	1.7	24.1	45.3	121.3
20	0.6	18.2*↓	41.7↓	118.0
80	-3.7*↓	13.5*↓	31.5*↓	101.9*↓
400	-23.5*↓	-7.5*↓	10.0*↓	73.0*↓

* significant difference ($P < 0.05$), ↓ decreased, GD = gestation day

▨ = administration period

Food consumption (dams):

There was a slight decrease in food consumption in the MD group and a moderate decrease at the HD.

Toxicokinetics:

Geometric means of $C_{max,ss}$ and AUC_{0-24h} and medians of $t_{max,ss}$ on GD 12 were

Dose [mg/kg/day]	N	$t_{max,ss}$ [h]	$C_{max,ss}$ [ng/ml]	AUC_{0-24h} [(ng/ml)×h]
20	6	1	1108	7131
80	6	1	3785	31592
400	6	2	8447	86643

Human AUC_{0-t} is 2080 ng.h/ml

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

One HD animal had complete resorption.

Parameter (mean)	Control	20 mg/kg	80 mg/kg	400 mg/kg	Historical range#
Fetal body weight [g]	5.5	5.5	5.2*	4.7*	4.8 - 5.6 g

* significant difference ($P < 0.05$), # = from vehicle controls and unaffected dosages

Offspring (malformations, variations, etc.):

There was one runt (fetuses weighing less than 65% of the weighted control mean values) in the MD and 10 in the HD groups. Skeletal and visceral abnormalities were seen in controls and treated animals with equal distribution. In the HD group, there were a greater number of fetuses with reduced ossification of fore limbs and a greater number with lumbar ribs.

Malformations were seen only in the treated groups. They included frequent malformations like cleft or fused vertebrae (2 at MD, 2 at HD) or a single malformation (agnathia) at the LD. In the HD, there were 2 fetuses with anophthalmia and one with hydrocephalus, all within one litter.

Fetal findings (%)

Findings	Control	Dose mg/kg			Historical Data %
	G 0	20 G 1	80 G 2	400 G 3	
Runts			1 (0.40)	10 (3.29)	0.19
Variations:					
retinal fold	24 (17.91)	31 (20.26)	16 (13.44)	21 (14.19)	0.13*
dilated renal pelvis	6 (4.48)	4 (2.61)	1 (0.84)	-	0.84*
distance between A.carotis communis sinistra and A.subclavia sinistra enlarged	1 (0.75)	-	-	-	-
cervical ribs	2 (1.38)	4 (2.53)	-	1 (0.64)	1.74*
lumbar ribs	1 (0.69)	1 (0.63)	4 (3.12)	9 (5.77)	0.35*
delayed ossification of fore limbs	1 (0.69)	-	-	11 (7.05)	2.56*
delayed ossification of sternebrae	1 (0.69)	1 (0.63)	1 (0.78)	1 (0.64)	2.68*
delayed ossification of vertebral body	1 (0.69)	1 (0.63)	1 (0.78)	1 (0.64)	0.43 (ossification delay in general)*
split or displaced sternebrae	3 (2.07)	1 (0.63)	3 (2.34)	1 (0.64)	0.34*
rudimentary sternebrae	-	-	-	1 (0.64)	-
hypoplastic 13th rib	-	-	1 (0.78)	-	0.43*
short ribs	-	-	-	1 (0.64)	-
Malformations:					
dilated ventricle of telencephalon (hydrocephalus)	-	-	-	1 (0.33) ⁺	0.09*
agnathia	-	1 (0.32) ⁺	-	-	-
anophthalmia	-	-	-	1 (0.33) ⁺ 1 (0.67)	-
cleft vertebrae	-	-	2 (1.56)	1 (0.64)	0.11
fused vertebra	-	-	-	1 (0.74)	0.02

+ calculated for all living fetuses

* These data origin from an internal historical data set which is filed in the Teratology Laboratory at Dr. (b)(4) GmbH, Biberach

The complete resorption in one HD dam and the decreased fetal body weights may be attributable to the significant reduction in maternal body weight in the HD group.

The two fetuses in the high dose group with anophthalmia one of them also with hydrocephalus, occurred in the one litter.

Study title: Study for effects on embryo-fetal development in rabbits by oral administration, gavage

Key study findings: No teratology findings. Increased fetal resorptions at HD.

Study no.: 68S; U95-2267

Volume #, and page #:

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. (b) (4) GmbH, Biberach an der Riss, Germany

Date of study initiation: January, 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9402-P;

Methods

Doses: 20, 40, 80 mg/kg

Species/strain: Chbb:HM (SPF) rabbits

Number/sex/group: 21 pregnant females/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 5 ml/kg

Satellite groups used for toxicokinetics: 6/gp

Study design: Pregnant females were dosed from GD 6-18. Animals were sacrificed on GD 29.

Results

Mortality (dams): One high dose dam died on GD 21, cause of death not stated.

Clinical signs (dams): The number of pregnant rabbits/group were 17, 17, 20 and 21 in control to high dose, respectively. Complete resorptions occurred in one MD dam and in 5 HD dams. There were abortions in one MD dam (2 fetuses) and one HD dam (5 fetuses).

Coprostasis (fecal impaction) was seen in 2/20 MD rabbits and in 14/21 HD rabbits.

I assume that the dams were culled to 17/group except for the HD which had only 15 pregnant dams remaining.

Body weight (dams):

Dose (mg/kg)	n	Mean of Body Weight Gain [g] relative to GD 6				
		GD 7	GD 8	GD 18	GD 21	GD 28
control	17	5.6	9.2	128.1	150.5	272.7
20	17	-5.1	-24.3	113.8	149.3	300.9
40	17	-27.7↓*	-55.7↓*	32.7↓*	84.2	238.5
80	15	-57.5↓*	-104.0↓*	-94.7↓*	-14.4↓*	180.1↓*

* significant difference (P<0.05) , ↓ decreased

Food consumption (dams):

FC was decreased during treatment in the MD and HD group.

Toxicokinetics:

Geometric means of $C_{max,ss}$ and AUC_{0-24h} and medians of $t_{max,ss}$ on GD 13/14:

Dose [mg/kg/day]	N	$t_{max,ss}$ [h]	$C_{max,ss}$ [ng/ml]	AUC_{0-24h} [(ng×h/ml)]
20	3	2	1286	7535
40	3	2	1821	15720
80	3	2	4852	53930

Human AUC_{0-t} is 2080 ng.h/ml

Terminal and necropsic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Gestation index was 100% in controls and LD, 85% in MD and 71% in HD due to aborted fetuses in one MD and one HD dam, complete abortion in one HD dam and to complete resorptions in one MD and 5 HD dams

Parameter (mean)	Control	20 mg/kg	40 mg/kg	80 mg/kg	Historical range#
Corpora lutea	6.8	7.3	8.1*	8.0	7.5-9.1
Late resorptions	0.1	0.1	0.1	0.8*	0-0.1
Resorption rate	5.8	6.6	2.4	17.0*	4.6-14.4

* significant difference (P<0.05) , # = from vehicle controls

Offspring (malformations, variations, etc.):

Five fetuses of the HD group were classified as runts (less than 65% of control value).

Parameter (mean)	Control	20 mg/kg	40 mg/kg	80 mg/kg	Historical range#
Fetal body weight [g]	39.0	38.5	36.2*	35.9*	34.6-39.6

* significant difference (P<0.05) , # = from vehicle controls

There were no variations or malformations in the treated groups that were considered treatment related. None occurred more than once or at a significantly greater frequency than concurrent controls and all were within the historical control range.

Findings	Control	Dose mg/kg)			Historical Data* / %
		20	40	80	
Runts		-	-	5 (5.0%)	1.2
Variations:					
Flexure	2 (1.9%)	-	1 (0.8%)	-	0.9
Ventricular septal defect (VSD)	3 (2.8%)	3 (2.7%)	4 (3.1%)	2 (2.0%)	38.3
short 12th rib	-	1 (0.9%)	-	-	0.1
13th rib	-	1 (0.9%)	1 (0.8%)	-	0.4
Malformations:					
Hydrocephalus	-	-	1 (0.8%)	-	0.05
Synostosis of sternebrae	2 (1.9%)	-	-	1 (1.0%)	0.25

* These data origin from an internal historical data set which is filed in the Teratology Laboratory at Dr. ^{(b) (4)} GmbH, Biberach

Prenatal and postnatal development

Study title: **Study for effects on pre- and postnatal development including maternal function in rats by oral administration, gavage**

Key study findings: Decreased number of offspring, some developmental effects.

Study no.: 96B026; U97-2294

Volume #, and page #:

Conducting laboratory and location:

Date of study initiation: March, 1996

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403-P

Methods

Doses: 20, 80, 200 mg/kg

Species/strain: Chbb: (b)(4) (SPF) rats

Number/sex/group: 24 females/group

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg

Satellite groups used for toxicokinetics: none

Study design: Dosing began on GD 6 and continued until postpartum (pp) day 21

Results

F₀ in-life: Twenty control and low dose and 21 mid and high dose females became pregnant. One HD dam died after the first treatment. Slight treatment related sedation was seen in the LD on treatment day 1 and in the MD from treatment day 1 to 10. In the HD group there was slight to severe sedation and somnolence, reduction of spontaneous activity and occasional pilo-erection, chromorhinodacryorrhea and dyspnea. Gestation period was 22 days for the control group, 3 dams in the LD and MD had gestations of 23 days and 8/20 dams in the HD group had a gestation of 23 days.

Body weight gain of pregnant dams of all treated groups was dose dependently and significantly decreased during gestation. However, by lactation day (LD) 21, only the HD group had decreased body weight.

Dose [mg/kg]	Mean of body weight gain (relative to GD 6) during gestation [g]				
	GD 7	GD 10	GD 17	GD 20	GD 21
control	4.4	15.6	62.9	109.4	127.5
20	-0.3*↓	8.6*↓	47.7*↓	84.8*↓	100.0*↓
80	-1.0*↓	6.1*↓	38.5*↓	73.7*↓	88.4*↓
200	-11.0*↓	1.0*↓	25.4*↓	55.9*↓	69.6*↓

* significant difference (P<0.05), ↓ decreased, GD=gestation day

Dose [mg/kg]	Mean of body weight gain (relative to LD 1) during lactation [g]			
	LD 4	LD 7	LD 14	LD 21
control	10.6	16.2	39.1	42.9
20	5.8	14.0	36.2	47.6
80	5.3	11.1	30.5*↓	44.0
200	0.8*↓	4.2*↓	23.0*↓	25.7*↓

* significant difference (P<0.05), ↓ decreased, LD=lactation day

F₀ necropsy:

No macroscopic changes were seen in the dams at weaning. All litter parameters were comparable in control, LD and MD groups. The HD group had significantly reduced numbers of implantations and newborns, due primarily to postimplantation loss.

Parameter (mean; range)	Control	20 mg/kg	80 mg/kg	200 mg/kg	Historical range#
Implantations	15.8	14.3	14.6	12.5*↓	14.3; 13.5-15.0
Number of offspring	14.1	12.4	12.7	10.8*↓	12.8; 12.8-12.9
Postimplantation loss [%]	10.7	13.3	12.8	16.6	12.2; 10.0-14.0

* significant difference (P<0.05), # = from vehicle controls

At birth anophthalmia was seen in two pups in the high dose group, exceeding the historical control range for the strain. No variations were seen in controls or treated groups.

In the 200 mg/kg dose group viability rate was decreased to 49.3% and weaning rate to 85.5%. In the 80 mg/kg dose group the effect on viability was 82%. All offspring survived after weaning.

At delivery the body weights of the F₁ pups from the 80 and 200 mg/kg groups were significantly lower than the control and below the mean historical control mean. Body weight gain of these groups remained significantly decreased during lactation.

F₁ physical development:

Dose [mg/kg]	Mean of body weight [g]	Mean of body weight gain (relative to LD 1) [g]			
	LD 1	LD 4	LD 7	LD 14	LD 21
Control	5.9	1.4	6.4	22.0	38.1
20	5.9	1.2	6.0	21.0	36.6
80	5.6*↓	0.7*↓	4.7*↓	18.0*↓	32.6*↓
200	5.3*↓	0.1*↓	2.6*↓	14.4*↓	27.7*↓

* significant difference (P<0.05), ↓ decreased, LD=lactation day

Time points for incisor eruption, growth of fur, opening of the ear canals, opening of the eyes, correct running, descent of the testes and vaginal opening were similar for controls, LD and MD. In the HD, growth of fur, opening of the ear canals and vaginal opening were significantly delayed for one day in 10/72 (fur), 12/72 (ear) and 5/21 (vagina) pups or more than one day in 6/72, 3/72 and 3/21 pups.

Pupillary reflex, air-righting reflex and hearing were not affected by treatment.

F₁ behavioral evaluation:

Biel Water T-maze Test: Results of the learning approach in wk 6 and test of memory function in wk 7 were similar in all groups.

Test on motility (Actiframe): Time dependent activity was similar between all groups. In controls, locomotor activity could be seen at 4 pm due to switch off of room light. The reaction in the MD and HD groups was significantly decreased (less total activity). In the LD, only the females were affected.

F₁ reproduction:

No treatment related effects were seen. Copulation and fertility indices were not affected by treatment and there were no abortions, resorptions of entire litters or intercurrent deaths. Mean numbers of viable fetuses, resorption rate and preimplantation loss were comparable between treated and control groups.

2.6.6.7 Local tolerance

Not done

2.6.6.8 Special toxicology studies

Study title: **34-week oral (diet) toxicokinetic study in the mouse**

Key study findings: no increase in serum prolactin levels

Study no.: 06R074

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim, Pharm, Ridgefield, CT

Date of study initiation: Sept, 2006

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, lot 05071202M, purity not stated

Methods

Doses: 10, 80, 200, 1000 (males) 1200 (females) mg/kg

Species/strain: CD-1 mice

Number/sex/group or time point (main study): 35/sex/gp

Route, formulation, volume, and infusion rate: oral in diet

Satellite groups used for toxicokinetics or recovery: none

Results:

Mortality: none drug related

Clinical signs: none drug related

Body weights: At the end of the study, HD males weighted about 15% less than controls. There were non-significant decreases in BW in the other male groups as well. No effects on BW in females.

Toxicokinetics:

Summary Table 1 Mean toxicokinetic parameters of flibanserin after dietary administration to mice

Parameter	Week	Gender	10 mg/kg	80 mg/kg	200 mg/kg	1000 mg/kg	1200 mg/kg
C(max)	3	m	39.1	176	376	1430	-
[ng/mL]	3	f	47.6	192	313	-	1710
AUC(0-24h)	3	m	591	3150	6020	16300	-
[ng-h/mL]	3	f	671	2370	5680	-	20800
C(1000)	3	m	18.5	115	217	628	-
[ng/mL]	3	f	18.6	66.1	188	-	477
C(0800)^a	13	m	18.0	207	247	787	-
	13	f	16.2	101	197	-	558
[ng/mL]	26	m	23.0	150	233	748	-
	26	f	16.5	88.6	311	-	764

^a actual collection between 0800 and 1050 hours

In humans taking flibanserin 100 mg, the AUC_{0-ss} is 2080 ng.h/ml.

Prolactin levels - means

Prolactin ng/ml	control	10 mg/kg	80 mg/kg	200 mg/kg	1000/1200 mg/kg
wk 14	129 ± 58*	128 ± 107	134 ± 42	115 ± 55	177 ± 104
wk 34	106 ± 153	33 ± 31	146 ± 152	103 ± 66	104 ± 77

* ± SD

Prolactin levels (ng/ml), week 14, individual data, summary statistics and t-tests

Animal	Control	Low-Dose	Low-Mid-Dose	High-Mid-Dose	High-Dose
1	136.65	77.93	93.94	70.27	163.47
2	179.06	142.06	160.03	200.57	185.50
3	169.86	315.66	169.87	102.10	114.38
4	185.08	156.46	180.49	175.00	140.99
5	105.97	4.24	155.71	54.58	126.31
6	19.53	177.52	93.57	121.59	101.86
7	107.31	19.75	81.15	79.38	404.58
N	7	7	7	7	7
Mean	129.066	127.660	133.537	114.784	176.727
SD	58.257	106.628	42.087	54.851	104.468
Geom. Mean	107.951	69.869	127.407	104.120	158.790
Geom. SD	2.200	4.546	1.403	1.612	1.584
p value	-	0.3363	0.7124	0.9359	0.3930

Prolactin levels (ng/ml), week 34, individual data, summary statistics and t-tests

Animal	Control	Low-Dose	Low-Mid-Dose	High-Mid-Dose	High-Dose
1	51.47	21.94	18.84	80.63	238.68
2	3.85	20.19	161.12	141.14	111.34
3	51.75	11.25	147.24	199.12	50.52
4	444.08	12.50	17.50	16.00	155.58
5	100.53	48.02	186.28	128.04	33.89
6	87.98	97.23	39.61	53.91	115.12
7	5.36	19.75	451.20		20.97
N	7	7	7	6	7
Mean	106.431	32.983	145.970	103.140	103.729
SD	153.368	30.839	152.206	66.030	77.078
Geom. Mean	41.608	24.784	82.512	79.384	77.694
Geom. SD	5.357	2.148	3.510	2.479	2.404
p value	-	0.4077	0.2760	0.3225	0.3196

Analyzed by ANOVA followed by Dunnett's test. Sponsor also did statistical tests on the geometric means. There were no significant effects.

In female mice given flibanserin, there were no effects on prolactin levels at either 14 or 34 wks at doses up to 1200 mg/kg or approximately 10 times the human exposure.

As quoted by sponsor

Prolactin levels measured in Drug Weeks 14 and 34 did not show any evidence of drug-related differences as compared to vehicle Control animals, nor were any dose-related changes observed. In Drug Week 34, estrus stage as evaluated by vaginal smear showed no correlation to prolactin levels or flibanserin dose level.

Summary: TK study with flibanserin gave exposure multiples in female mice of approximately 0.3, 1, 3 and 10 times the human exposure in humans taking 100 mg. The doses had no effect on plasma prolactin levels and cannot explain the increase in mammary tumors seen in mice given flibanserin

Study title: Four week oral (gavage) immunotoxicity study in female rats

Key study findings:

Study no.: 04B105

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim Pharma, Biberach/Riss, Germany

Date of study initiation: May, 2004

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 99111078M, 99.4% pure

Formulation/vehicle: 0.5% methylcellulose

Methods

Doses: 0, 20, 100, 250 mg/kg/day

Study design: Flibanserin was administered daily for at least 28 days by oral gavage to groups of 10 female Chbb. (b)(4) rats (gps 1-4). A fifth group of 10 females was gavaged with 25 mg/kg cyclosporin A. An additional 10 animals received either 0 or 250 mg/kg flibanserin for 28 days and were kept for a 4 week recovery period.

Group No.	Daily dose of BIMT 17 BS [mg/kg]	Females	
		Main study	Recovery
1	0 (vehicle)	151-160	161-170
2	20	251-260	-
3	100	351-360	-
4	Day 1: 400 Day 2- 28: 250	451-460	461-470
5	Cyclosporin A 25 mg/kg	551-560	-

High dose was reduced due to unexpected severe catalepsy in individual animals and inter-individual aggressiveness in all animals at 400 mg/kg, the HD was reduced from day 2 on to 250 mg/kg. Neither the catalepsy nor the aggressiveness had been seen in other studies at 400 mg/kg. Apparently, the aggressiveness was due to a combination of flibanserin treatment and group housing. On day three, animals were housed in individual cages.

Results:

Semiquantitative comparison of cell markers in Cyclosporin-A treated animals versus control

Cell marker	CD 2	CD 3	CD 8	CD 45RA
Lymphocyte subset	T-subset	T-subset	T-subset	B-subset
Thymus				
Cortex	unchanged	unchanged	unchanged	unchanged
Medulla	reduced	reduced	reduced	reduced*
Spleen				
Follicle	unchanged	unchanged	unchanged	unchanged
PALS	reduced	reduced	reduced	reduced*
Marginal zone	unchanged	unchanged	unchanged	unchanged
Red pulp	unchanged	unchanged	unchanged	unchanged
Lymph node, mesenterial				
Follicle	unchanged	unchanged	unchanged	unchanged
Paracortex	reduced	reduced	reduced	reduced*
Sinusoidal lymphocytes	unchanged	unchanged	unchanged	unchanged

unchanged: Similar or equal to Control / No noteworthy difference when compared to Control

reduced: Reduced volume of the respective anatomical zone as a consequence of the reduced number of positive staining lymphocytes. Note: the remaining lymphocytes may stain positive.

reduced* Note: there are generally only a few or no B-lymphocytes within the respective anatomical zone.

Results of flow cytometry of peripheral blood cells (means)

Blood, spleen and thymus cells were placed into flow cytometry tubes containing combinations of antibodies to rat leukocyte cell surface proteins. These antibodies had been conjugated to different fluorochrome dyes to allow detection and discrimination of the different cell populations.

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclo- sporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.97	99.98	99.95	99.99*	99.96	100.00	100.00
T-lymphocytes (CD45 ⁺ CD3 ⁺ cells) (% of leukocytes)	44.15	43.75	42.64	42.32	28.36*	50.26	48.16
T-helper-lymphocytes (CD3 ⁺ CD4 ⁺ cells) (% of T-lymphocytes)	83.80	83.84	82.87	82.12	80.41	80.70	79.58
Double positive T-lymphocytes (CD3 ⁺ CD4 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	1.54	0.70*	1.00	1.01	0.66*	2.16	2.83
Cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	16.86	15.79	17.34	18.01	18.90	20.60	22.28
B-lymphocytes (CD45 ⁺ CD45RA ⁺ cells) (% of leukocytes)	45.60	45.93	47.30	46.47	58.73*	35.78	33.54
Monocytes (CD3 ⁺ CD4 ⁺ cells) (% of analysed cells)	3.59	3.15	2.81*	2.88*	6.14*	9.04	11.58
Total Natural Killer Cells (CD45 ⁺ CD161 ⁺ cells) (% of leukocytes)	4.80	4.11	3.97	4.16	5.36	5.51	6.47
Ratio of T-lymphocytes (CD3 ⁺) to B-lymphocytes (CD45RA ⁺) (calculated)	1.00	1.04	0.96	0.96	0.50*	1.46	1.51
Ratio of T-helper-lymphocytes (CD3 ⁺ CD4 ⁺) to cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺) (calculated)	5.22	5.58	5.15	4.72	4.53	4.08	3.63

* Statistically significant differences (p<0.05) versus Control

Cyclosporine A treatment tended to decrease T-cells and increase B-cells in peripheral blood. There was an isolated effect of flibanserin treatment to decrease monocytes in the MD and HD.

Results of flow cytometry of spleen cells (immune cell activity)

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.97	99.98	99.99*	99.99*	99.98*	100.00	100.00
T-lymphocytes (CD45 ⁺ CD3 ⁺ cells) (% of leukocytes)	44.67	44.01	45.11	44.73	37.95	45.47	45.58
T-helper-lymphocytes (CD3 ⁺ CD4 ⁺ cells) (% of T-lymphocytes)	67.05	67.66	66.83	68.68	60.48	67.77	73.15*
Double positive T-lymphocytes (CD3 ⁺ CD4 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	0.87	0.95	0.91	1.07	0.87	1.06	1.23
Cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	28.38	28.92	30.98	28.88	35.69*	29.52	24.31
B-lymphocytes (CD45 ⁺ CD45RA ⁺ cells) (% of leukocytes)	44.62	43.45	44.10	43.76	46.60	27.17	31.37*
Monocytes (CD3 ⁺ CD4 ⁺ cells) (% of analysed cells)	4.18	5.30	4.92	5.77	6.93*	12.81	12.26
Total Natural Killer Cells (CD45 ⁺ CD161 ⁺ cells) (% of leukocytes)	6.50	6.45	6.75	6.43	8.29	16.03	11.94*
Ratio of T-lymphocytes (CD3 ⁺) to B-lymphocytes (CD45RA ⁺) (calculated)	1.06	1.02	1.11	1.14	0.84	1.73	1.49
Ratio of T-helper-lymphocytes (CD3 ⁺ CD4 ⁺) to cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺) (calculated)	2.54	2.43	2.41	2.53	1.72*	2.38	3.14

* Statistically significant differences (p<0.05) versus Control

Cyclosporin administration resulted in a decrease of both the ratio of T to B lymphocytes and the ratio of T-helper to cytotoxic T-lymphocytes. There was a relative increase in both monocytes and natural killer cells in these animals.

Results of flow cytometry of thymus cells

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Number of thymus cells (x 10 ⁻⁷)	27.10	25.57	30.87	30.80	23.59	25.35	30.88
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.51	99.73	99.82	99.30	99.80	99.56	99.64
CD3 ⁺ thymus cells (% of thymus cells)	21.76	18.14	20.91	18.61	6.97*	14.39	20.13
CD4 ⁺ thymus cells (% of analysed cells)	95.81	95.29	94.69*	94.94	95.46	96.66	96.92
CD8 ⁺ thymus cells (% of analysed cells)	91.93	92.59	91.79	92.60	98.45*	95.83	97.06
CD4 ⁺ CD8 ⁻ thymus cells (% of analysed cells)	6.79	5.96	6.66	5.97	0.72*	3.34	2.48
CD4 ⁺ CD8 ⁺ thymus cells (% of analysed cells)	3.03	3.39	3.90*	3.77*	3.81	2.60	2.72
CD4 ⁺ CD8 ⁺ thymus cells (% of analysed cells)	88.90	89.20	87.90	88.82	94.64*	93.23	94.35
CD4 ⁺ CD8 ⁻ thymus cells (% of analysed cells)	1.28	1.45	1.55	1.44	0.83*	0.83	0.46*
CD3 ⁺ CD4 ⁺ CD8 ⁻ thymus cells (% of CD3 ⁺ cells)	26.50	28.59	28.93	27.52	3.23*	19.03	9.37
CD3 ⁺ CD4 ⁺ CD8 ⁺ thymus cells (% of CD3 ⁺ cells)	7.03	5.91	7.48	8.18	5.30	5.93	4.87
CD3 ⁺ CD4 ⁺ CD8 ⁺ thymus cells (% of CD3 ⁺ cells)	63.35	62.72	60.99	61.49	89.57*	73.44	85.08*
CD3 ⁺ CD4 ⁺ CD8 ⁻ thymus cells (% of CD3 ⁺ cells)	3.12	2.79	2.60	2.81	1.90*	1.61	0.69*
Ratio of CD4 ⁺ to CD8 ⁺ thymus cells (calculated)	1.04	1.03*	1.03	1.03	0.97*	1.01	1.00*

* Statistically significant differences (p<0.05) versus Control

Cyclosporine treatment reduced the number of isolated thymocytes and shifted the thymocyte subsets. There was a reduction in more mature CD3⁺ and CD4⁺CD8⁻ thymocytes whereas the relative number of immature double positive CD4⁺CD8⁺ was increased. As a consequence, the relative number of mature CD3⁺CD4⁺CD8⁻ cells was markedly reduced. There was a small reduction in mature CD3⁺CD4⁺CD8⁺ thymocytes and the relative number of CD3⁺CD4⁺CD8⁺ thymocytes increased.

Natural Killer Cell activity of spleen cells

Daily Dose [mg/kg]	Mean Specific Release [%] at various effector to target cell ratios					
	ratio 200:1	ratio 100:1	ratio 50:1	ratio 25:1	ratio 12:1	ratio 6:1
Main study 0 (Control)	9.2	6.5	4.9	3.1	1.6	1.2
Main study 20 BIMT 17 BS	11.6	8.3	6.1	3.7	2.0	1.3
Main study 100 BIMT 17 BS	14.3*	10.3*	7.1	4.5	2.4	1.5
Main study 400/250 BIMT 17 BS	14.6*	10.5*	7.4	4.2	2.0	1.2
Main study 25 Cyclosporine A	25.7*	18.9*	12.7*	7.6*	4.1	2.6
Recovery: 0 (Control)	27.3	22.2	15.7	9.7	5.5	3.0
Recovery: 400/250 BIMT 17 BS	36.7*	30.3	20.9	12.6	7.0	4.3

* Statistically significant differences (p<0.05) versus Control

In the main study, animals treated with flibanserin had an increased killing of YAC-1 target cells compared to controls at both 100 and 250 mg/kg. At the higher dose, this increase was still seen after the recovery period. Treatment with Cyclosporin-A resulted in a pronounced increase in spleen cell NKC activity along with an increased phenotypic presence of NKC's in the spleen.

The sponsor believes that the increase activity with HD flibanserin was due to the abnormally low control activity level. Hard to say although the NKC activity of the control for the recovery group was considerably higher. However, if control activity was higher, then the Cyclosporine A activity would not have shown an increase.

I'm no expert on immunotoxicity studies but it seems clear that cyclosporin A had activity in altering the numbers of T and B cells in blood, spleen and thymus. In contrast, flibanserin treatment clearly had no effects on immune cell numbers or ratios. However, flibanserin treatment at both 100 and 250 mg/kg did increase natural killer cell activity. Whether this was due to abnormally low activity in controls is not clear.

Overall, it seems that the effect of flibanserin administration on immune activity is minimal at most.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Flibanserin is a 5-HT_{1A} agonist and 5-HT_{2A} antagonist that is intended to treat women with hypoactive sexual desire disorder (HSSD). In studies in mice, chronic flibanserin administration decreased serotonin levels in the median prefrontal cortex and the nucleus accumbens shell while increasing norepinephrine levels. Effects on dopamine appear mixed.

Flibanserin had a number of neurological effects, some severe at high doses but not unexpected for a centrally acting drug. In rats, flibanserin produced mild elevations of

blood pressure and heart rate but had no effect on the ECG rhythm or waveform morphology. There were no respiratory effects in rats.

Flibanserin is metabolized extensively but is considered the active moiety since most of the metabolites are pharmacologically inactive and only two active metabolites cross the blood brain barrier, and neither are near the concentration of flibanserin.

In chronic toxicity studies in rats and dogs, flibanserin produced clear clinical signs of discomfort at high doses (exposures 28 times higher in rats and 100 times higher in dogs than human exposure). In dogs there were dose-related ophthalmologic and histopathologic changes of the eyes characterized by discrete, focal, smoky opacity or by focal thickening of the corneal epithelium. This effect was also seen in a shorter, 13 week study in dogs but was not seen in any study in rats.

Also in dogs, flibanserin produced fatty changes in the liver of males and females after 26 and 52 weeks. This effect seemed both dose and duration related with an increase in severity and numbers of dogs affected. Hepatocellular fatty changes were also seen in the 13 and 26 week rat toxicity studies.

There was an increase in fatty accumulation in the myocardium of dogs of both sexes without an increase in severity or number of dogs affected from 26 to 52 weeks.

This fat deposition occurred in both the MD and HD with the no effect level being 3 mg/kg or approximately 0.6 times the human exposure. The mechanism for the fat deposition is unknown but it did not produce measurable changes in cardiac electrical patterns measured throughout the study. Significantly, there was no increase in cardiac fat deposition in rats at any dose. There were fatty changes in the livers of both rats and dogs.

Because of the ophthalmologic findings in dogs, the Sponsor was asked to include ophthalmologic endpoints in a clinical trial. In A 24 week, randomized, double blind placebo controlled trial with an open-label extension, exams were performed at screening and end of treatment and included the best corrected distance visual acuity, tonometry (intraocular pressure measurement) and with pupils dilated, slit lamp evaluation of the anterior segment, including the cornea and lens. There were no increased findings or corneal abnormalities with flibanserin treatment.

Flibanserin was not mutagenic in vitro in bacteria or Chinese hamster ovary cells but was positive in the in vitro human lymphocyte assay. It was negative in the in vivo rat micronucleus assay and the Comet assay for DNA damage. Based on the weight of evidence, I consider flibanserin to be non-genotoxic.

When administered orally to mice and rats for two years, flibanserin increased the incidence of liver tumors. In male mice there was a significant increase in hepatocellular carcinomas. In females, there was an increase in the incidence of hepatocellular carcinomas and combined carcinomas and adenomas in the three higher dosed groups that was significant pairwise and for trend at the high dose group. In rats, there was a modest increase in the incidence of hepatocellular carcinomas in males but not in females

(significant for trend against one control group only). Flibanserin induced centrilobular hepatocellular hypertrophy probably by inducing hepatic cytochrome P-450 enzymes. Sponsor believes that flibanserin is acting like phenobarbital in promoting liver tumors. Because flibanserin is an enzyme inducer in rodents and is essentially non-genotoxic, this seems like a reasonable explanation.

In the mammary gland of female mice, there was a clear dose-related increase in adenocarcinomas and malignant adenoacanthomas. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes. This effect was not seen in rats.

In reproduction studies in rats and rabbits, flibanserin administration did affect the duration of the estrus cycle in rats but had no effect on female fertility as measured by copulation index, fertility index or gestation index. When given during the period of organogenesis, flibanserin produced some visceral and skeletal variations in rats at the 400 mg/kg dose (approximately 42 times human exposure) and there were different malformations in pups from all treated groups without a discernable pattern. There were resorptions in rabbits treated with 80 mg/kg (26 times human exposure) but there was no increase in fetal variations or malformations. In a pre- and postnatal development study in rats, flibanserin produced maternal toxicity in all groups with a significant reduction in number of implantations and newborns from dams treated with the HD (200 mg/kg or roughly 30 times human exposure). Dams treated with the HD showed poor maternal care leading to failure to thrive and high mortality. There were some development delays in these pups but fertility and the F₂ generation seemed unaffected.

In general, flibanserin administration produced no clear signal for adverse effects on fetal development. Although almost all malformations occurred in fetuses from treated rats they affected different organ systems without an obvious common mechanism. In both the embryofetal and the prenatal and postnatal development study, flibanserin administration resulted in pups with anophthalmia. In the rat embryofetal development study, two pups from one litter from the HD had anophthalmia and in the postnatal study, there were two affected fetuses from the high dose group (drug blood levels not determined but probably around 30 times human exposure extrapolating roughly from the rat embryofetal development toxicokinetic data). There was one fetus with hydrocephalus in both treated rats (42 times human exposure) and rabbits (8 times human exposure). Considering the different types of malformations, lack of dose response and the fairly high drug exposure levels in which they occurred, the animal data do not indicate to me that the offspring are at significant risk if flibanserin is inadvertently taken by a pregnant woman.

In special toxicology studies, flibanserin increased natural killer cell activity but had no other effects on the rat immune system and did not seem to produce self-administration behavior in rats.

In general, flibanserin produced few significant toxicological effects in rats or dogs. In reproduction studies, flibanserin did not affect fertility and was not obviously teratogenic.

In carcinogenicity studies, flibanserin produced malignant mammary tumors in mice but not rats.

Unresolved toxicology issues (if any): none

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/s/

ALEXANDER W JORDAN
09/06/2013

Comments on NDA 22-526 flibanserin

Date 8/26/10

From: A Jacobs AD

1. Comments on approvability: There are no outstanding pharm/tox issues.
2. I could not find any evidence of the exec-cac minutes for the results, which would be the CDER opinion on what neoplasms are drug related.
3. I have conveyed some other comments to the reviewer who will address them as appropriate.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22526	ORIG-1	BOEHRINGER INGELHEIM PHARMACEUTICA LS INC	FLIBANSERIN

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/s/

ABIGAIL ABBY C C JACOBS
08/26/2010



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-526
SERIAL NUMBER: 001
DATE RECEIVED BY CENTER: November 10, 2009
PRODUCT: Trade Name (flibanserin; BIMT 17 BS)
INTENDED CLINICAL POPULATION: Women with hypoactive sexual desire disorder
SPONSOR: Boehringer Ingelheim
DOCUMENTS REVIEWED: ECT
REVIEW DIVISION: Division of Reproductive and Urological Drug Products
PHARM/TOX REVIEWER: Alex Jordan, PhD
PHARM/TOX SUPERVISOR: Lynnda Reid, PhD
DIVISION DIRECTOR: Scott Monroe, MD
PROJECT MANAGER: Charlene Williamson

Date of review submission to Division File System (DFS): July 7, 2010

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: I recommend approval of flibanserin (Trade Name) for women with hypoactive sexual desire disorder. Because of the mammary tumors in mice, I would recommend limiting the patient population to women who are not at an increased risk for breast cancer, i.e., women without previous breast cancer or a family history of breast cancer. Post-approval tracking of patients for breast cancer should be implemented, if possible.

B. Recommendation for nonclinical studies: none

C. Recommendations on labeling

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C

In reproduction toxicity studies in rats given flibanserin during the period of organogenesis, there were sporadic malformations at exposures ≥ 3 times human exposure based on drug blood levels (AUC). In rabbit reproduction studies, there was an increase in resorptions at 26 times human exposure but there was no increase in fetal malformations. There are no adequate and well controlled studies of flibanserin in pregnant women. Trade Name should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

8.2 Nursing Mothers

It is not known if flibanserin is excreted in human milk. However, flibanserin is excreted in rat milk. Because many drugs are excreted in human milk, caution should be exercised when Trade Name is administered to a nursing mother.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility:

Carcinogenesis

Oral administration of flibanserin for 2 years produced a significant dose-related increase in the incidence of malignant mammary gland carcinomas in female mice at drug levels approximately 3 and 10 times the levels in women taking the recommended dose. In male and female mice, flibanserin administration increased the incidence of liver tumors at exposures approximately 10 times the exposure for women taking 100 mg. There was a non-significant increase in liver tumors in male rats.

Mutagenesis

Flibanserin was negative for mutagenesis in vitro in *Salmonella typhimurium* (Ames test) and in Chinese hamster ovary cells. It was positive for chromosomal aberrations in cultured human lymphocytes but was negative for

chromosomal aberrations in vivo in the rat bone marrow micronucleus assay and for DNA damage in rat liver in the Comet assay.

Impairment of fertility

Flibanserin administration to female rats 2 weeks before mating until gestation day 6 resulted in slight changes in the duration of the estrus cycle, decreases in body weight gain and some neurological signs at high doses. Flibanserin administration had no effect on fertility or early embryonic development.

13.2 Animal Toxicology

In oral repeat-dose toxicity studies over a maximum of 13 weeks in mice, 26 weeks in rats and 52 weeks in dogs, severe signs of toxicity were only observed at doses far in excess of the clinical dose of 100 mg once a day. Hepatocellular hypertrophy was found in mice and rats and is considered the morphological correlate to enzyme induction that has been shown for flibanserin in rodents. Changes in lipid storage in the liver were present in rodents at high drug exposures. There were no unequivocal lipid changes in the livers of dogs but there was lipid droplet accumulation in the myocardium of the left ventricle. The no effect dose for fat deposition in the heart was 3 mg/kg or approximately 0.6 times the exposure in women taking 100 mg. This effect was not seen in rodents. Also in dogs there were dose related ophthalmological and histological changes of the eyes characterized by discrete, focal, smoky opacity or by focal thickening of the epithelium. The no effect drug exposure was approximately equal to the human exposure.

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings: In rats, chronic administration of flibanserin resulted in an increase in weights of the thymus, adrenal and liver with a reduction in fat deposition in the liver of males and an increase in hepatocellular hypertrophy in females treated with 400 mg/kg daily (approximately 29 times human exposure). In dogs, chronic flibanserin administration produced corneal opacities, increased heart rate and an increase in the frequency and intensity of fatty liver and heart which seemed dose and duration dependent. The fatty accumulation in the heart occurred only in dogs at exposure levels in females of approximately 5 times the exposure in women taking 100 mg. In the adrenal, there was an increase in fat accumulation which was dose but not duration dependent. There was a degeneration of the mucous membranes of the trachea in dogs treated with 15 or 75 mg/kg/day (5 to 53 time human exposure). In mice, there was a dose-related statistically significant increase in malignant mammary tumors. This was not seen in rats. The weight of evidence indicates that flibanserin is not genotoxic. Flibanserin had no significant effects on fertility in male or female rats. When flibanserin was given to rats during embryogenesis, there was an increase in fetal variations at the high dose and there were sporadic non dose-related fetal malformations. There were no adverse fetal effects in rabbits other than an

increase in resorptions at the high dose. In a pre-and postnatal development study in rats, flibanserin reduced the number of implantations and newborns from dams treated with the HD (200 mg/kg or roughly 30 times human exposure). Dams treated with the HD showed poor maternal care leading to failure to thrive and high mortality. There were some development delays in these pups but fertility and the F₂ generation seemed unaffected.

- B. Pharmacologic activity: Flibanserin acts on the serotonin receptor and functions as a 5-hydroxytryptamine (5-HT)_{1A} agonist 5-HT_{2A} antagonist that decreases extracellular serotonin and increases norepinephrine in rat prefrontal cortex, medial preoptic area and nucleus accumbens. There are also regional, dose-related effects on dopamine. These effects are believed to be related to its effect on increased sexual desire in females.
- C. Nonclinical safety issues relevant to clinical use: The major issue is the increased incidence of mammary tumors in mice. Flibanserin is a new molecular entity with a neurochemical mechanism indicated for hypoactive sexual desire disorder in women. Mammary tumors are not particularly common in mice so there is some concern about an increased risk of breast cancer. However, flibanserin does not seem to be genotoxic and there was no increased incidence of mammary tumors in rats. The incidence of mammary tumors in mice while significantly elevated compared to concurrent controls, was only somewhat higher than the historical control maximum, thus the risk, if any, for breast cancer in women seems slight. I recommend that flibanserin be contraindicated in women with breast cancer or with a family history of breast cancer and that some form of post-approval tracking of flibanserin treated women be utilized, if possible.

[Please limit to 1-3 pages]

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-526

Review number: 1

Sequence number/date/type of submission: EDR

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Boehringer Ingelheim

Manufacturer for drug substance: (b) (4)

Reviewer name: Alex Jordan, PhD

Division name: Reproductive and Urological Products

HFD #:

Review completion date:

Drug:

Trade name:

Generic name: flibanserin

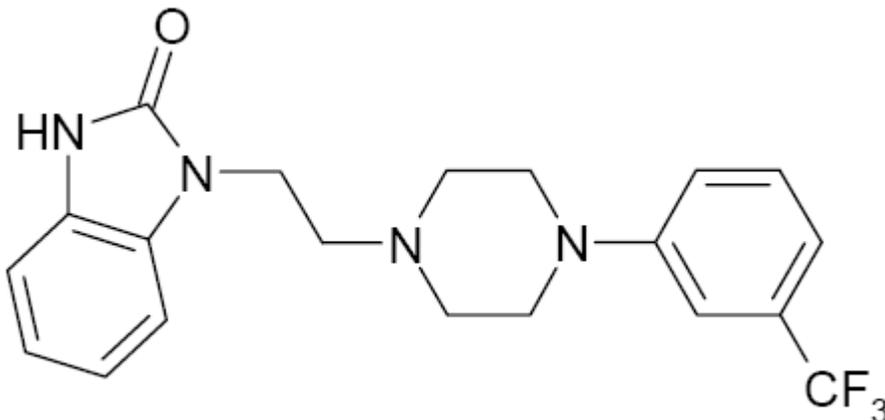
Code name: BIMT 17 BS

Chemical name: 2H-benzimidazol-2-one, 1,3-dihydro-1-[2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl].

CAS registry number:

Molecular formula/molecular weight: 390.4 g/mol

Structure:

Relevant INDs/NDAs/DMFs: IND (b) (4)

Drug class: Serotonin modulator

Intended clinical population: Women with hypoactive sexual desire disorder

Clinical formulation:

Table 1 Qualitative and quantitative composition of flibanserin film-coated tablets, 100 mg

Ingredient	mg per Tablet	Function	Reference to Standards
Flibanserin	100.000	Active ingredient	Company Standard
Lactose monohydrate (b) (4)	(b) (4)	(b) (4)	NF/Ph. Eur./JP
Microcrystalline cellulose (b) (4)			NF/Ph. Eur./JP
Hypromellose (b) (4)			USP/Ph. Eur./JP
Croscarmellose sodium			NF/Ph. Eur./JP
Magnesium stearate (b) (4)			NF/Ph. Eur./JP
(b) (4)			USP/Ph. Eur./JP
(b) (4) Pink (b) (4)			Company Standard
Total			347.0

Route of administration: oral

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Flibanserin acts preferentially as a post-synaptic 5-hydroxytryptamine (HT) [serotonin]_{1A} receptor agonist and a 5-HT_{2A} receptor antagonist. Flibanserin had high affinity for human 5-HT_{1A} recombinant receptors ($K_i = 6.59$ nM) and was a full agonist on 5-HT_{1A} receptors when receptors were expressed in Chinese hamster ovary (CHO) cells. Flibanserin had high affinity ($K_i = 15.3$ nM) and was a full antagonist for human 5-HT_{2A} receptors when expressed in CHO cells. Flibanserin also had moderate affinity for 5-HT_{2B}, 5-HT_{2C} and dopamine (DA) D₄ receptors. Flibanserin does not have physiologically significant affinity for human monoamine transporters, does not inhibit reuptake of these neurotransmitters into synaptosomes expressing rat monoamine transporters and does not inhibit monoamine oxidase type A or B in rat brain.

Flibanserin was originally developed to treat depression. In Phase 2 depression trials, flibanserin was not associated with sexual dysfunction, which is unusual for serotonergic antidepressants. A multi-dimensional measure of sexual dysfunction, the Arizona Sexual Experiences Scale (ASEX), was included in the four Phase 2b depression studies for comparison of flibanserin, standard antidepressants, and placebo. In one of these trials,

flibanserin was superior to both the positive comparator and placebo on the ASEX scale, mainly on the "sex drive" item in women.

Hypoactive sexual desire disorder (HSDD) is defined in the DSM-IV-TR as a deficiency or absence of sexual fantasies and desire for sexual activity that causes marked distress or interpersonal difficulty and is not due to the physiological effects of a substance or general medical condition. The biological causes of HSDD are only poorly understood and to date there are no approved pharmacological treatments in the US. Clinical development and off-label usage has focused on hormone products including a testosterone transdermal system that has been approved for surgically postmenopausal women in Europe.

The therapeutic mechanism of action of flibanserin in HSDD is unknown but there is evidence for the involvement of serotonin. Serotonin reuptake inhibitors (SSRIs) produce a number of sexual side effects with the most common being anorgasmia or delayed orgasm. There are also reports of spontaneous orgasm in conjunction with fluoxetine treatment and reports of hypersexual effects following treatment with fluoxetine or the serotonin releaser, fenfluramine suggesting that both inhibited and enhanced sexual responses to serotonergic agents may be produced. Flibanserin has been shown to stimulate 5-HT_{1A} receptors while blocking 5-HT_{2A} receptors and has moderate affinity to 5-HT_{2B,2C} receptors. These 5-HT receptor mediated mechanisms directly affect serotonergic neurotransmission but also have some indirect effects on dopaminergic and noradrenergic neurotransmitter signaling. Flibanserin decreases extracellular serotonin levels and increases the extracellular dopamine concentration in the medial prefrontal cortex in rats. This effect is completely abolished by the selective 5-HT_{1A} antagonist WAY100.635.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Flibanserin binding studies to various receptors in vitro revealed a high affinity for 5-HT_{1A} ($K_i = 6.59$ nM) and 5-HT_{2A} ($K_i = 15.3$ nM). Flibanserin has a moderate affinity for 5-HT_{2B} ($K_i = 89.3$ nM), 5-HT_{2C} ($K_i = 88.3$ nM), and dopamine D₄ receptors ($K_i = 167$ nM). Flibanserin does not have physiologically significant affinity for monoamine transporters 5-HT: ($K_i = 2.63$ uM), DA: ($K_i = 2.63$ uM), NA ($K_i = 2.63$ uM), and does not inhibit monoamine A and B oxidase.

In human post mortem studies, flibanserin significantly reduced the activity of forskolin-stimulated adenylyl cyclase postsynaptically (prefrontal cortex), and in the hippocampus, but had no effect in the raphe nuclei (presynaptic level).

Flibanserin acts as a full agonist on 5-HT_{1A} receptors in reducing forskolin-stimulated adenylyl cyclase activity. This was observed in all tissues in the human dorsal raphe (largest serotonergic nucleus where some antidepressants are believed to act) which contrasts with other 5-HT_{1A} receptor agonists, which are inactive in reducing forskolin-stimulated AC activity in the cortex.

Flibanserin 5-HT_{1A} agonist activities result in many of its pharmacological properties in vivo. Flibanserin decreases firing rates of 5-HT neurons in the rat dorsal raphe nucleus and alters firing rates in the hippocampus. Flibanserin also reduces neuronal cortical activity and these decreases can be inhibited by 5-HT_{1A} antagonists.

Drug activity related to proposed indication:

Effect of acute and chronic treatment with flibanserin 15 and 45 mg/kg po on extracellular serotonin, norepinephrine, dopamine, GABA and glutamate in the medial prefrontal cortex and in nucleus accumbens shell of freely moving female rats.

This study was conducted at (b) (4) under study key 191 (parts A and B). Rats (280-350 g) were from (b) (4). Drug was flibanserin batch 05071202M.

Female Wistar rats in the nonreceptive stage (not in estrous) were anesthetized and placed in a stereotaxic frame and I shaped probes were inserted in the medial prefrontal cortex (mPFC) and Nucleus Accumbens shell (NAcc).

The rats were orally dosed with 0, 15 or 45 mg/kg flibanserin two times a day for 21 days. On the day of the experiment, the probes were connected with flexible PEEK tubing to a microperfusion pump and perfused with artificial CSF at a flow rate of 1.5 ul/min. Microdialysis samples were collected at 30 min intervals. After the experiment the rats were sacrificed and the brains removed and the position of each probe was verified histologically.

Concentrations of 5-HT, NA, DA, GABA and Glu were determined by HPLC separation and electrochemical and fluorometric detection.

Effects of chronic treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle

	5-HT	NA	DA	GABA	Glu
mPFC (15 mg/kg)	Decrease +	Increase +	----	----	----
NAcc (15 mg/kg)	----	----	----	----	----
mPFC (45 mg/kg)	Decrease +	Increase +	Increase +	----	----
NAcc (45 mg/kg)	Decrease +	----	(Decrease +)	----	----

---- no statistical significance
(significant only at a single time point)
+ statistically significant

Sponsor concludes: At an oral dose of 15 and 45 mg/kg, extracellular serotonin and norepinephrine were significantly changed in the medial prefrontal cortex compared to controls.

At an oral dose of 45 mg/kg extracellular dopamine in the medial prefrontal cortex and serotonin in the nucleus accumbens were significantly changed compared to vehicle.

Basal absolute values of the neurotransmitters vary between the doses given. This variation is an effect of the compound when given chronically and is dose dependent in the prefrontal cortex with respect to dopamine and norepi.

Effects of acute and chronic treatment with flibanserin on extracellular serotonin, norepinephrine, dopamine, GABA and Glutamate in the Medial Preoptic Area of freely moving female rats.

Nonreceptive female Wistar rats were anesthetized and placed in a stereotaxic frame and I shaped probes were inserted into the medial preoptic area (MPOA).

Rats were given flibanserin orally twice a day for 21 days. On the day of the experiment, the probes were connected to flexible PEEK tubing and perfused with artificial CSF. On the day of the experiment the animals were challenged with flibanserin at different doses depending on the pretreatment dose. Animals treated with vehicle were challenged with vehicle on the first day. On the second day they received an acute dose of 45 mg/kg flibanserin.

Effect of chronic treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle.

	5-HT	NA	DA	GABA	Glu
MPOA (15 mg/kg)	Decrease +	Increase +	Decrease (+)	----	----
MPOA (45 mg/kg)	Decrease +	Increase +	----	----	----

---- not statistically significant

+ statistically significant

(+) significant only at a single time point

Effect of acute treatment with flibanserin on extracellular neurotransmitter levels compared to vehicle.

	5-HT	NA	DA	GABA	Glu
MPOA (45 mg/kg)	Decrease +	Increase +	Increase (+)	----	----

---- not statistically significant

+ statistically significant

Flibanserin reduced 5-HT release in the mPFC, mPOA and Nacc, but not in the hippocampus. Flibanserin increased extracellular DA in the mPFC and in the mPOA of the hypothalamus, but not in the Nacc or hippocampus. Repeated treatment of flibanserin induced selective effects. In rats treated twice daily for 15 or 45 mg/kg for 21 days, flibanserin administration decreased serotonin and increased NA in the mPFC and mPOA. DA levels were increased only in the mPFC (45 mg/kg) and not in the mPOA or Nacc. Serotonin was decreased in the Nacc (45 mg/kg) but no changes in NA or DA were observed. Repeated treatment increased basal levels of DA and NA in the mPFC but only NA was increased in the Nacc.

Animal model for female sexual behavior

The bilateral chamber test is used to examine sexual incentive motivation. The chamber consists of an upper and lower level which allows the female to escape the male and thus pace mating. Female Long-Evans rats (13 per group) were ovariectomized and rendered sexually receptive with injections of estradiol and progesterone. Flibanserin was administered orally at doses of 5, 15 and 45 mg/kg for 28 days. Sexual behavior was noted during 30 minute period tests on days 1, 8, 15, 22 and 29. Proceptivity was defined as gestures such as solicitations (headwise orientation toward the male followed by abrupt runaway terminated by the assumption of a crouching posture), hops and darts and ear wiggings. Receptivity or readiness to allow copulation is represented by lordosis.

Flibanserin induced a dose-dependent increase in solicitations on days 15 and 22 but not on the other days. There were no other effects of sexual motivation such as ear wiggles or hops and darts. Similarly, there was no effect on lordosis behavior.

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects:

In mice, flibanserin administration at an oral dose of 64 mg/kg resulted in a significant reduction in locomotor activity and hypothermia. Four of eight mice exhibited hindlimb abduction, one exhibited flaccid abdominal tone, two had reduced grip strength and one showed grasping in the traction test. After 128 mg/kg flibanserin, a complete inhibition of locomotor activity, an increase in hot plate reaction time and hypothermia were observed. All treated animals had marked hindlimb abduction and head weaving. One mouse showed straub-tail (erect tail often seen with opiate treatment), three had ptosis, six had reduced grip strength, six exhibited grasping in the traction test and two mice were cataleptic.

Cardiovascular effects:

Arterial blood pressure, heart rate and ECG (lead II) were measured in two male and two female Beagles which had previously been chronically implanted with telemetry transmitters. Following a predose recording for 30 minutes, the animals were orally dosed with 3, 10 or 30 mg/kg flibanserin or vehicle control.

Flibanserin treatment induced mild but statistically significant elevations in mean arterial blood pressure at the high dose. Time to peak effect was approximately 1-2 hrs after dosing and these effects had abated after about 5 hrs.

Mean heart rate was mildly elevated approximately 3 hrs after dosing at 30 mg/kg.

Flibanserin produced no adverse changes to the ECG rhythm or waveform morphology at any dose. There were treatment-related changes in the RR, PR and uncorrected QT interval which would be anticipated from mild elevation of heart rate at the high dose. There was no evidence of QT prolongation at doses (3, 10 and 30 mg/kg) that produced geometric overall mean plasma concentrations at one hour of 250, 549 and 1190 ng/ml, respectively.

Maximum changes in arterial pressure in comparison with mean pre-dose values and the approximate times of peak effect

Treatment & dose (mg/kg)	Maximum increase from pre-dose values at time measured, for:					
	Systolic (mmHg)		Diastolic (mmHg)		Mean (mmHg)	
	Pre*	Change	Pre*	Change	Pre*	Change
Vehicle	137	+14 (1 hr)	87	+15 (1 hr)	104	+15 (1 hr)
Flibanserin (3 mg/kg)	139	+17 (3.17 hr)	91	+14 (4.17 hr)	107	+14 (3.17 hr)
Flibanserin (10 mg/kg)	138	+23 (1 hr)	89	+18 (1 hr)	105	+20 (1 hr)
Flibanserin (30 mg/kg)	134	+27 (1.17 hr)	87	+24 (2 hr)	103	+26 (2 hr)

Flibanserin was shown to block hERG-mediated potassium current in HEK293 cells with an IC₅₀ of 1.18 μ M.

Fraction of hERG current (I/I_0) at four different concentrations of flibanserin

Concentration	individual experiment			mean	SD
0.1 μ M	0.90	0.87	0.93	0.90	0.03
0.3 μ M	0.61	0.73	0.64	0.66	0.06
1.0 μ M	0.46	0.44	0.58	0.49	0.08
3.0 μ M	0.37	0.31	0.27	0.32	0.05

Flibanserin has no effect on the action potential configuration in guinea pig papillary muscles at concentrations up to 10 uM. Increasing the concentration up to 30 uM produced a shortening of the action potential.

Pulmonary effects:

Flibanserin was investigated for effects on respiratory rate, tidal volume and minute volume following a single oral administration to rats at doses of 20, 100 and 250 mg/kg.

The low dose of 20 mg/kg had no effect on any parameter. At 100 mg/kg and above, a moderately delayed decrease in respiratory rate and moderate to marked delay of the normal decrease in tidal volume were observed.

Renal effects: not done

Gastrointestinal effects: not done

Abuse liability:

Effects of flibanserin and amphetamine in the self-administration paradigm in male Wistar rats

Flibanserin was compared to amphetamine and saline in the self-administration paradigm in male rats. Rats were trained to lever-press in the operant chambers with sucrose pellets as reinforcement, on a fixed ratio (FR)-one schedule of reinforcement.

Operant chambers were equipped with two levers – one designated active, the other inactive. Drug delivery was accomplished via a jugular vein catheter through an injector mounted on a swivel-arm apparatus, which was connected to a syringe pump delivering a 10 uL drug infusion upon active lever responding. Each drug delivery was followed by a 20 second time-out period in which lever pressing produced no consequence.

During the acquisition/maintenance phase, all animals were trained to lever press for infusions of their assigned drug (amphetamine, flibanserin or saline) on an FR-1 schedule of reinforcement to establish self-administration behavior.

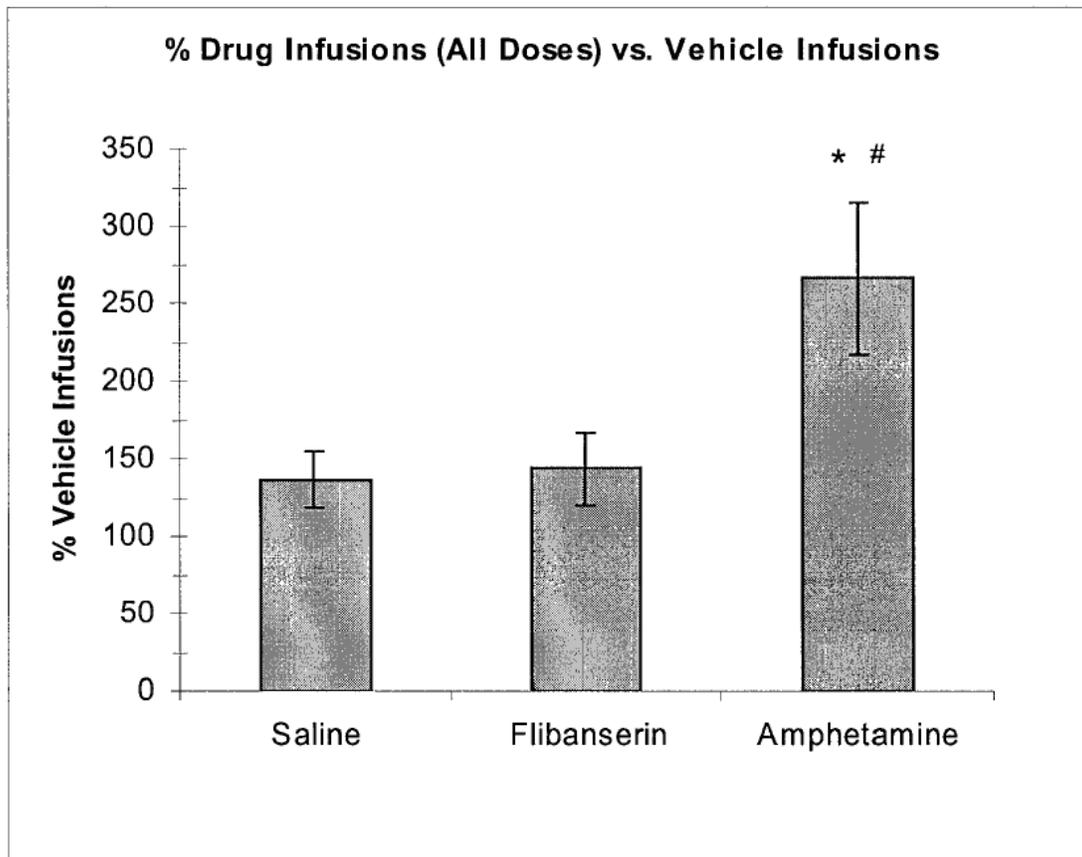
Flibanserin was dissolved in 25% polyethylene glycol and administered at doses of 0, 25, 50 and 100 ug/infusion. D-amphetamine was given at doses of 0, 5, 10 and 20 ug/infusion.

Dosing Schedule for Dose-Response Self-Administration Phase

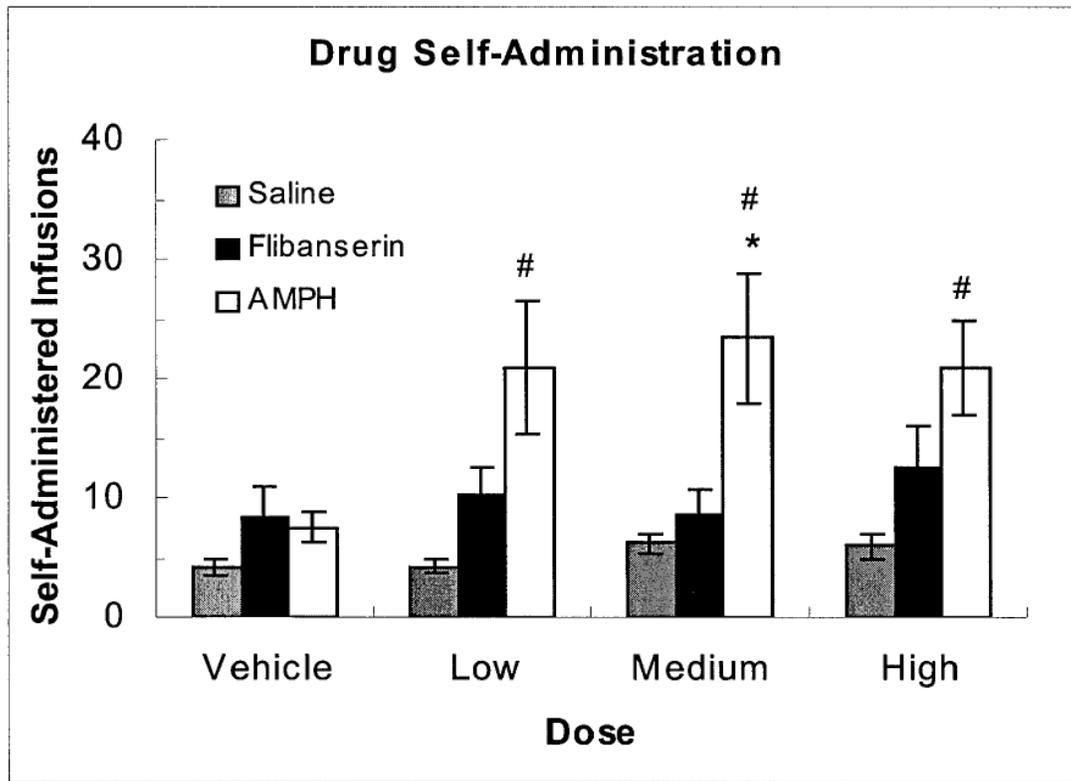
Dose-Response Test Days	Amphetamine Group	Flibanserin Group	Saline Group
Day 1-4	0.5 mg/mL (5 µg/infusion)	2.5 mg/mL (25 µg/infusion)	0.9% saline
Day 5-8	1 mg/mL (10 µg/infusion)	5.0 mg/mL (50 µg/infusion)	0.9% saline
Day 9-12	2 mg/mL (20 µg/infusion)	10 mg/mL (100 µg/infusion)	0.9% saline
Day 13-15	0 mg/mL (0 µg/infusion)	0 mg/mL (0 µg/infusion)	0.9% saline

- The total duration of the Dose-Response Phase was 15 days.
- Testing of the vehicle (0 µg/infusion) was limited to 3 days, rather than 4, to reduce the likelihood of extinction of self-administration behaviour.
- Animals responded on an FR-1 schedule of reinforcement.
- One priming infusion of the appropriate drug commenced all sessions
- Infusion volume for all reinforcers was 10 µL, with a 20 second time-out (during which lever pressing produced no consequence) following each infusion

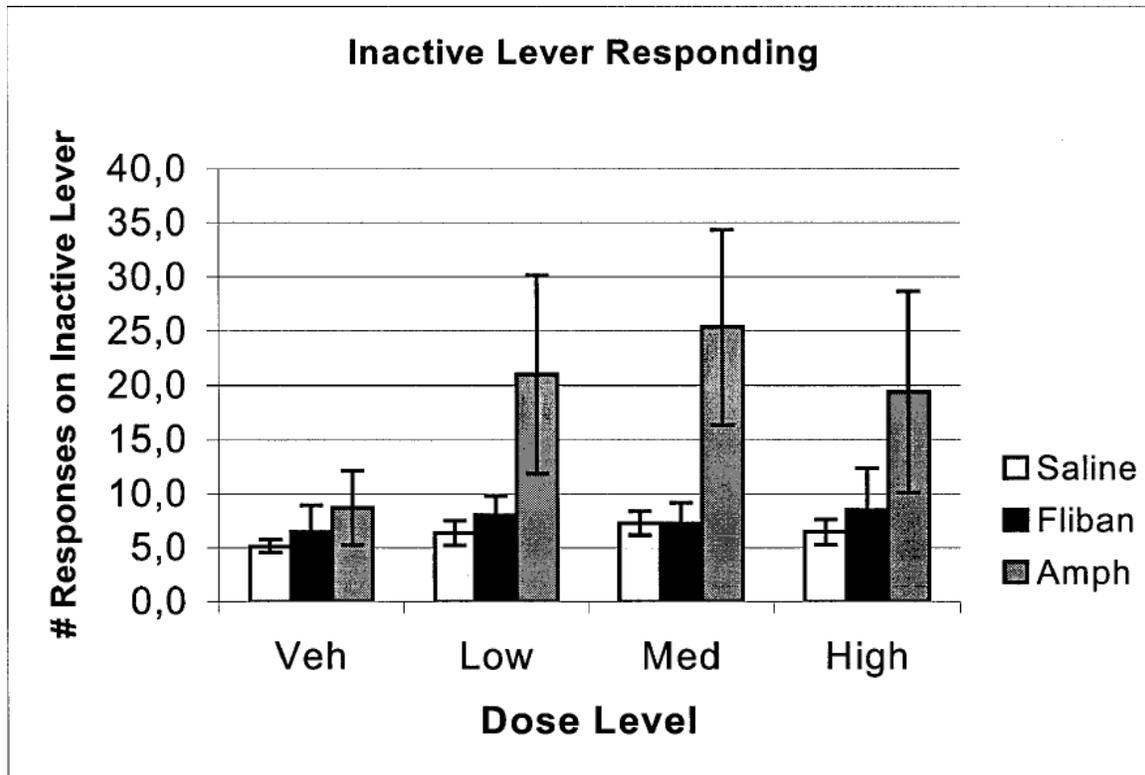
Sample sizes were amphetamine n=6, flibanserin n=5 and saline n=4.



Bars represent the mean (\pm SEM) number of infusions delivered for each dose group, expressed as a percentage of the mean number of vehicle administrations. Flibanserin did not differ significantly from saline.



Mean \pm SEM of number of self-administered infusions of each test drug at each dose levels. None of the test doses of flibanserin differed significantly from saline.



Relative to vehicle, animals had a significantly higher number of self-administered infusions of amphetamine than flibanserin or saline in the first days of the experiment. However, amphetamine treated rats had a higher number of inactive lever pulls than controls, indicating that amphetamine increased overall activity such that the self administration of amphetamine may be a response to hyperactivity and not a signal of self-reward.

All data are highly variable and amphetamine showed an overall tendency to be self-administered, the results with flibanserin seem to indicate that this compound may not be self-administered, even if no definitive conclusions can be drawn due to the small sample size and the failure of the positive control to clearly elicit self-administration behavior.

2.6.2.5 Pharmacodynamic drug interactions

Flibanserin was active in several animal models sensitive to antidepressants and anxiolytics but it did not elicit depression- or anxiety-like behavior. Flibanserin had antinociceptive activity in two models of acute pain which was antagonized by naloxone and potentiated by morphine. Flibanserin has a low affinity for opioid receptors so the action may be indirect. Flibanserin did not impair learning and memory in a water maze test in rats.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

In male and female rats, absorption was relatively fast after oral administration of a suspension with a T_{max} of approximately 1 h. Absolute bioavailability (F) was 35% in males and 65% in females despite almost complete absorption suggesting a moderate first pass effect. After IV administration the clearance (CL) was 27 ml/min/kg in males and 18 ml/min/kg in females indicating a fast clearance of parent drug. Plasma t_{1/2} of parent was approximately 1-2 hrs at steady state. In contrast, the t_{1/2} for radioactivity in plasma was 66 h after oral dosing indicating the presence of long-lived metabolite(s). In humans, the t_{1/2} is 10-11 hrs for parent drug and 66 hrs for radioactivity.

In a pilot study in the cynomolgus monkey, drug bioavailability was only about 4% resulting in low blood levels and the decision not to use cynomolgus monkeys for toxicity studies. The AUC_{0-∞} for flibanserin in monkeys following oral administration of 3 mg/kg of flibanserin was 100 ng.h/ml compared to 2100 ng.h/ml in dogs following a similar dose.

Absorption in humans

Pharmacokinetics of flibanserin in healthy women following a single dose. Study no. 511.97 U07-1869

Table 11.5.2.2: 1 Comparison of geometric mean (gCV in %) key pharmacokinetic parameters of Flibanserin by treatment

Flibanserin		1 x 25 mg (F1_25) (N=22)	2 x 25 mg (F2_25) (N=22)	1 x 50 mg (F1_50) (N=22)	2 x 50 mg (F2_50) (N=22)	1 x 100 mg (F1_100) (N=20)
AUC _{0-∞}	[ng·h/mL]	515 (44.9)	1140 (38.6)	1140 (39.2)	2250 (34.2)	2320 (32.8)
AUC _{0-∞,norm}	[ng·h/mL/mg]	20.6 (44.9)	22.8 (38.6)	22.8 (39.2)	22.5 (34.2)	23.2 (32.8)
%AUC _{tz-∞}	[%]	5.74 (42.5)	3.36 (67.5)	3.58 (60.3)	2.78 (63.5)	2.48 (59.6)
AUC ₀₋₁₂	[ng·h/mL]	403 (39.4)	831 (32.9)	827 (31.5)	1610 (29.6)	1670 (33.7)
AUC _{0-12,norm}	[ng·h/mL/mg]	16.1 (39.4)	16.6 (32.9)	16.5 (31.5)	16.1 (29.6)	16.7 (33.7)
AUC ₀₋₂₄	[ng·h/mL]	472 (41.4)	999 (35.0)	1000 (34.6)	1960 (31.4)	2020 (32.9)
AUC _{0-24,norm}	[ng·h/mL/mg]	18.9 (41.4)	20.0 (35.0)	20.0 (34.6)	19.6 (31.4)	20.2 (32.9)
C _{max}	[ng/mL]	140 (34.8)	282 (35.8)	253 (27.2)	483 (38.6)	540 (36.5)
C _{max,norm}	[ng/mL/mg]	5.59 (34.8)	5.64 (35.8)	5.07 (27.2)	4.83 (38.6)	5.40 (36.5)
t _{max} ¹	[h]	0.750 (0.500-2.03)	0.750 (0.500-1.50)	0.750 (0.500-2.00)	0.750 (0.500-2.00)	0.750 (0.500-3.00)
t _½	[h]	8.22 (24.5)	9.95 (27.4)	9.77 (25.7)	10.1 (21.0)	10.5 (16.1)

¹ median and range

The AUC_{0-∞} for flibanserin in women taking a single dose of 100 mg was 2320 ng.h/ml, C_{max} was 540 ug/ml and the T_{1/2} was 10.5 hrs.

Human pharmacokinetics in patients (single dose). Study no. 511.105 U07-1871

Single dose	50 mg q.d. (N=30)		100 mg q.d. (N=28)		25 mg b.i.d. (N=33)		50 mg b.i.d. (N=32)	
	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]
AUC₀₋₁₂ [ng·h/mL]	685	36.4	1150	52.4	391	50.5	745	45.9
AUC_{0-12,norm} [ng·h/mL/mg]	13.7	36.4	11.5	52.4	15.7	50.5	14.9	45.9
AUC₀₋₂₄ [ng·h/mL]	817	39:4	1420	54.7	---	---	---	---
AUC_{0-24,norm} [ng·h/mL/mg]	16.3	39:4	14.2	54.7	---	---	---	---
AUC_{0-tz} [ng·h/mL]	816	39.3	1420	54.6	391	50.5	744	45.8
AUC_{0-∞} [ng·h/mL]	904	43.5	1630	54.6	474	53.2	919	52.0
AUC_{0-∞,norm} [ng·h/mL/mg]	18.1	43.5	16.3	54.6	19.0	53.2	18.4	52.0
%AUC_{tz-∞} [%]	8.34	56.5	11.0	59.8	16.4	36.8	17.3	42.5
C_{max} [ng/mL]	217	40.8	336	50.7	136	41.9	250	40.0
C_{max,norm} [ng/mL/mg]	4.33	40.8	3.36	50.7	5.45	41.9	4.99	40.0
t_{max} ¹ [h]	0.875	0.500-3.00	1.00	0.500-3.00	0.750	0.500-3.00	1.00	0.500-3.00
t_{1/2} [h]	8.45 ²	23.3 ²	9.33	27.8	5.93 ³	24.8 ³	6.06	27.0
MRT_{po} [h]	8.46	29.2	10.0	35.4	6.47	24.6	6.79	30.2
CL/F [mL/min]	922	43.5	1020	54.6	878	53.2	907	52.0
V_Z/F [L]	676	33.2	827	62.6	457	58.2	476	43.7

Source Data: Section 15, [Table 6.3: 1](#), [Table 6.2.1: 1](#) and [Table 6.2.1: 15](#)

¹ median and range

² N=31; ³ N=34

AUC_{0-∞} was 1630 ng.h/ml in patients following a single dose of 100 mg

Human pharmacokinetics in patients (steady state) Study no. 511.105 U07-1871

Steady state	50 mg q.d. (N=30)		100 mg q.d. (N=29)		25 mg b.i.d. (N=33)		50 mg b.i.d. (N=31)	
	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]	gMean	gCV [%]
AUC_{τ,ss} [ng·h/mL]	991	47.7	2080	46.6	653	60.1	1400	46.9
AUC_{τ,ss,norm} [ng·h/mL/mg]	19.8	47.7	20.8	46.6	26.1	60.1	28.1	46.9
R_{A,AUC} []	1.20 ²	19.3 ²	1.44 ³	63.5 ³	1.70 ⁴	26.4 ⁴	1.84	21.4
C_{max,ss} [ng/mL]	234	41.2	469	42.7	168	50.9	346	34.4
C_{max,ss,norm} [ng/mL/mg]	4.68	41.2	4.69	42.7	6.72	50.9	6.91	34.4
R_{A,Cmax} []	1.09 ²	23.5 ²	1.36 ³	58.0 ³	1.28 ⁴	40.1 ⁴	1.37	31.4
t_{max,ss} ¹ [h]	1.00	0.417-4.00	1.00	0.500-3.00	1.00	0.500-3.00	0.750	0.500-3.00
t_{½,ss} [h]	10.1	23.3	11.4	24.3	11.9	25.5	11.6	21.4
MRT_{po,ss} [h]	9.44	34.5	11.3	27.8	11.1	30.2	12.1	29.5
CL/F_{ss} [mL/min]	841	47.7	803	46.6	638	60.1	593	46.9
V_Z/F_{ss} [L]	734	33.9	795	53.9	655	46.8	594	38.4

Source data: Section 15 [Table 6.3.1: 1](#)

¹ median and range

² N=28; ³ N=27; ⁴ N=32

The steady state drug level (AUC_i) in patients taking 100 mg was 2080 ng.h/ml. This is the figure I have used for all cross-species comparisons of drug exposure.

Pharmacokinetic parameters for various species following a single dose

Route	Parameter	Unit	Mouse (m)	Rat (m/f)	Rabbit (f)	Dog (m&f)	Cynomolgus Monkey (m&f)	Man ^{a, d} (m)
	Dose	mg/kg	5	5 / 5	3	3.0 p.o. 3.3 i.v.	3	50 mg p.o. 20 mg i.v.
oral	C(max)	µg/mL	0.17	0.42 / 0.61	nd	0.64	0.057	0.25
	t(max) ^b	h	1.0	0.67 / 1.0	nd	0.5	1.0	0.5
	AUC(0-∞)	(µg/mL)·h	1.1	1.1 / 3.0	nd	2.1	0.1	0.75
	MRT	h	7.0	2.7 / 3.8	nd	3.3	1.7	5.9
	t(½)	h	5.5	nd / 2.5	nd	4.4	0.73	6.8
	MAT	h	6.1	1.2 / 1.0	nd	0.17	-0.1	nd
	F(a)	%	nd	119 ^c / 111	>72 ^c	105 ^c	nd	90
	F	%	84	35 / 65	nd	44	4.1	33
i.v.	AUC(0-∞)	(µg/mL)·h	1.3	3.1 / 4.7	3.5	5.1	2.4	0.91
	MRT	h	0.92	1.5 / 2.8	3.0	3.2	1.8	6.3
	t(½)	h	0.75	0.9 / 2.1	3.5	2.9	2.0	7.2
	CL	(mL/min)/kg	62	27 / 18	15	11	21	366 mL/min
	V(ss)	L/kg	3.4	2.3 / 3.0	2.6	2.0	2.3	134 L
<i>in vitro</i>	F(b)	%	98	97 / nd	98	98	98	98

^a body weight = 72 to 86 kg^b median^c from urinary data^d geometric means reported for human except of t(max) (median) and plasma protein binding (arithmetic mean)

2.6.4.4 Distribution

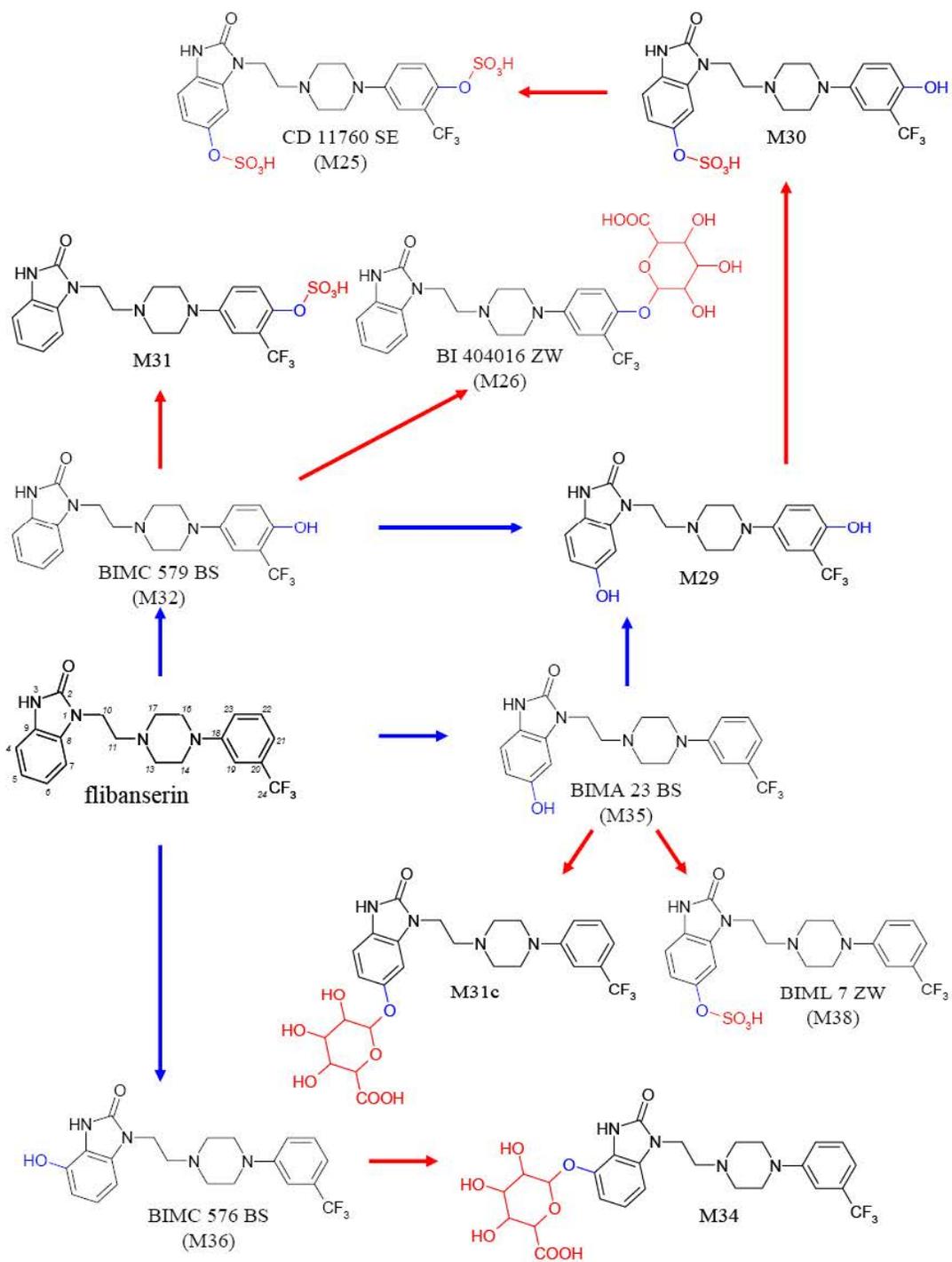
Flibanserin exhibited a high volume of distribution (approximately 2-3 L/kg) in all species examined. Thus, there is a species independent distribution of flibanserin from plasma into tissues.

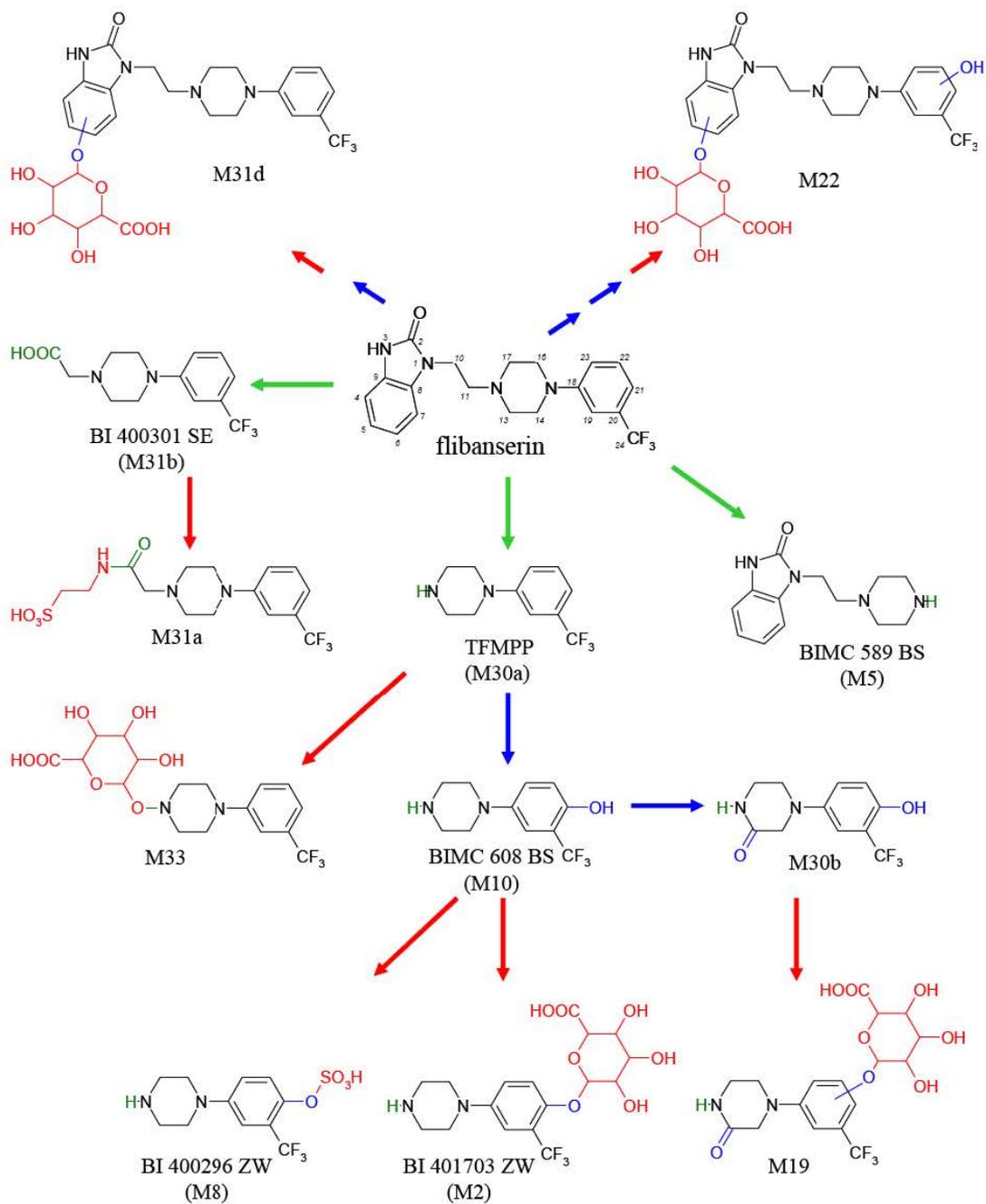
Tissue distribution was investigated in rats by whole body autoradiography. After repeated dosing, radioactivity was present in high concentrations in the liver, kidney, pancreas and brown fat. Pigmented rats showed higher concentrations of radioactivity in skin and eyes in comparison with non-pigmented ones, suggesting melanin binding of flibanserin or its metabolites. Flibanserin and its active minor metabolite M30a crossed the blood brain barrier to a large extent, whereas the active metabolites M35 and M38 did not.

2.6.4.5 Metabolism

After oral administration, flibanserin is almost completely metabolized in mice, rats, rabbits, dogs and humans to at least 35 metabolites. In humans, flibanserin is extensively metabolized by CYP3A4 and to a lesser extent by CYP2D6.

Flibanserin is mainly metabolized by aromatic hydroxylation to several mono- and di-alcohols of flibanserin. In addition, three cleavage pathways exist.





Metabolite Peak No.	BI-Code	mouse (m)	rat (m)	rabbit (f)	dog (m/f)			man (m)	
		5 mg/kg 2 h	5 mg/kg 2 h	3 mg/kg 2 h	3 mg/kg			50 mg	
					2 h (m)	2 h (f)	5.5 h (f)	1 h	4 h
M25/M26 ^a	BI 404016 ZW CD 11760 SE	55	72	826	235	219	682	940	139
M38 ^b	BIML 7 ZW	22	168	29	39	34	81	373	50
M39	flibanserin	237	161	274	1198	1318	230	308	166
M31b	BI 400301 SE	17 ^c	458 ^c	199 ^c	99 ^c	93 ^c	73 ^c	159	69
M8	BI 400296 ZW	nd	nd	nd	nd	nd	nd	138	89
M2	BI 401703 ZW	25	98	307	11	7	nd	23	132
M21		6	nd	nd	nd	nd	15	69	33
M1a		nd ^d	nd ^d	nd ^d	26	39	46	13	39
M30 ^e		6	20	25	nd	13	9	39	7
M1		52	128	110	124	139	66	13	39
M22		9	11	146	7	6	26	36	35
M41		78	43 ^f	144	377 ^f	750 ^f	571 ^f	13	31
M35	BIMA 23 BS	47	15	6	131	162	90	29	27
radioactivity		318	1447	2865	2791	3432	2333	2240	925

^a M25 and M26 could not be separated in plasma samples. Investigations by LC-MS showed that M25 dominated in animals, whereas M26 dominated in man

^b originally reported as M37 or M38, but shown to be only one metabolite (M38 = BIML 7 ZW)

^c in animal samples, M31 could not be separated from M31a, M31b, M31c, M31d

^d might be included in M1

^e in animal samples, M30 could not be separated from M30a (TFMPP) and M30b

^f M41/42

nd = not detected

Table 7.2.1: 3 Overview of geometric mean (gCV) key pharmacokinetic parameters of flibanserin and relevant metabolites after single oral administration of 100 mg flibanserin to healthy female subjects and HSDD patients (Datasets 1 + 3)

100 mg single dose	Flibanserin		BIMA 23 BS		BIML 7 ZW		TFMPP	
	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])
C_{max} (ng/mL)	100	399 (47.8)	51	30.6 (55.0)	51	452 (89.7)	50	5.47 (63.6)
AUC₀₋₁₂ (ng·h/mL)	81	1380 (44.8)	51	153 (45.7)	51	1000 (63.7)	50	36.7 (63.2)
AUC_{0-∞} (ng·h/mL)	100	2050 (47.6)	51	258 (49.3)	51	1460 (62.4)	50	58.6 (75.3)
RAUC_{0-∞,Met} ()	NC	NC	51	0.137 (34.7)	51	0.647 (80.0)	50	0.0530 (74.4)

NC = not calculated

Table 7.2.1: 4 Overview of geometric mean (gCV) key pharmacokinetic parameters of flibanserin and relevant metabolites after multiple oral administration of 50 mg flibanserin twice daily to healthy female subjects and HSDD patients (Datasets 1 + 3)

50 mg b.i.d.	Flibanserin		BIMA 23 BS		BIML 7 ZW		TFMPP	
	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])	N	gMean (gCV [%])
$C_{max,ss}$ (ng/mL)	79	298 (41.7)	79	31.1 (37.9)	79	268 (48.4)	79	5.19 (75.8)
$AUC_{\tau,ss}$ (ng·h/mL)	79	1320 (46.6)	79	206 (45.8)	79	897 (34.2)	79	36.7 (82.9)
$C_{pre,ss}$ (ng/mL)	292	48.4 (81.0)	292	10.2 (75.7)	292	26.8 (54.5)	277	1.82 (93.2)
$RAUC_{\tau,ss,Met}$ ()	NC	NC	79	0.150 (42.7)	79	0.544 (53.5)	79	0.0470 (84.0)

NC = not calculated

Based on pharmacological tests including a receptor screen, three metabolites were considered potentially active, M35, M38 and M30_a (TFMPP). Considering the human plasma exposure at therapeutic doses and the brain penetration in rat in addition to the receptor binding data of flibanserin and its metabolites, sponsor concludes that flibanserin in the CNS active substance at therapeutic doses.

The metabolite M8 (BI 400296 ZW) was detected in humans but not in the species used in the toxicology studies. The toxicity of the metabolite was investigated in a 2 week IV dose range finding study, a 4 week GLP study and in in vitro and in vivo genotoxicity assays.

Flibanserin was administered IV to 10 male and 10 female Chbb ^{(b)(4)} rats at daily doses of 0, 0.4, 4.0 and 40 mg/kg for four weeks.

There was no drug related mortality or clinical signs at any dose.

There were no clear drug-related effects on body weight or food consumption, ophthalmology, clinical chemistry and urinalysis. There was a dose related decrease in WBC (mainly lymphocytes) in males only but it was mild and not considered biologically significant (no effect in females).

There were no drug related organ weight changes or test item related microscopic changes.

The NoAEL was considered to be 40 mg/kg corresponding to a mean C_{max} of 64.2 ug/ml in males and 58.9 ug/ml in females. Corresponding mean AUC (0-24h) values were 48.9 and 44.5 ug.h/ml, respectively.

Based on a receptor screen, M8 was considered inactive. M8 was negative in the Ames test at doses up to 5000 ug/plate and the rat micronucleus assay in vivo when given at 40 mg/kg IV for 4 weeks. Plasma protein binding of the metabolite was fairly low (~50%) in both rats and humans.

Human exposure, plasma protein binding, receptor binding and brain/plasma ratios of flibanserin and metabolites

Code	Human C(max)ss at 100 mg q.d. [nM]	PPB man [%]	lowest K _i [nM]	C(max)/lowest K _i	Brain/plasma ^a ratio
Flibanserin	1200	98%	6.59	182	3.71
BIML 7 ZW	1320	97%	207	6.38	0.02
CD 11760 SE ^c	1050 ^b	nd	>10000	<0.105	nd
BI 401703 ZW	282	nd	>10000	<0.028	nd
BI 400296 ZW	281	52%	1760	0.160	nd
BI 400301 SE	159 ^c	nd	>10000	<0.016	nd
BIMA 23 BS	105	95%	7.65	13.7	0.08
BI 404016 ZW	36.6	nd	>10000	<0.004	nd
TFMPP	27.8	73%	32	0.869	2.80

PPB plasma protein binding in man

nd not determined

a based on total radioactivity data in tissues at the end of a 4h i.v. infusion of the respective radiolabelled compound to rats

b C(max) after single oral administration of 100 mg flibanserin to man [U09-1167, Module 5.3.5.3]

c based on C(max) after single oral dosing of 50 mg [¹⁴C]-flibanserin to man determined from pooled 1 h and pooled 4 h plasma samples [U99-1776]

In the above table, it can be seen that the drug concentration (Cmax) divided by the potency (K_i) gives essentially an activity ratio with a higher number denoting greater relative activity. Take into consideration the ability to penetrate the blood brain barrier and it can be seen that flibanserin is basically the only active substance.

Plasma protein binding of flibanserin at a concentration of 0.1 ug/ml was 97% in rat and 98% in mouse, rabbit, dog, monkey and human. Most of the binding was to serum albumin with some binding to α_1 -acid glycoprotein.

2.6.4.6 Excretion

Flibanserin excretion is primarily via feces in mice, rats and dogs with most of the rest through the urine. It is 50/40 feces/urine in humans.

Parameter	Units	Mouse p.o.	Rat p.o.	Rat i.v.	Rabbit p.o.	Dog p.o.	Dog i.v.	Man ^a p.o.	Man ^a i.v.
N/Gender		5m/5f	4m/4f	4m/4f	3f	2m/2f	2m/2f	6m	6m
Dose	mg/kg	5	5	5	3	3	3	50 mg	20 mg
Bile	% of dose	nd	nd/25.3	57.0/39.9	nd	nd	nd	nd	nd
Urine	% of dose	21.6/21.4	19.1/32.5	16.0/18.6	71.9	15.1	15.0	44.1	40.7
Feces	% of dose	70.8/50.0	73.3/70.0	79.1/78.3	19.9	82.4	80.9	50.9	56.0
Recovery	% of dose	92.5/71.4	92.5/105.4	95.2/98.4	92.4	98.7	97.6	95.5	97.0

^a geometric mean nd = not determined

Bile sampling period: male rat (0-5h), female rat (0-6h)

Urine sampling period: mouse (0-48h); male rat (0-96h); female rat, rabbit, dog (0-168h); man (p.o. 0-264h, i.v. 0-192h)

Feces sampling period: mouse (0-48h); male rat (0-96h); female rat, rabbit, dog (0-168h); man (p.o. 0-384h, i.v. 0-288h)

2.6.4.7 Pharmacokinetic drug interactions

See biopharmaceutics review

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

See final discussion

2.6.4.10 Tables and figures to include comparative TK summary

See above

02.6.5 PHARMACOKINETICS TABULATED SUMMARY

See above

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

See final discussion

Genetic toxicology:

See final discussion

Carcinogenicity:

See final discussion

Reproductive toxicology:

See final discussion

Special toxicology:

See final discussion

2.6.6.2 Single-dose toxicity

Single dose studies were conducted in mice and rats. Oral flibanserin was given at doses up to 4000 mg/kg in mice and rats. When given IV, the high doses were 80 mg/kg in mice and 90 mg/kg in rats. Additionally, an acute oral toxicity study was conducted in dogs at 50 and 100 mg/kg.

Oral doses resulted in significant mortality at 2000 mg/kg in mice and greater than 4000 mg/kg in rats. After IV administration, significant mortality was seen at 50 mg/kg for mice and 70 mg/kg for rats. Dogs had significant clinical signs after both 50 and 100 mg/kg.

2.6.6.3 Repeat-dose toxicity

Sponsor performed a number of toxicity tests in rats, mice and dogs. In a 13 week toxicity test in mice with a single dose of 1000 mg/kg/day, flibanserin treatment resulted in an increase in liver weight and hepatic lipidosis. Mean plasma flibanserin AUC_{0-24h} values for the mice were 18 ug.h/ml for males and 12 ug.h/ml for females. In a 13 week oral dose ranging study with doses of 20, 100 and 400 mg/kg (raised to 1200 and 2400 mg/kg in drug weeks 6 and 11 due to lack of toxicity), oral administration of flibanserin to CD-1 mice produced hepatocellular hypertrophy in MD and HD animals of both sexes with fatty accumulation.

In a 13 week toxicity study, rats were dosed with 20, 100 and 400 mg/kg which resulted in significant neurological signs at the HD with a reduction in body weight gain at all doses in males only. There was slight but significant decrease in RBC's with an increase in MCH in males. There was an increase in liver and ovary weights of females with reversible periacinar hypertrophy in the liver of the MD (males) and HD (males and females) at week 13. Mild reversible fatty changes were seen in livers of HD females.

In the 13 week rat study, serum prolactin levels were measured

PROLACTIN (ng/ml)				
	Dose mg/kg	MEDIAN	Interquartile Range	NI
CONTROL		52.45	27.05 - 67.41	10
BIMT17 BS	20	65.37	40.76 - 79.52	10
BIMT 17 BS	100	98.27 *	83.42 - 137.43	10
BIMT17 BS	400	174.00 **	142.27 - 275.93	10

* P < 0.05

** P < 0.01

In a 6 month toxicity study in rats at oral doses of 20, 100 and 400 mg/kg flibanserin administration produced hepatic hypertrophy. This change occurred in males at the two higher doses and in females at the highest dose only. In females at the highest dose, there was a mild increase in hepatic lipid content. No other histopathological changes were noted.

Tab. A: Geometric mean C_{max} - and AUC-values and median t_{max} -values (data of both sexes are combined).

Day	1			9			last		
	t_{max} h	C_{max} ng/ml	$AUC_{0-\infty}$ ng/ml*h	$t_{max,ss}$ h	$C_{max,ss}$ ng/ml	AUC_{ss} ng/ml*h	$t_{max,ss}$ h	$C_{max,ss}$ ng/ml	AUC_{ss} ng/ml*h
20	1.5	552	2663	1	520	3321	1	521	3334
100	2	2791	50523	2	1617	17464	2	2321	20662
400	8	7315	- *	2	2755	31712	2	3069	41386

* not calculable due to the late C_{max}

This study showed very little toxicity in rats at exposures up to approximately 20 times the human exposure (2080 ng.h/ml) in women taking 100 mg.

In a 13 week study in 3 male and 3 female beagles per group, flibanserin given orally at doses of 3, 15 and 100 mg/kg/day produced significant clinical signs and lower body weight in males and females of the highest dose group. There was an increase in intraocular tension in the eyes of 3 of 6 dogs treated with 100 mg/kg. No other significant findings were noted.

Study title: 6-month oral toxicity study in the rat

Key study findings: Minimal toxicity

Study no.: Internal study no. I48

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim KG, Ingelheim, Germany.

Date of study initiation: Nov. 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: FIMT 17 BS, batch 9403-P, purity not stated

Methods

Doses: 15, 80, 400 mg/kg/day

Species/strain: Chbb: ^{(b)(4)} strain rats

Number/sex/group or time point (main study): 20/sex/gp

Route, formulation, volume, and infusion rate: oral gavage, flibanserin suspended in 0.5% Methocel-solution, 10 ml/kg.

Satellite groups used for toxicokinetics or recovery: none

Age: 57 days

Weight: 174-258 gg

Sampling times:

Unique study design or methodology (if any): None

Results

Mortality:

Group Sex	1 (control)		2		3		4	
	m	f	m	f	m	f	m	f
Died	1	1	0	1	1	0	2	0
Sacrificed	0	0	0	0	0	1	1	0
Total	1	1	0	1	1	1	3	0
%	5	5	0	5	5	5	15	0

The eight animals that died or were sacrificed all had congestion of the parenchymal organs, pulmonary edema and emphysema, intraorbital hemorrhages (one animal) and indications of gavage error (one animal). The cause of death was not determined.

Clinical signs:

Group Sex	1 (control)		2		3		4	
	m	f	m	f	m	f	m	f
Observation								
Ataxia	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20
Convulsions	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20
Dyspnoea/altered respiration	0/20	0/20	0/20	0/20	1/20	0/20	1/20	0/20
Lateral recumbency	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20
Sternal recumbency	0/20	0/20	0/20	0/20	0/20	0/20	2/20	9/20
Phobia	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Resistance to administration	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Sedation	0/20	0/20	0/20	0/20	0/20	0/20	20/20	20/20
Poor condition/ruffled coat	0/20	0/20	0/20	0/20	1/20	0/20	2/20	2/20

Body weights:

Males

Week	Group			
	1	2	3	4
13	163	167 (102)	149 (92)	122 (75)
26	223	215 (97)	193 (86)	145 (65)

Females

Week	Group			
	1	2	3	4
13	68	72 (105)	77 (113)	84 (124)
26	89	94 (105)	100 (113)	105 (118)

() = percent of control body weight

For males, the decrease in body weight gain was statistically significant for the mid and high dose. In females, flibanserin treatment resulted in a significant increase in body weight in mid dose animals at weeks 10 and 11 and a significant increase in body weight in high dose rats from week 5 onward.

Food consumption: Animals of the two higher dosed groups demonstrated a statistically significant tendency to consume more food than the controls.

Ophthalmoscopy: No drug induced lesions could be determined with slit-lamp exams.

Hematology: From week 6 onwards high dose males and females showed slightly decreased RBC counts and hemoglobin values.

Clinical chemistry:

GPT: Females in the mid and high dose had a slight but significant increase in GPT (ALT) values during the entire treatment phase. There was no dose dependency.

AP: There was a slight reduction in enzyme activity in mid and high dose males during the entire treatment.

Triglycerides: Moderate to marked dose dependent decrease in serum triglycerides was seen in the mid and high dose males and females during the entire treatment.

Cholesterol: Slight elevations of serum levels were seen in high dose females during the entire treatment and in mid dose females during weeks 6 and 26 and, as a tendency, in low dose females at the end of the study.

Bilirubin: Minimal reductions in serum values in the high dose animals were within the normal range.

Glucose: Slight decreases in mid and high dose animals of both sexes.

Inorganic phosphate: Mid and high dose females showed a slight, dose dependent increase in serum levels. There was also a small increase in low dose females at the end of the study.

Prolactin: Levels were measured in all animals 2 hours after drug administration in weeks 8 and 23 of the study. In week 15 prolactin was determined 24 hours after dosing. There was a dose dependent increase of serum prolactin 2 hours after dosing in both males and females of the mid and high dose groups in week 8 and week 23. No change was seen after 24 hours in the low and mid dose groups and a slight decrease was seen in the high dose group.

Dose mg/kg	Male Rats	Week 8 2 h p. Appl.	Week 15 24 h p. Appl.	Week 23 2 h p. Appl.
Control	Median NI	65.43 20	66.32 20	93.09 19
15	Median NI	91.01 20	60.86 20	97.50 20
80	Median NI	**134.82 20	39.32 20	**173.98 20
400	Median NI	**198.99 20	**9.94 19	**249.14 17

Dose mg/kg	Female Rats	Week 8 2 h p. Appl.	Week 15 24 h p. Appl.	Week 23 2 h p. Appl.
Control	Median NI	103.86 19	15.24 19	48.63 18
15	Median NI	59.31 20	10.30 20	89.09 19
80	Median NI	132.67 19	10.60 19	**202.08 18
400	Median NI	290.11 20	<4.40 20	**335.22 18

** = significantly different from the control group (p < 0.01)

Urinalysis: There were no biologically relevant differences between control and treated animals.

Gross pathology: No biologically significant findings.

Organ weights (specify organs weighed if not in histopath table):

In males there was an increase in absolute thymus weights in all dosed groups and a significant increase in relative thymus weights in the low and high dose groups only. Group 2, 3 and 4 mean absolute values were 20, 18, and 30% higher than control values.

There was an increase in adrenal weights in the mid and high dose groups of males only. Group 2, 3 and 4 mean absolute values were 2, 14 and 19% higher than control.

In males, there was an increase in the relative liver weights in the high dose group. No change in absolute weight.

In females there was an increase in both absolute and relative liver weights in the mid and high dose groups. Group 2, 3 and 4 mean absolute weights were 9, 17 and 44% higher than control values.

Histopathology: Adequate Battery: yes (x), no ()—explain
Peer review: yes (x), no ()

Only changes were seen in the liver of the high dose group. There was a reduction of the regularly occurring fat deposition with the lobuloperipheral hepatocytes in males which may explain the decrease in relative liver weights in males. In contrast, high dose females demonstrated a slight to mild hypertrophy of the lobulocentrally located hepatocytes which probably correlates with the increased liver weight.

Liver changes	G1		G2		G3		G4	
	0 mg/kg		15 mg/kg		80 mg/kg		400 mg/kg	
	M	F	M	F	M	F	M	F
Fatty change	18/20	20/20	20/20	8/20	19/20	14/20	4/20	17/20
Hepatocellular hypertrophy	0/20	0/20	0/20	0/20	0/20	0/20	0/20	20/20

There was no increase in myocardial fat deposition in rats at any dose.

Toxicokinetics

Geometric mean; males and females combined; n = 7-10.

Week	2			26		
Dose mg/kg	T _{max} h	C _{max} ng/mL	AUC _{ss} ng/mL*h	T _{max} h	C _{max} ng/mL	AUC _{ss} ng/mL*h
15	1.0	722	3820	1.1	791	3840
80	1.4	2020	17700	1.1	2690	22300
400	2.8	6040	61800	1.3	7100	59800

Exposures are 10 fold and 29 fold the human exposure (2080 ng.h/ml at 100 mg) at the mid and high doses, respectively.

Study title: 52-week oral (gavage) toxicity study in beagle dogs with interim autopsy after 26 weeks.

Key study findings: Severe neurological signs in HD with clear animal suffering, mild toxicities in LD and MD dogs

Study no.: U99-1576; internal study no. I49

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim KG, Ingelheim, Germany.

Date of study initiation: Dec. 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403-P & 9605-P, purity not stated.

Methods

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

Species/strain: Beagles

Number/sex/group or time point (main study): 3/sex/gp in the 6 month part of the study and 4/sex/gp in the 12 month part.

Route, formulation, volume, and infusion rate: oral gavage, flibanserin suspended in 0.5% Methocel-solution.

Satellite groups used for toxicokinetics or recovery: none

Age: 6-18 months

Weight: 8.9 – 14.5 kg

Unique study design or methodology (if any): None

Results

Mortality: One HD group 4 dog, sacrificed moribund on week 7.

Clinical signs: Salivation and ataxia in group 3 (15 mg/kg) on isolated occasions. In group 4 dogs salivation occurred after dose administration, the animals became recumbent, vocalized and were ataxic or convulsive from about 1 hour after drug administration until beyond 6 hours after administration. Dogs also exhibited dry eyes and various behavioral traits such as biting the metal drinking bowls or feeding racks, standing with head forward, and forcing head or limbs between the bars of the door or the enclosure. Symptoms were seen from 2 hours after administration onwards beyond 6 hours. In the course of the study the number of tremor and vocalization episodes decreased and the duration of vocalization became shorter.

Extreme aggression was seen in one high dose dog that also suffered from salivation, vocalization, sedation, ataxia, convulsions, refusal to eat, no defecation, and poor general condition. The dog was sacrificed on humane grounds.

Isolated cases of reduced locomotion, absence of feces or abnormal feces and miosis were also seen in the high dose group.

There was an increase in neurological signs (compulsive gnawing) with dose.

The continued treatment of the high dose dogs seems to me to be clear animal abuse. I'm not sure why the animal welfare monitor did not demand lowering the dose for these animals.

Body weights: Significant decrease BW gain in group 2 and 3 males but not in females. Group 4 males and females also gained less weight than control dogs but the difference for females was not significant.

Food consumption: No drug related effects.

Ophthalmoscopy: One MD dog and 7 HD dogs demonstrated dose-related ophthalmological and/or histopathological changes of the eyes. They were characterized by discrete, focal, smoky opacity or by focal facet (focal thickening of the cornea epithelium) in the central or peripheral region of the cornea. There was no increase in intensity with time and two of the opacities disappeared with time and did not reappear. Similar ocular findings were seen in the 13 week dog toxicity studies where 2/12 dogs at 100 mg/kg and 4/6 dogs at 100/75 mg/kg were affected.

EKG: There was an increase in heart rate in both male and female dogs of all treated groups at different time points during the study (mostly 3 hours after drug administration). From 18-58% in the low dose group to a mean maximum increase of 72 beats/minute (120%) in the male HD group. In general, males seemed more affected than females.

PQ-, QRS-, QT-intervals were within the normal historical range but there were increases in blood pressure in males and females of groups 2 and 3, 3 and 24 hours after administration in week 26 and 24 hours after administration in weeks 39 and 52 and in females of group 4, 24 hrs after administration in week 52. There was no dose response and the relationship to treatment is questionable.

Hematology: Individually reduced RBC counts and hemoglobin values in treated dogs without a dose response. Platelet counts were increased slightly, biologically and statistically significantly in group 4 dogs at all weeks tested. Thrombin and partial thrombin times were slightly reduced in these animals.

There were no differences in bone marrow smears from group 4 and control animals.

Clinical chemistry: Alkaline phosphatase values were slightly but significantly increased in group 4 dogs during the entire treatment phase.

Prolactin levels for groups 3 and 4 were elevated both for males and females. The increase was slight and not consistent, being less pronounced in males but not in females during later stages of the study.

There was a slight increase in cortisol values in both sexes of group 4 during the course of the study.

There was a slight increase in the α_2 -globulin fraction in group 4 animals which was accompanied by a corresponding change in the albumin-globulin ratio.

Urinalysis: There were no biologically relevant differences between control and treated animals.

Gross pathology: there was an increase in findings in treated groups but no particular lesion seemed significant. 2/14 high dose dogs had pale livers, two had some kidney changes (pale areas, abnormal brown yellowish color) compared to no liver or kidney effects in control dogs.

Organ weights (specify organs weighed if not in histopath table):

There were no significant changes in organ weights in treated animals compared to controls.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (x), no ()

Liver, fatty change in males after 26 and 52 weeks

Group:	Incidence	Males 26WK	Incidence	Males 52WK
Dose (mg/kg/d)	26WK	Animal No./Fatty Changes	52WK	Animal No./Fatty Changes
<i>controls:</i>	2/3	102, mild	4/4	104, mild
0		103, mild		105, very mild
				106, mild
				107, very mild
<i>low dose:</i>	0/3	none	4/4	204, very mild
3				205, moderate*
				206, mild to moderate
				207, very mild
<i>mid dose:</i>	3/3	301, mild	4/4	304, mild to moderate
15		302, moderate		305, moderate*
		303, moderate to marked*		306, mild to moderate
				307, mild
<i>high dose:</i>	2/2	401, moderate to marked*	4/4	403, moderate to marked*
75		402, moderate		404, mild
		(405 premature decedent)		406, mild to moderate*
				407, mild to moderate

Liver, fatty change in females after 26 and 52 weeks

Group:	Incidence	Females 26WK	Incidence	Females 52WK
Dose (mg/kg/d)	26WK	Animal No./Fatty Changes	52WK	Animal No./Fatty Changes
<i>controls:</i>	1/3	152, mild	1/4	157, very mild
0				
<i>low dose:</i>	1/3	253, moderate*	1/4	254, mild
3				
<i>mid dose:</i>	3/3	351, mild to moderate	2/4	355, mild
15		352, moderate*		356, mild to moderate*
		353, moderate		
<i>high dose:</i>	2/3	451, moderate	4/4	454, mild
75		452, mild		455, very mild
				456, very mild
				457, very mild

* Macroscopical findings consisted in some kind of discoloration of the liver.

Frequency and intensity of fatty liver seemed dose and possibly duration related. It was characterized as mild to marked with an increase in intensity in the mid and high dose groups. The fat droplets were present in the periportal and midzonal areas of the liver and did not coalesce into larger droplets.

Hepatocellular fatty change has also been observed in a 13 week toxicity study in rats which may have been an adaptive change perhaps due to the induction of metabolizing enzymes. Flibanserin is not an enzyme inducer in dogs.

Heart, fatty changes in males and females after 26 and 52 weeks

Dose Group	Weeks	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
control	26	0/3	no	0/3	no
control	52	0/4	no	0/4	no
low	26	0/3	no	0/3	no
low	52	0/4	no	0/4	no
mid	26	1/3	303, very mild	0/3	no
mid	52	1/4	306, mild	2/4	355, mild 356, moderate
high	26	1/3	402, mild	0/3	no
high	52	3/4	403, mild to moderate 404, very mild 407, mild to moderate	3/4	454, moderate 456, moderate to marked 457, moderate

There was a fine, focal or multifocal lipid droplet accumulation in the myocardium of the left ventricle. The frequency and severity seemed dose and duration dependent. Sponsor attributed the fatty disposition to a relative hypoxia due to the increased heart rate and/or the reduced physical condition (I assume due to the CNS toxicity). The no effect dose for fat deposition in the heart is 3 mg/kg or approximately 0.6 times the exposure of women taking 100 mg.

Trachea, degeneration of mucous membrane in males and females after 26 and 52 weeks

Study Group (mg/kg/d)	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
controls	0/4	no	0/4	no
3	0/4	no	0/4	no
15	3/4	304, moderate 305, mild 307, moderate	1/4	355, mild
75	4/4	403, mild 404, moderate 406, moderate 407, mild	3/4	454, moderate 455, moderate 456, moderate

The tracheal degeneration consisted of decreased size and number of goblet cells, mild and diffuse granulocytic infiltration in the superficial mucosal layers and in the lamina submucosa, loss of cilia and regenerative changes of the respiratory epithelium. The changes were drug and dose dependent without gender differences. The mechanism and relevance for humans for this effect is unknown.

Thymus, accelerated involution in males and females after 52 weeks.

Study Group (mg/kg/d)	Males Nos./ Group	Animal Nos., Degree in Males	Females Nos./ Group	Animal Nos., Degree in Females
controls	0/4	no	0/3	no
3	1/4	207, moderate	2/3	256, mild 257, moderate
15	3/4	304, moderate to marked 305, moderate 307, moderate to marked	1/4	357, moderate
75	3/4	403, mild 406, moderate to marked 407, moderate to marked	2/4	454, mild 455, moderate

There was thymic involution in animals of all groups including the controls. It was accelerated in animals of the dosed groups and considered by the sponsor to be a nonspecific stress response.

Histology conclusion: Administration of flibanserin for one year to dogs resulted in a dose and time dependent, mild increase of hepatocellular fatty change in all dose groups.

A dose-related focal or multi-focal, very mild to marked myocardial fatty change in the left ventricle in the mid and high dose groups.

A dose-related degeneration of the mucous membrane of the trachea in the mid and high dose groups.

A mild to marked, accelerated thymus involution in all dosed groups.

A dose-related, but not time dependent, focal thickening of the corneal epithelium.

Toxicokinetics:

Geometric C_{max}; AUC_{0-24h} and median T_{max} values in the male dogs (n = 3-7)

Gender	Week	Dose	[mg/kg]	3	15	75
male	1	T _{max}	[h]	1	1	6
		C _{max}	[µg/mL]	0.5	2.9	9.0
		AUC	[µg/mL·h]	1.3	12.1	114.0
	14	T _{max}	[h]	1	1	2
		C _{max}	[µg/mL]	0.4	3.2	10.2
		AUC	[µg/mL·h]	1.0	11.9	77.3
	25	T _{max}	[h]	1	1	3
		C _{max}	[µg/mL]	0.5	3.5	13.2
		AUC	[µg/mL·h]	1.5	14.9	135.0
	51	T _{max}	[h]	1	1	2.5
		C _{max}	[µg/mL]	0.6	3.3	13.2
		AUC	[µg/mL·h]	1.2	15.9	108.0
female	1	T _{max}	[h]	1	2	10
		C _{max}	[µg/mL]	0.3	0.8	5.9
		AUC	[µg/mL·h]	0.8	4.8	72.0
	14	T _{max}	[h]	1	2	3
		C _{max}	[µg/mL]	0.4	1.6	9.7
		AUC	[µg/mL·h]	1.1	6.6	73.9
	25	T _{max}	[h]	1	1	3
		C _{max}	[µg/mL]	0.5	2.8	14.1
		AUC	[µg/mL·h]	1.3	13.6	140.0
	51	T _{max}	[h]	1	2	2
		C _{max}	[µg/mL]	0.6	2.9	15.1
		AUC	[µg/mL·h]	1.9	13.7	161.0

Plasma AUC is nonlinear for dose and seems to be more pronounced at weeks 25 and 51. Human AUC_{0-24h} for women taking 100 mg is 2080 ng.h/ml.

Aside from the moderate increase in heart rate, the NoAEL for this study was 3 mg/kg.

2.6.6.4 Genetic toxicology

Study title: **Study of the capacity of the test article to induce gene mutations in strains of Salmonella typhimurium**

Key findings: Study was negative

Study no.: (b) (4) no. 910474; U93-0573

Volume #, and page #:

Conducting laboratory and location: (b) (4)

Date of study initiation: Nov. 1991

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 CL, lot and purity not stated

Methods

Strains/species/cell line: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100

Doses used in definitive study: 5, 15, 50, 150, 500 ug/plate

Basis of dose selection: Initial study used doses of 1, 10, 100, 1000 and 5000 ug/plate. Doses above 100 ug/plate were toxic

Negative controls: DMSO 0.1 ml/plate

Positive controls: Hydrazine sulfate 500 ug/plate, 9-aminoacridine 40 ug/plate, 2-nitrofluorene 2.5 ug/plate, doxorubicine 4 ug/plate, 2-aminofluorene 5 ug/plate

Incubation and sampling times: 72 h incubation

Results

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

Plates run in triplicate in two experiments. If the number of colonies reverted was at least double the number of spontaneously reverted colonies the test was considered positive.

Study outcome:

Toxicity was apparent from 100-300 ug/plate without S9 and 500-800 ug/plate with S9.

No mutagenic effect at concentrations up to 500 ug/plate with or without S9

Positive controls were active in all experiments

Study title: **Mutagenicity study in the V79 (HPRT) forward mutation assay**

Key findings: Study was negative

Study no.: U95-2191

Volume #, and page #:

Conducting laboratory and location: Dept Exp Path Tox, Dr. (b) (4) Gmbh,
Boehringer Ingelheim

Date of study initiation: March, 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9402-P, purity not stated

Methods

Strains/species/cell line: V79 Chinese hamster cell line

Doses used in definitive study: 10, 20, 30, 40, 50 ug/ml

Basis of dose selection: Dose range finding study from 5-500 ug/ml without metabolic activation. Concentrations above 50 ug/ml had no cells surviving. In the activation system, cellular toxicity was evident (59.6% survival) at a dose concentration of 40 ug/ml.

Negative controls: DMSO

Positive controls: ethylmethane sulfonate (EMS); methyl-N-nitro-nitrosoguanidine (MNNG); 7,12-dimethylbenz(a)anthracene (DMBA)

Incubation and sampling times: Cells were treated with the control and test article for 3 hrs. Experiments consisted of one negative control (DMSO), one positive control (EMS, MNNG or DMBA) and four compound-treated cultures. In the activation system the S9 was added during the incubation procedure.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Positive response is defined as a reproducible and concentration dependent increase in mutant frequency for a minimum of two successive concentrations.

COMPOUND ($\mu\text{g/ml}$)	% SURVIVORS		HPRT-MUTANTS/ 1 MIO. SURVIVORS	
	EXP. 1	EXP. 2	EXP. 1	EXP. 2
CONTROLS NEGATIVE DMSO	100.0	100.0	7.9	1.0
POSITIVE EMS 500 MNGG 0.2	99.9 -	- 43.8	161.6* -	- 155.0*
BIMT 17 BS				
10	96.4	96.0	6.9	0.4
20	86.4	94.6	7.1	0.6
30	53.4	57.8	7.5	5.0
35	-	0.6	-	-
40	0	-	-	-

COMPOUND ($\mu\text{g/ml}$)	% SURVIVORS		HPRT-MUTANTS/ 1 MIO. SURVIVORS	
	EXP. 1	EXP. 2	EXP. 1	EXP. 2
CONTROLS NEGATIVE DMSO	100.0	100.0	2.5	4.0
POSITIVE DMBA 5	32.8	84.1	117.1*	170.9*
BIMT 17 BS				
10	91.6	88.0	5.1	8.8
20	104.0	83.5	0	5.6
30	87.3	-	3.6	-
40	59.6	69.8	1.7	12.4*
50	-	14.1	-	0

P: PRECIPITATION

HISTORICAL DATA FOR MUTANT FREQUENCY: MEAN 6.0/1 MIO. (RANGE 0-35.2)

* SIGNIFICANT INCREASE ($P \leq 5\%$)

Study outcome: Study was negative. The positive at 40 $\mu\text{g/ml}$ in the activated experiment was considered not treatment related mainly because it fell within the historical control range. Sponsor also stated that there was no consistent, dose-related or reproducible increase in the mutant frequency compared to vehicle control. True, but since the next highest dose was toxic with very low % survival, it would be hard to detect a dose-response. Nevertheless, the dose is in the toxic range (% survivors are decreased)

and the result is within historical control range so I would agree with sponsor on their interpretation.

Study title: **Mutagenicity study for chromosomal aberrations in human lymphocytes in vitro**

Key findings: Study was positive

Study no.:

Volume #, and page #:

Conducting laboratory and location: Dr. (b) (4), Boehringer Ingelheim, Biberach an der Riss, Germany

Date of study initiation: January, 1997

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9507-P, purity not stated

Methods

Strains/species/cell line: Human peripheral blood lymphocytes

Doses used in definitive study: Nonactivation: 10, 20, 40, 50 ug/ml

Activation trial 1: 10, 100, 150 (175) ug/ml

Activation trial 2: 10, 100, 150 ug/ml

Basis of dose selection: Dose range finding study showed a marked toxicity at a dose concentration range of 50-500 ug/ml (without S9) and 100-500 ug/ml (with S9). Based on this the doses between 10 and 200 ug/ml were selected.

Negative controls: DMSO

Positive controls: Cyclophosphamide (CP), Adriamycin (ADR)

Incubation and sampling times:

Test	Culture Initiation	Treatment	Colcemid	Harvesting	
				Regular	Delayed
- S9	0	48 - 72	70 (94)	72	96
+ S9	0	48 - 52	70 (94)	72	96

Structural chromosome aberrations (% aberrant cells excluding gaps)

Metabolic activation: without

COMPOUND ($\mu\text{g/ml}$)	EXPERIMENT 1	EXPERIMENT 2	
	72. hr	72 hr	96 hr
CONTROLS			
NEGATIVE DMSO	1.0 NT	1.0 NT	1.0 NT
POSITIVE ADR 0.05	23.0 * LT	8.0 * NT	16.0 * NT
BIMT 17 BS			
10	0 NT	2.0 NT	ND
20	3.0 LT	1.0 LT	ND
40	0 ¹ HT	(0 ²) HT	ND
50	0 ¹ HT	(0 ²) HT	1.0 NT

ND: not done

1: <100 metaphases scored (toxic)

2: < 50 metaphases scored (toxic)

NT: no toxicity (MI $75 \geq 100$ %)

LT: low toxicity (MI 50-74 %)

HT: high toxicity (MI ≤ 49 %)*: Significantly different from the vehicle control ($P \leq 5\%$)

Historical negative control values (% aberrant cells excl. gaps): 0.5 (range 0-4)

Structural chromosome aberrations (% aberrant cells excluding gaps)

Metabolic activation: rat liver S9

COMPOUND ($\mu\text{g/ml}$)	EXPERIMENT 1	EXPERIMENT 2	
	72. hr	72 hr	96 hr
CONTROLS			
NEGATIVE DMSO	3.0 NT	1.0 NT	1.0 NT
POSITIVE CP 7	27.0 * NT	27.0 * HT	18.0 * LT
BIMT 17 BS			
10	1.0 NT	0 NT	ND
100	7.0 LT	5.0 NT	ND
150	8.5 ¹ LT	3.8 ¹ LT	4.0 LT
175	N.E. HT	ND	ND

¹: < 100 metaphases scored (toxic)

N.E.: Not evaluable

N.D.: Not done

NT: no toxicity (MI ≥ 100 %)

LT: low toxicity (MI 50-74 %)

HT: high toxicity (MI ≤ 49 %)

*: Significantly different from the vehicle control ($P \leq 5\%$)

Historical negative control values (% aberrant cells excl. gaps): 0.6 (range 0-3.0)

Study outcome: Study was negative without metabolic activation. Slightly increased aberration frequencies were observed in the activation system without dose dependency. Although the difference between treated and negative controls did not reach statistical significance, individual values were outside the historical control range (0-3%). The effects were reproducible and partly associated with cell toxicity as indicated by mitotic inhibition or poor metaphase quality. Because of the cellular toxicity, the sponsor suggests that the effect may be due to an indirect mechanism and not the result of a direct DNA mutation. This is total speculation and the study met the sponsor's criteria for a positive test.

Study title: **Rat bone marrow micronucleus test after oral administration**

Key findings: Result was negative

Study no.: MUT 265; U96-2432

Volume #, and page #:

Conducting laboratory and location: Dept Exp Path Tox, Boehringer Ingelheim, Ingelheim, Germany

Date of study initiation: Sept, 1994

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403A, purity 99.9%

Methods

Strains/species/cell line: Chbb: (b)(4) rats

Doses used in definitive study: 600, 1200, 1800 mg/kg

Basis of dose selection: Maximum tolerated dose using same dosing schedule

Negative controls: 0.5% methocel

Positive controls: Cyclophosphamide

Incubation and sampling times:

Doses were administered orally by gavage. Sampling was performed at 24 and 48 hrs after dosing.

Dose	Sampling time	Sex	Animal number
0.5% Methocel solution	24 h	m/f	001-005/051-055
BIMT 17 BS, 1800 mg/kg	24 h	m/f	101-105/151-155
BIMT 17 BS, 1800 mg/kg	48 h	m/f	201-205/251-255
BIMT 17 BS, 1800 mg/kg	--	m/f	301-302/351-352*
BIMT 17 BS, 1200 mg/kg	24 h	m	401-405
BIMT 17 BS, 600 mg/kg	24 h	m	501-505
Cyclophosphamide, 20 mg/kg	24 h	m/f	601-605/651-655

* Additional animals, to substitute animals in the test substance groups, in case of death.

Results

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

Study was valid

Study outcome:

Dose ^a	Sampling time	Sex	Number of rats	Micronucleated PE (‰) Mean ± standard deviation	p-value
Vehicle control	24 h	male	5	1.4 ± 0.9	
		female	5	1.1 ± 0.5	
BIMT 17 BS 1800 mg/kg	24 h	male	5	1.2 ± 0.3	0.758
		female	5	0.5 ± 0	1.000
BIMT 17 BS 1800 mg/kg	48 h	male	5	1.8 ± 0.4	0.262
		female	5	1.3 ± 1.3	0.433
BIMT 17 BS 1200 mg/kg	24 h	male	5	1.2 ± 0.8	0.714
BIMT 17 BS 600 mg/kg	24 h	male	5	1.0 ± 0.6	0.841
Cyclophosphamide 20 mg/kg	24 h	male	5	22.8 ± 7.0*	0.004
		female	5	21.4 ± 3.0*	0.004

^a Vehicle control, 20 ml 0.5% Methocel solution per kg.

* Significantly different from the vehicle control (p<0.05).

PE = polychromatic erythrocytes.

NE = normochromatic erythrocytes.

Study was negative

Study title: **In vivo comet assay for measurement of DNA damage in the liver of rats after repeat oral administration (gavage)**

Key findings: Study was negative

Study no.: 08B162

Volume #, and page #:

Conducting laboratory and location: Non-clinical drug safety, Boehringer Ingelheim, Biberach an der Riss, Germany

Date of study initiation: October, 2008

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 04040593M, 99.9% pure

Methods

Strains/species/cell line: CrI:WI(Han) male rats

Doses used in definitive study: 5 male rats/group were given either 400 or 1500 mg/kg flibanserin 28 and 4 hrs prior to necropsy. Vehicle controls received 0.5% hydroxyethylcellulose and the positive controls were given 200 mg/kg ethyl methanesulfonate, concurrently.

Basis of dose selection: Dose response study, 1800 mg/kg was significantly toxic.

Negative controls: 0.5% hydroxyethylcellulose

Positive controls: Ethyl methanesulfonate/EMS

Incubation and sampling times:

Results

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

Sponsors summary:

At necropsy samples from the target organ liver (right medial lobe) were immersed in chilled tissue buffer (Hank's balanced salt solution containing 20 mmol/L ethylenediaminetetraacetic acid disodium salt (EDTA) and 10 % dimethylsulfoxide (DMSO), pH 7.5).

(b) (4)

(b) (4)

The slides were coded in a random manner to allow an objective evaluation. The coverslipped slides were examined at 200 x magnification using fluorescent microscopy immediately after staining with ethidium bromide. A total of 150 cells (75 cells/slide) was analyzed. Images were visualized by a CCD camera and measured using the Kinetic Imaging software system "Komet 6 GLP". The head and tail areas of the image were identified and the light intensity of each was quantified and expressed as Olive Tail Moment (OTM) which is calculated automatically and saved to file. $OTM = (Tail.mean - Head.mean) \times Tail\%DNA/100$. Among different parameters including %DNA in tail used for comparison purposes, the OTM is regarded as the most relevant parameter to measure comet induction. Extensively damaged cells or cell clusters, so called "hedgehogs" were excluded from analysis. However, as a sign for toxicity, these "hedgehogs" (necrotic and apoptotic nuclei) were estimated in parallel. Pieces from the livers were taken adjacent to the sites used for comet analysis and fixed in 4 % neutral buffered formaldehyde solution. Since the comet result was negative, these samples were not processed and evaluated.

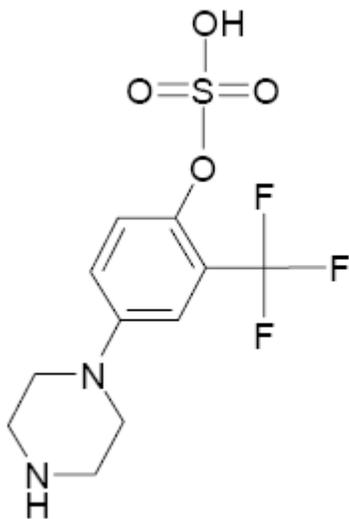
Study outcome:

	Flibanserin (mg/kg)			Pos. Control EMS (mg/kg)
	Control	400	1500	200
OTM:				
	0.18 (0.16-0.21)	0.20 (0.16-0.23)	0.22 (0.17-0.26)	0.41
%DNA in tail:				
	2.36 (2.21-2.65)	2.69 (2.02-2.99)	2.91 (2.50-3.32)	4.11
% Hedgehogs:				
	~2.4	~2.4	~2.4	~4.5

Flibanserin did not induce single strand breaks and alkaline-labile sites in the liver in this assay.

Flibanserin was negative in the in vitro Ames and HPRT assays but induced a non-significant increase in chromosomal aberrations in human peripheral erythrocytes in the presence of a metabolic activation system. However, flibanserin was negative in in vivo assays for clastogenicity (rat bone marrow micronucleus) and for DNA reactivity (Comet) when tested up to toxic doses. Based on the weight of evidence, I consider flibanserin negative for genotoxicity.

Metabolite BI 400296 ZW (M8)



The metabolite was tested for mutagenicity in the Ames assay against TA 1535, TA 1537, TA 98, TA 100 and TA102 with and without metabolic activation (study no. 06B221)

It was negative for all strains at concentrations up to 5000 ug/plate.

Mutagenic activation of M8 in *S. typhimurium* without metabolic activation**Experiment 1 (Plate test)**

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	10	50	58	131	434
BI 400296 ZW					
100	12	42	63	133	434
300	6	43	75	132	436
1000	7	47	66	128	392
3000	9	45	65	129	422
5000	9	42	63	139	434
Positive Controls					
NaN ₃ 5	<u>1054</u>	-	-	<u>1145</u>	-
9-AA 50	-	<u>558</u>	-	-	-
2-NF 10	-	-	<u>642</u>	-	-
MMC 0.5	-	-	-	-	<u>1160</u>

Experiment 2 (Preincubation)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	7	16	42	88	397
BI 400296 ZW					
100	7	18	44	79	376
300	7	14	45	81	403
1000	6	17	45	84	367
3000	7	13	39	88	381
5000	6	13	43	79	343
Positive Controls					
NaN ₃ 5	<u>761</u>	-	-	<u>1172</u>	-
9-AA 50	-	<u>340</u>	-	-	-
2-NF 10	-	-	<u>526</u>	-	-
MMC 0.5	-	-	-	-	<u>1667</u>

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Historical Range	6 – 22	3 - 30	16 - 68	49 - 150	249 - 458
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Mutagenic activation of M8 in *S. typhimurium* with metabolic activation

Experiment 1 (Plate test)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	12	55	59	139	528
BI 400296 ZW					
100	12	42	53	146	524
300	16	42	60	145	539
1000	12	44	55	156	510
3000	13	46	56	163	533
5000	15	49	61	170	536
Positive Controls					
2-AA 4	<u>133</u>	<u>158</u>	<u>986</u>	<u>1067</u>	-
2-AA 10	-	-	-	-	<u>1148</u>

Experiment 2 (Preincubation)

Test substance (µg/plate)	Mean Revertants/Plate				
	<i>S. typhimurium</i>				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	10	16	39	107	486
BI 400296 ZW					
100	8	14	36	111	479
300	12	19	34	111	495
1000	9	16	39	108	472
3000	11	18	36	120	505
5000	11	14	37	112	502
Positive Controls					
2-AA 4	<u>162</u>	<u>376</u>	<u>1616</u>	<u>1411</u>	-
2-AA 10	-	-	-	-	<u>668</u>

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Historical Range	12 - 22	3 - 39	20 - 61	74 - 164	279 - 531
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2.6.6.5 Carcinogenicity

Study title: **Carcinogenicity study in rats by oral administration (dietary admixture) over a period of 2 years**

Key study findings: borderline positive for liver tumors in males

Adequacy of the carcinogenicity study and appropriateness of the test model:

Acceptable, doses and methods approved by exec CAC in fax dated 23 December 1997.

Evaluation of tumor findings:

Study no.: 97B090

Volume #, and page #: Electronic submission

Conducting laboratory and location: Dept. non-clinical drug safety, Boehringer Ingelheim Pharm, Biberach, Germany

Date of study initiation: March, 1998

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS (flibanserin), batch 720618, 99.44% pure

CAC concurrence: yes

Methods

Doses: 10, 30, 100 mg/kg

Basis of dose selection (MTD, MFD, AUC etc.):

Species/strain: rat/ Wistar Chbb: (b)(4)

Number/sex/group (main study): 50/sex/gp

Route, formulation, volume: oral in food, admixed in diet

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: 5/sex/gp

Age: 38-42 days

Animal housing: Up to 5/cage

Restriction paradigm for dietary restriction studies: no

Drug stability/homogeneity: stable

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: none

Results

Mortality:

Mortality	Daily dose of BIMT 17 BS [mg/kg]									
	0 (control 1)		0 (control 2)		10		30		100	
	M	F	M	F	M	F	M	F	M	F
Sacrificed	6	14	12	12	6	9	9	10	7	13
Found dead	3	0	3	1	0	3	0	2	4	2
Total	9	14	15	13	6	12	9	12	11	15
% mortality	18	28	30	26	12	24	18	24	22	30

M, F: males, females

Survival rate was equal or greater than 70% for all gps.

Clinical signs:

In parentheses: time period (first to final observation day during study)

Group	control 1		low dose		mid dose		high dose		control 2	
BIMT 17 BS [mg/kg]	0		10		30		100		0	
	M	F	M	F	M	F	M	F	M	F
No. of animals/group	50	50	50	50	50	50	50	50	50	50
No changes observed	18	19	20	19	28	24	25	18	12	23
Eye, milky, opaque	5 (482-722)	4 (279-722)	5 (510-722)	1 (475-722)	2 (412-722)	2 (356-722)	3 (489-723)	0	5 (615-723)	2 (601-723)
Hair loss	1 (700-722)	5 (398-722)	1 (608-671)	9 (447-722)	0	3 (622-722)	1 (447-630)	4 (398-723)	1 (629-723)	3 (398-723)
Pale appearance	0	7 (419-681)	0	6 (608-722)	0	6 (420-712)	0	5 (125-699)	1 (678-684)	2 (548-615)
Rough coat	2 (576-581)	9 (412-728)	0	7 (623-722)	4 (615-666)	10 (293-722)	4 (475-688)	8 (545-708)	4 (454-707)	4 (411-615)
Testes, increased size	1 (664-722)	---	7 (384-722)	---	5 (545-722)	---	6 (307-723)	---	12 (447-723)	---
Thickening, limbs	5 (412-643)	2 (580-663)	4 (405-663)	1 (195-722)	4 (426-722)	0	0	0	3 (384-650)	0
Vaginal discharge, red	---	4 (419-722)	---	7 (384-722)	---	6 (489-722)	---	4 (552-723)	---	1 (538-723)

M, F: males, females

No treatment related clinical signs.

Body weights:

	Group	control 1	low dose	mid dose	high dose	control 2
Time	Dose [mg/kg]	0	10	30	100	0
Week -1	Males	168.80	171.29 (1.5)	170.93 (1.3)	169.36 (0.3)	171.93 (1.9)
	Females	137.35	140.49 (2.3) ↑	137.45 (0.1)	140.80 (2.5) ↑	138.91 (1.1)
Week 12	Males	428.34	424.97 (-0.8)	418.07 (-2.4)	403.03 (-5.9) ↓	437.71 (2.2)
	Females	267.93	265.09 (-1.1)	254.11 (-5.2) ↓	241.09 (-10.0) ↓	263.52 (-1.6)
Week 52	Males	584.20	566.21 (-3.1)	563.29 (-3.6) ↓	521.94 (-10.7) ↓	575.38 (-1.5)
	Females	313.64	309.81 (-1.2)	300.72 (-4.1) ↓	276.97 (-11.7) ↓	308.72 (-1.6)
Week 80	Males	612.26	597.89 (-2.4)	585.34 (-4.4) ↓	525.63 (-14.2) ↓	612.19 (0)
	Females	317.53	313.99 (-1.1)	300.71 (-5.3) ↓	283.49 (-10.7) ↓	318.93 (0.4)
Week 104	Males	640.72	612.93 (-4.3)	607.78 (-5.1) ↓	525.87 (-17.9) ↓	616.29 (-3.8)
	Females	323.85	321.84 (-0.6)	306.94 (-5.2) ↓	285.44 (-11.9) ↓	327.56 (1.1)

↑, ↓: significantly increased, decreased compared with control 1; p < 0.05, many-to-one t-test, two-sided

There was a dose related decrease in BW gain that gradually increased over time. For males, the difference in absolute weight in the LD and MD were small, 4-5% when compared to control 1 (heaviest). The HD gp was reduced by ~18%

For females, the decrease in BW was negligible for the LD, 5% for MD and 12% for HD.

Food consumption:

	Group	control 1	low dose	mid dose	high dose	control 2
Time	Dose [mg/kg]	0	10	30	100	0
Week -1	Males	150.74	149.51 (-0.8)	150.83 (0.1)	151.57 (0.6)	152.63 (1.2)
	Females	124.17	122.43 (-1.4)	120.57 (-2.9) ↓	119.74 (-3.6) ↓	119.89 (-3.4) ↓
Week 12	Males	204.75	200.84 (-1.9)	203.64 (-0.5)	199.07 (-2.8)	213.34 (4.2)
	Females	152.48	149.22 (-2.1)	141.40 (-7.3) ↓	130.09 (-14.7) ↓	156.50 (2.6)
Week 52	Males	182.77	178.86 (-2.1)	180.17 (-1.4)	177.43 (-2.9)	179.34 (-1.9)
	Females	123.33	123.06 (-0.2)	121.20 (-1.7)	113.94 (-7.6) ↓	132.12 (7.1) ↑
Week 80	Males	165.08	162.20 (-1.7)	164.04 (-0.6)	162.73 (-1.4)	166.78 (1.0)
	Females	120.03	116.49 (-3.0)	111.39 (-7.2) ↓	110.01 (-8.4) ↓	119.46 (-0.5)
Week 104	Males	174.87	164.82 (-5.8)	167.21 (-4.4)	152.98 (-12.5) ↓	173.44 (-0.8)
	Females	125.11	122.61 (-2.0)	117.43 (-6.1) ↓	113.04 (-9.7) ↓	129.73 (3.7)

↑, ↓: significantly increased, decreased compared with control 1; p < 0.05, many-to-one t-test, two-sided

Non-consistent decrease in FC for both MD and HD males and females.

Hematology: No treatment related changes in hematology.

Organ Weight:

Daily dose of BIMT 17 BS [mg/kg]	0		10		30		100	
Gender (M = male, F = female)	M	F	M	F	M	F	M	F
Number of organs weighed	76	73	44	38	41	38	38	35
Body weight [g]	608.0	307.3	582.8	300.9	577.4	286.7	502.7	265.9
Liver								
Absolute weight [g]	16.0	9.9	16.2	10.2	17.5↑	9.9	15.7	9.7
Liver weight to body weight ratio [%]	2.64	3.23	2.78↑	3.39↑	3.05↑	3.46↑	3.16↑	3.64↑
Liver weight to brain weight ratio [%]	672	460	691	480↑	747↑	465	673	465

↑, ↓: significantly increased, decreased compared with control 1; p< = 0.05, many-to-one t-test, two-sided

Drug related slight increase in relative liver wt in all treated animals. The increase correlates with the histopath findings of centrilobular hepatocellular hypertrophy.

No other organ wt changes were considered toxicologically significant.

Gross pathology:

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
Gender (M = male, F = female)	M	F	M	F	M	F	M	F	M	F
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver:										
Nodule	1	2	1	3	5	2	5	0	1	0
Uterine cervix:										
Enlargement	-	5	-	7	-	13	-	10	-	5
Hard consistency	-	3	-	8	-	14	-	15	-	5

In the liver, there was an increase in nodules in MD and HD males but not in females. In the uterus, the enlargement and hard consistency may be due to fibrosis (see under non-neoplastic histopath)

Histopathology:Non-neoplastic:

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)	10	30	100	0 (control 2)
Number of animals examined	50	50	50	50	50
Uterine cervix:					
Fibrosis/fibroplasia	34 (2.4)	36 (2.7)	37 (2.5)	39 (2.4)	35 (2.3)
moderate to severe,	14	23	17	17	13
with/without necropsy	3/11	5/18	8/9	13/4	4/9
correlate					
Hyperplasia squamous cell	7 (2.6)	8 (2.0)	7 (2.6)	7 (2.6)	2 (1.5)

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
	M	F	M	F	M	F	M	F	M	F
Gender (M = male, F = female)										
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver										
Hypertrophy hepatoc. centrilobular / slight to moderate	12	1	13	0	24	0	32	4	14	0
Vacuolation centrilobular hepatoc. / moderate to severe	1	0	2	0	6	0	18	1	4	0
	14	2	18	0	26	0	26	0	12	0
	2	0	3	0	9	0	10	0	2	0
Kidneys										
Mineralisation tubular	0	8	0	4	0	1	0	30	1	1
Dilatation of tubules	0	4	1	1	2	3	0	9	1	1
Nephrosis, chronic-progressive	28	5	23	0	22	4	16	1	24	2
Rectum										
Edema	7	5	5	6	8	6	13	3	1	4
Infiltration, inflammatory	9	2	5	2	6	4	13	2	2	0
Spleen										
Hemosiderosis	16	26	10	29	16	30	14	43	15	29
Adrenal gland										
Peliosis / angiectasis	14	43	23	41	25	39	23	38	13	35

Neoplastic:

Group	control 1		low dose		mid dose		high dose		control 2	
	0		10		30		100		0	
Daily dose of BIMT 17 BS [mg/kg]	M	F	M	F	M	F	M	F	M	F
Gender (M = male, F = female)										
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Liver										
Carcinoma, hepatocellular	0	0	1	1	4	1	5	0	2	1
Adenoma, hepatocellular	2	2	1	0	1	2	1	0	1	0
Combined adenoma/carcinoma hepatocellular	2	2	2	1	5	3	6	0	3	1
Focus eosinophilic	2	2	2	1	1	0	3	4	3	1
Focus basophilic tigroid	18	10	18	10	11	16	6	2	16	21
Slight or more	10	4	9	3	4	8	0	1	5	10
Focus basophilic diffuse	2	2	4	3	2	3	4	1	4	4
Focus basophilic NOS	14	4	19	5	13	5	9	2	15	4
Focus clear cell	39	13	39	6	43	7	40	11	38	14
Adrenal gland										
Adenoma, cortical	6	1	3	5	3	3	0	4	2	3
Hyperplasia, cortical	32	29	32	28	26	19	20	19	30	28
Moderate to severe	16	8	11	9	5	7	5	7	11	14
Ovary										
Tumor sex cord stromal mixed [B]	-	13	-	7	-	3	-	2	-	8
Hyperplasia sex cord stromal	-	29	-	24	-	23	-	14	-	28
- severe	-	6	-	6	-	7	-	0	-	8

NOS: not otherwise specified

In the liver, the incidence of hepatocellular carcinomas was slightly higher in HD males. Two HD males died due to intra-abdominal hemorrhage from liver tumors in weeks 90 and 86. The carcinogenic finding was not significant when compared to pooled controls. There were no effects in females.

Statistical evaluation of hepatocellular carcinomas (Sponsors statistics) in males

Dose BIMT 17 BS	0 mg/kg (control 1)	0 mg/kg (control 2)	10 mg/kg	30 mg/kg	100 mg/kg	Trend
Examined (N)	50	50	50	50	50	
Incidences	0	2	1	4	5	
p	-	-	0.7460	0.1088	0.0430	0.0116
p1	-	0.2033	0.5116	0.0551	0.0253	0.0044 ^b
p2		-	0.9172	0.4162	0.2541	0.0788

p p value including pooled controls
p1 p value versus control 1
p2 p value versus control 2
^b significance level $p < 0.005$ for trend

Statistical evaluation of hepatocellular carcinomas and adenomas (combined tumors) in males

Dose BIMT 17 BS	0 mg/kg (control 1)	0 mg/kg (control 2)	10 mg/kg	30 mg/kg	100 mg/kg	Trend
Examined (N)	50	50	50	50	50	
Incidences	2	3	2	5	6	
p	-	-	0.7967	0.2330	0.1166	0.0408
p1	-	0.6543	0.7094	0.2493	0.1165	0.0308
p2		-	0.8834	0.4075	0.2895	0.0860

p p value including pooled controls
p1 p value versus control 1
p2 p value versus control 2

Other organs/tissues with significant numbers of tumors

Daily dose of BIMT 17 BS [mg/kg]	0 (control 1)		10		30		100		0 (control 2)	
	M	F	M	F	M	F	M	F	M	F
Number of animals examined	50	50	50	50	50	50	50	50	50	50
Adrenal gland Tumor medullary [B]	4	2	4	2	6	8	2	2	5	1
Mammary gland Fibroadenoma	0	2	0	4	0	3	0	9	0	7
Pancreas Infiltration, inflammatory	4	0	6	0	5	4	10	0	2	2
Skin Fibromas ^a	0	1	1	2	0	0	0	3	1	0
Combined fibromas ^a and fibrosarcomas	1	1	1	3	0	0	1	3	2	1
Combined fibromatous tumors ^b	5	1	3	3	3	1	3	5	3	3
Thyroid gland Adenoma, follicular cell [B]	0	0	0	0	0	2	0	1	0	0

^a Including fibroma [B] and fibroma fibromatosis type [B]

^b Including fibroma [B], fibroma fibromatosis type [B], fibrosarcoma [M], fibrous histiocytoma [B], fibrous histiocytoma [M]

Other than possibly the liver, there were no treatment related tumor increases.

Toxicokinetics:

Group	low dose		mid dose		high dose	
	10 mg/kg		30 mg/kg		100 mg/kg	
Dose	Males	Females	Males	Females	Males	Females
Week 13						
AUC(0-24h)	1960	1670	4610	5910	9670	18700
C(6:00 h)	70.6	108	235	322	584	1080
C(16:00 h)	89.2	28.9	145	147	196	400
Week 26						
AUC(0-24h)	1270	2400	4600	5440	8930	22200
C(6:00 h)	60.8	130	276	335	444	1170
C(16:00 h)	43.2	67.7	103	111	294	623
Week 52						
AUC(0-24h)	1330	1800	4300	4440	7520	16500
C(6:00 h)	48.5	91.9	217	202	389	803
C(16:00 h)	59.9	53.1	141	165	231	558
Week 104						
AUC(0-24h)	1780	1760	5960	7190	10000	17100
C(6:00 h)	96.7	108	311	353	516	1050
C(16:00 h)	50.8	36.9	180	245	314	278

	AUC of BIMT 17 BS (ng·h/mL)					
	10 mg/kg		30 mg/kg		100 mg/kg	
	M	F	M	F	M	F
Rats (week 104)						
AUC(0-24h)	1780	1760	5960	7190	10000	17100
Human (steady state)	2 mg/kg (50 mg BID)		4 mg/kg (100 mg BID)			
Geometric mean AUC _{ss}	2170 (2 x 1085)		5188 (2 x 2594)			
Safety margin	10 mg/kg		30 mg/kg		100 mg/kg	
Ratio rat/human (LD)	0.8	0.8	2.7	3.3	4.6	7.9
Ratio rat/human (HD)	0.34	0.34	1.1	1.4	1.9	3.3

Note: Human daily dose: 50 mg BID (LD, low dose), 100 mg BID (HD, high dose); individuals assumed with 50 kg bodyweight; U97-2256

Using 2080 ng·h/ml as the human exposure at a dose of 100 mg, the safety margins of exposure at the two higher doses for females are roughly 3 and 8, and for males 3 and 5, respectively.

Summary of rat study:

This was a 2 year carcinogenicity study in Wistar rats. The doses were 10, 30 and 100 mg/kg which gave multiples of human exposure to female rats of approximately 1, 3 and 8 for women taking 100 mg. These doses produced reductions in final BW of 18% for HD males and 12% for HD females. Decreased food consumption probably accounts for some of the weight decrease.

The doses were agreed to by the exec CAC in a meeting of Dec. 23, 1997.

The only tumorigenic effect was an increase in the incidence of hepatocellular carcinomas in males. Incidence was 0 and 2 in the controls, 1 in LD, 4 in MD and 5 in

HD. In a carcinogenicity study in mice, there was an increase in hepatocellular carcinomas in males and malignant mammary tumors in females. In the present study, the incidence of malignant mammary carcinomas was 1 and 2 in controls and 1 in LD, 0 in MD and 1 in HD and the incidence of mammary fibroadenomas was 2 and 7 in controls and 4, 3 and 9 in LD, MD and HD, respectively.

Historical controls in Wistar rats (hepatocellular carcinomas)

Study No.	Group No.	Start [m/y]	Duration [month]	Strain	Breeder	Males			Females		
						Animals exam.	with lesion	%	Animals exam.	with lesion	%
2	1	11/1984	24	WIST	A	50	2	4.0	50	0	0.0
13	1	05/1984	31	WIST	A	100	1	1.0	100	0	0.0
20	1	09/1986	24	WIST	A	100	4	4.0	100	0	0.0
21	1	06/1986	24	WIST	A	20	0	0.0	20	1	5.0
22	1	06/1986	25	WIST	A	50	3	6.0	50	0	0.0
28	1	02/1989	26	WIST	A	99	5	5.1	100	1	1.0
30	4	02/1989	26	WIST	A	100	2	2.0	100	0	0.0
49	4	05/1989	25	WIST	A	50	2	4.0	49	0	0.0
51	1	02/1990	25	WIST	A	50	0	0.0	50	0	0.0
65	6	02/1990	25	WIST	A	50	3	6.0	50	0	0.0
83	1	04/1993	25	WIST	A	20	2	10.0	20	0	0.0
89	1	09/1992	25	WIST	A	50	2	4.0			
93	1	09/1992	25	WIST	A				50	2	4.0
95	1	02/1989	26	WIST	A	100	8	8.0			
103	1	08/1994	25	WIST	A	50	2	4.0	50	0	0.0
117	1	08/1995	25	WIST	A	50	4	8.0	50	1	2.0
135	1	08/1995	24	WIST	A	20	1	5.0	20	0	0.0
139	1	05/1991	25	WIST	A	50	4	8.0	50	1	2.0
144	1	05/1991	25	WIST	A	20	0	0.0	20	0	0.0
156	1	10/1998	24	WIST	A	50	5	10.0	50	2	4.0
All 20 studies:						1079	50	4.6	979	8	0.8
Range MIN:								0.0			0.0
Range MAX:								10.0			5.0

m/y: month/year

The maximum percent was 10% in the historical controls and 10% for the HD in this study.

This study was borderline for liver tumors in males. Flibanserin increases the incidence and severity of centrilobular hepatocellular hypertrophy probably by inducing hepatic cytochrome P-450 enzymes. Sponsor believes that flibanserin acts similar to Phenobarbital in promoting liver tumors. It's a reasonable explanation since flibanserin was essentially negative in the available genotox studies and is probably not an initiator.

Combined with the data in mice (see below), flibanserin can be considered positive for liver tumors in male rodents.

Study title: **Twenty-four month oral (diet) carcinogenicity study in the mouse.**

Key study findings: mammary and hepatocellular carcinomas

Adequacy of the carcinogenicity study and appropriateness of the test model:

Deemed adequate by exec CAC

Evaluation of tumor findings:

Study no.: 98R003

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim Pharm, Ridgefield, CT

Date of study initiation: 3/1998

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 (flibanserin), lot 720618, purity not stated

CAC concurrence: yes

Methods

Doses: 10, 80, 200, 1000 (M), 1000/1200 (F). Doses for HD females increased from 1000 to 1200 mg/kg in drug week 23.

Basis of dose selection (MTD, MFD, AUC etc.): Doses were recommended by the Exec CAC in a July 14, 1998 meeting with the Division of Neuropharmacology. In a letter to the Sponsor they recommended that the high dose in females should be 1200 mg/kg based on MTD but apparently the sponsor decided initially on 1000 mg/kg and then increased the dose to 1200 mg/kg when little toxicity was apparent.

Species/strain: (b) (4) CrI:CD-1 (ICBR) mice

Number/sex/group (main study): 70/sex/gp

Route, formulation, volume: oral in feed

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: no

Age: 5-6 wks

Animal housing: individual cages

Restriction paradigm for dietary restriction studies: none

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: TK non GLP

Results

Mortality: Survivability similar between all gps including controls. 38-42 males survived and 22-41 females with no dose response.

Group	Flibanserin mg/kg/day	Males	Females
G1 Control I	0	40	22
G2 Control II	0	38	29
G3 Low	10	46	29
G4 Mid	80	42	41
G5 High-mid	200	44	28
G6 High	M: 1000 F: 1000/1200	40 -	- 32

- a Study termination started in Drug Week 105; numbers take into account all early deaths including animals found dead and sacrificed moribund, as well as dead due to accidental trauma.

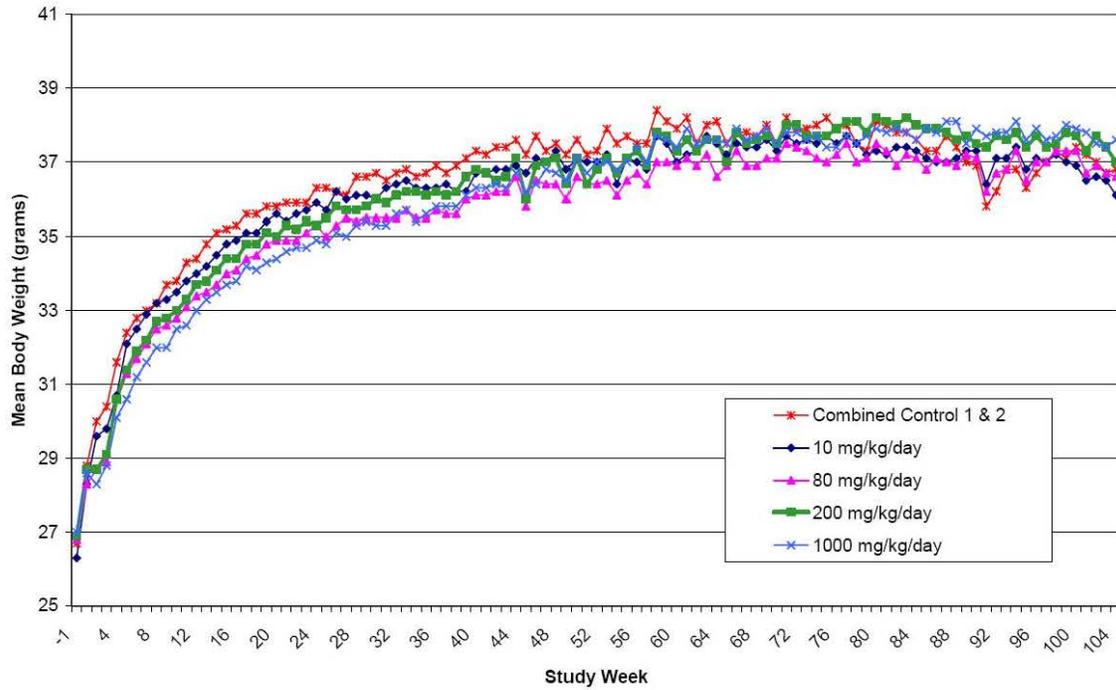
Clinical signs: Noted mainly in 1000 mg/kg gp males and included distended/discolored abdomens, dermal blanching and hair staining. Hair staining was also seen in 200 mg/kg males. Distended abdomens was observed at wk 85 and increased to 70% at study termination compared to combined controls which had 39%. The abdominal finding was only seen in males at 1000 mg/kg dose level and not in females at any dose or lower dose males.

Body weights: No difference between treated and control males at study end. Mean body wts for 1000/1200 mg/kg females were consistently increased over combined controls from wk 5 to 82 and considered treatment related. From wk 82 on, wts between treated and control females were similar.

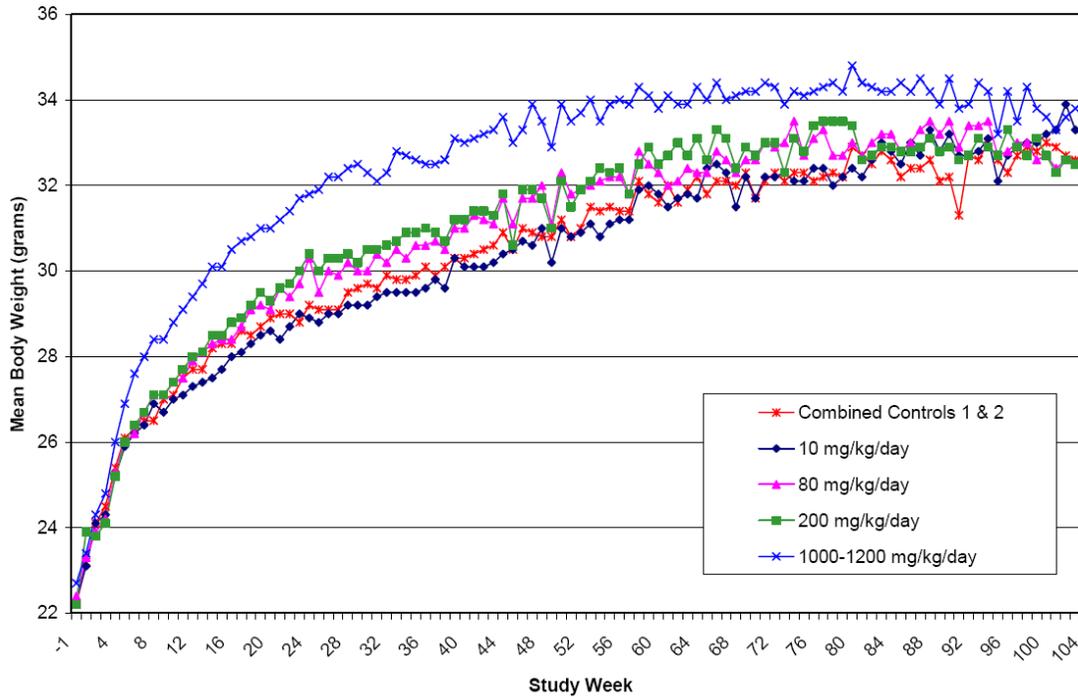
BW week	Control 1+2		10 mg/kg		80 mg/kg		200 mg/kg		1000/1200	
	M	F	M	F	M	F	M	F	M	F
104	36.4	31.9	36.0	33.6	36.8	32.7	37.6	32.0	37.3	32.5

Weekly body weight means

Figure 1: 98R003: Carcinogenicity Study in Mice on Flibanserin
Mean Body Weights, Combined Control Groups - Males



**Figure 2: 98R003: Carcinogenicity Study in Mice on Flibanserin
Mean Body Weights, Combined Control Groups - Females**



Body weight changes in both males and females were considered drug related. Mean male body weights were significantly decreased (3 to 6%) at the HD during study weeks 2 to 22, but were not decreased upon study termination. Mean body weights in the HD females were significantly increased over combined controls (3 to 11%) during weeks 5 to 82. There were no significant differences at study termination.

Food consumption: Greater than controls in HD males and females. Because of possible palatability issues, there was significant food spillage and food consumption may be overestimated.

Gross pathology: Only treatment related macroscopic change was increase in liver masses in HD males (15/70) versus controls (0/70, 9/70).

Histopathology:

Non-neoplastic: Increased incidence of hepatocellular (centrilobular and midzonal) hypertrophy in males treated with ≥ 80 mg/kg and HD females.

Neoplastic:

There was an increased incidence of tumors in the liver and mammary gland

Incidence and Percentage of Selected Proliferative Changes in the Mammary Gland of Female CD-1 Mice

Group description	Control 1	Control 2	Low	Mid 1	Mid 2	High
Dose Level (mg/kg/day) Flibanserin	0	0	10	80	200	1200
Number of Animals Examined	70	70	70	70	70	70
Adenocarcinoma	0 0%	1 1.4%	3 4.3%	3 4.3%	5 7.1%	5 7.1%
Metastasis (Lung)	0	0	1	1	4	5
Metastasis (Bronchial L. Nodes) ^a	0	0	0	0	2	3
Adenoacanthoma, Malignant ^b	0 0%	0 0%	0 0%	0 0%	1 1.4%	2 2.9%
Combined Carcinomas	0 0%	1 1.4%	3 4.3%	3 4.3%	6 8.6%	7 10%
Hyperplasia	2	0	0	2	0	2

^a Bronchial Lymph Nodes

^b Also known as Malignant Adenosquamous Carcinoma

Table 3.2.3:6

Statistical Analysis of Mammary Gland Adenocarcinomas, Malignant Adenoacanthomas, and Adenocarcinomas and Malignant Adenoacanthomas Combined (Females)

M-Adenocarcinomas (common)								
Dose mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1200	Trend
Examined	N	70	70	70	70	70	70	-
Incidence	I	0	1	3	3	5	5	-
p value versus	C1 + C2	-	-	0.0937	0.1013	0.0154	0.0134	0.0064
	C1	-	1.000	0.0827	0.0666	0.0246	0.0218	0.0185
	C2	-	-	0.3013	0.2695	0.1104	0.0951	0.0575
M-Adenoacanthomas (rare)								
Incidence	I	0	0	0	0	1	2	-
p value versus	C1 + C2	-	-	1.000	1.000	0.3544	0.1458	0.0194 ^d
	C1	-	1.000	1.000	1.000	0.5600	0.3466	0.0328
	C2	-	-	1.000	1.000	0.4912	0.2710	0.0287
M-Adenocarcinomas and M-Adenoacanthomas Combined (common)								
Incidence	I	0	1	3	3	6	7	-
p value versus	C1 + C2	-	-	0.0937	0.1013	0.0062 ^a	0.0024 ^a	0.0008 ^c
	C1	-	1.000	0.0827	0.0666	0.0138	0.0076 ^a	0.0033 ^c
	C2	-	-	0.3013	0.2695	0.0621	0.0323	0.0119

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.05, pairwise comparison, Peto test, rare tumor

c p < 0.005, trend, Peto test, common tumor

d p < 0.025, trend, Peto test, rare tumor

Table 1 Historical control data for mammary gland adenocarcinomas and malignant adenoacanthoma in CD-1 mice (from: (b) (4))

Study ID	Start [y]	Animals examined	Adenocarcinoma		Adenoacanthoma, malignant	
			with lesions	[%]	with lesions	[%]
35	1993	54	0	0.00	0	0.00
36	1993	64	0	0.00	0	0.00
37	1993	49	1	2.04	0	0.00
38	1993	62	2	3.23	0	0.00
39	1993	49	2	4.08	0	0.00
40	1994	50	2	4.00	0	0.00
41	1994	56	2	3.57	0	0.00
42	1994	57	1	1.75	0	0.00
43	1995	60	5	8.33	0	0.00
44	1995	38	2	5.26	0	0.00
45	1995	52	0	0.00	2	3.85
46	1995	68	2	2.94	0	0.00
47	1996	48	1	2.08	1	2.08
48	1996	46	0	0.00	0	0.00
49	1996	55	0	0.00	0	0.00
50	1996	53	0	0.00	0	0.00
51	1998	54	1	1.85	0	0.00
52	1999	65	2	3.08	0	0.00
53	1999	57	3	5.26	0	0.00
54	2000	55	1	1.82	0	0.00
all studies			27	2.5	3	0.3
			range min	0.00		0.00
			range max	8.33		3.85

Incidence and Percentage Summary of Selected Proliferative and Non-Proliferative Findings in the Liver

Group description	Control 1		Control 2		Low		Mid 1		Mid 2		High	
Dose Level (mg/kg/day) Flibanserin	0		0		10		80		200		1000/1200	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number of Animals Examined	70	70	70	70	70	70	70	70	70	70	70	70
PROLIFERATIVE FINDINGS												
Carcinoma, Hepatocellular	1 1.4%	0 0%	6 8.6%	0 0%	7 10.0%	0 0%	8 11.4%	5 7.1%	9 12.9%	1 1.4%	11 15.7%	4 5.7%
Adenoma, Hepatocellular	3 4.3%	1 1.4%	3 4.3%	0 0%	2 2.9%	0 0%	0 0%	1 1.4%	3 4.3%	0 0%	5 7.1%	2 2.9%
Combined Adenomas/Carcinomas, Hepatocellular	4 5.7%	1 1.4%	8 11.4%	0 0%	8 11.4%	0 0%	8 11.4%	5 7.1%	11 15.7%	1 1.4%	13 18.6%	6 8.6%
Foci, Acidophilic	0	0	1	0	0	0	0	0	3	0	6	1
Foci, Basophilic	0	0	1	0	0	0	2	1	2	0	8	3
Foci, Mixed	0	0	2	0	0	0	0	0	1	0	7	2
Foci, Vacuolated	0	0	0	0	0	0	0	0	0	1	3	0
Foci, Clear Cell	0	0	0	0	3	0	0	0	0	0	0	0
Foci, Combined	0	0	4	0	3	0	2	1	5	1	17	4
DRUG-RELATED NON- PROLIFERATIVE FINDINGS												
Hypertrophy Hepatocellular Centrilobular	0	0	0	0	0	0	2	0	6	0	13	3
Hypertrophy Hepatocellular Midzonal	0	1	0	0	3	1	6	0	6	0	25	6

Statistical Analysis of Combined Hepatocellular Adenomas and Carcinomas

Hepatocellular Adenomas and Carcinomas Combined (common)								
Dose Level mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1000 M 1200 F	Trend
No.Examined/ Sex	N	70	70	70	70	70	70	-
MALES								
Incidence	I	4	8	8	8	11	13	-
p value	C1+C2	-	-	0.3518	0.2859	0.1192	0.0239	0.0188
versus	C1	-	0.2452	0.2417	0.1987	0.0865	0.0113	0.0092
	C2	-	-	0.7020	0.6551	0.3475	0.1435	0.0851
FEMALES								
Incidence	I	1	0	0	5	1	6	-
p value	C1+C2	-	-	1.000	0.0599	0.5862	0.0046 ^a	0.0024 ^b
versus	C1	-	0.4314	1.000	0.3088	0.8114	0.0491	0.0153
	C2	-	-	1.000	0.0619	0.4912	0.0092 ^a	0.0025 ^b

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.005, trend, Peto test, common tumor

Table 3.2.3:3

Statistical Analysis of M-Hepatocellular Carcinomas in the Liver of Mice

M-Hepatocellular Carcinomas (common)								
Dose Level mg/kg/day Flibanserin	Stat	Control 1 0	Control 2 0	10	80	200	1000 M 1200 F	Trend
No.Examined/ Sex	N	70	70	70	70	70	70	-
MALES								
Incidence	I	1	6	7	8	9	11	-
p value	C1+C2	-	-	0.1661	0.0865	0.0764	0.0065 ^a	0.0062
versus	C1	-	0.1198	0.0367	0.0185	0.0162	0.0028 ^a	0.0035 ^b
	C2	-	-	0.5364	0.4189	0.3997	0.1111	0.0859
FEMALES								
Incidence	I	0	0	0	5	1	4	-
p value	C1+C2	-	-	1.0000	0.0152	0.3544	0.0045 ^a	0.0045 ^b
versus	C1	-	0.10000	1.0000	0.1066	0.5600	0.0248	0.0203
	C2	-	-	1.0000	0.0619	0.4912	0.0338	0.0180

a p < 0.01, pairwise comparison, Peto test, common tumor

b p < 0.005, trend, Peto test, common tumor

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Table 2 Historical control data for hepatocellular carcinomas in CD-1 mice 1981 to 2000

Males					Females				
Study ID ^{1,2}	Start [y]	total examined	with lesions	[%]	Study ID ^{1,2}	Start [y]	total examined	with lesions	[%]
CQ	1985	50	14	28.00	CQ	1985	50	1	2.00
CR	1985	50	0	0.00	CR	1985	50	2	4.00
CP	1985	50	1	2.00	CP	1985	51	1	1.96
BX	1981	52	9	17.31	BX	1981	52	1	1.92
DN	1988	48	4	8.33	DN	1988	49	0	0.00
DX	NS	50	8	16.00	DX	NS	50	1	2.00
CX	1983	72	12	16.67	CX	1983	71	0	0.00
DU	1989	50	7	14.00	DU	1989	50	2	4.00
EG	1989	50	5	10.00	EG	1989	49	1	2.04
DZ	1990	49	8	16.33	DZ	1990	49	0	0.00
27	1989	50	3	6.00	30	1992	50	0	0.00
28	1992	49	5	10.20	31	1990	60	2	3.33
29	1990	60	4	6.67	32	1991	70	1	1.43
30	1991	67	10	14.93	33	1991	58	2	3.45
31	1991	60	2	3.33	34	1992	117	0	0.00
32	1993	59	8	13.56	35	1993	59	1	1.69
33	1993	70	4	5.71	36	1993	70	3	4.29
34	1993	50	3	6.00	37	1993	50	0	0.00
35	1993	65	1	1.54	38	1993	65	0	0.00
36	1993	50	8	16.00	39	1993	51	0	0.00
37	1994	50	2	4.00	40	1994	50	0	0.00
38	1994	65	0	0.00	41	1994	65	0	0.00
39	1994	65	5	7.69	42	1994	65	1	1.54
40	1995	60	0	0.00	43	1995	60	0	0.00
41	1995	60	2	3.33	44	1995	41	0	0.00
42	1995	60	4	6.67	45	1995	59	0	0.00
43	1995	70	6	8.57	46	1995	70	0	0.00
44	1996	50	4	8.00	47	1996	50	0	0.00
45	1996	50	5	10.00	48	1996	50	0	0.00
46	1996	90	5	5.56	49	1996	60	0	0.00
47	1996	60	2	3.33	50	1996	70	1	1.43
48	1996	60	7	11.67	51	1998	60	0	0.00
49	1998	65	6	9.23	52	1999	65	0	0.00
50	1999	70	7	10.00	53	1999	60	1	1.67
51	1999	60	9	15.00	54	2000	55	0	0.00
52	2000	55	2	3.64					
March 1995*¹		471	54	11.46			521	9	1.73
March 2005*²		1570	114	7.26			1530	12	0.78
1993 – 2000*		1284	90	7.00			1175	7	0.60
all studies*		2041	168	8.23			2051	21	1.02

Study ID with letters: Spontaneous Neoplastic Lesions in the CrI:CD-1[®] (ICR)BR Mouse. March 1995, (b)(4) information prepared by (b)(4) Appendix A.

Study ID with numbers: Spontaneous Neoplastic Lesions in the CrI:CD-1[®] (ICR)BR Mouse in Control Groups from 18 Month to 2 year Studies. March 2005, (b)(4) information prepared by (b)(4) Appendix B.

Study CQ excluded for males due to exceptionally high incidence(shaded grey)

**Table 3 Historical control data for hepatocellular carcinomas in CD-1 mice
December 1993 (92 - 104 weeks)**

Males					Females				
Study ID ¹	Start [y] ²	total examined	with lesions	[%]	Study ID ¹	Start [y] ²	total examined	with lesions	[%]
618	ns	50	8	16.00	618	ns	50	1	2.00
670	ns	49	8	16.33	670	ns	49	0	0.00
617	ns	50	5	10.00	617	ns	50	0	0.00
293	ns	50	7	14.00	293	ns	50	2	4.00
694	ns	50	0	0.00	694	ns	50	0	0.00
483	ns	50	5	10.00	483	ns	49	1	2.04
056	ns	50	2	4.00	056	ns	50	0	0.00
001	ns	50	5	10.00	001	ns	50	0	0.00
total		399	40	10.03			398	4	1.01
range			min	0.00				min	0.00
			max	16.33				max	4.00

Background tumour incidences from carcinogenicity studies in CRL:CD one Swiss mice, OA39/BTI(mice).1297, (b)(4) Appendix C.
ns = not specified

Historical control data were from the same strain and same vendor ((b)(4)), four different production sites). Studies were initiated between 1993 and 2000 for mammary tumors and 1981 to 2000 for hepatocellular tumors.

Toxicokinetics:

Table 3.1.4:1 Mean Plasma Concentrations of Flibanserin in Mice

Group	Dose (mg/kg/day)	Gender	Flibanserin Plasma Concentration (ng/mL)		
			Week 24	Week 53	Week 78
G1	0 (Control)	Male	0	24.6 ± 55.0	2.32 ± 5.19
		Female	0	0	0
G2	0 (Control)	Male	0	0	0
		Female	0	0	0
G3	10	Male	60.4 ± 69.2	0 ^a	12.5 ± 14.4
		Female	6.62 ± 14.80	0 ^a	21.5 ± 12.6
G4	80	Male	124 ± 60	131 ± 79 ^a	142 ± 69
		Female	123 ± 48	47.4 ± 65.0 ^a	203 ± 181
G5	200	Male	211 ± 47	228 ± 67	286 ± 169
		Female	181 ± 120	204 ± 79	185 ± 89
G6	1000 (males)	Male	807 ± 635	1253 ± 301	1218 ± 407
	1200 (females) ^b	Female	571 ± 215	735 ± 482	571 ± 276

a NOTE: The LOQ of the assay for these samples was higher (100 ng/mL) than for samples in Weeks 24 and 78 (10 ng/mL). As a consequence, and because a concentration of zero ng/mL was used in place of BLQ for calculations of mean±SD, the mean concentrations for the 10 and 80 mg/kg/day dose group in Week 53 are biased to be lower than if an LOQ of 10 ng/mL had been possible. Therefore, comparisons to other groups may be inaccurate.

b The initial dose in Group G6, 1000 mg/kg/day, was escalated to 1200 mg/kg/day in Week 23 for females only.

No AUC data were available from the mouse carcinogenicity study. Direct exposure comparisons with humans cannot be made.

Serum drug and prolactin levels were determined in a 34 week study in the same strain of mice and at the same doses as the carcinogenicity study. For additional information on the prolactin measurements, see under special toxicology studies, below.

Summary Table 1 Mean toxicokinetic parameters of flibanserin after dietary administration to mice

Parameter	Week	Gender	10 mg/kg	80 mg/kg	200 mg/kg	1000 mg/kg	1200 mg/kg
C(max)	3	m	39.1	176	376	1430	-
[ng/mL]	3	f	47.6	192	313	-	1710
AUC(0-24h)	3	m	591	3150	6020	16300	-
[ng·h/mL]	3	f	671	2370	5680	-	20800
C(1000)	3	m	18.5	115	217	628	-
[ng/mL]	3	f	18.6	66.1	188	-	477
C(0800)^a	13	m	18.0	207	247	787	-
	13	f	16.2	101	197	-	558
[ng/mL]	26	m	23.0	150	233	748	-
	26	f	16.5	88.6	311	-	764

^a actual collection between 0800 and 1050 hours

In humans taking 100 mg, drug AUC_{0-ss} was 2080 ng·h/ml. These TK data are used to compare mouse and human exposures for the carcinogenicity study.

Prolactin levels - means

Prolactin ng/ml	control	10 mg/kg	80 mg/kg	200 mg/kg	1000/1200 mg/kg
wk 14	129 ± 58*	146 [^] ± 107	134 ± 42	115 ± 55	177 ± 104
wk 34	106 ± 153	33 ± 31	146 ± 152	103 ± 66	104 ± 77

* ± SD

[^] In another table, the value was given as 128

Summary: Flibanserin was tested for carcinogenicity in a two year study in mice. Mortality was similar for males and females among all treated groups and controls.

Only sign of toxicity was distended abdomen in HD males. Changes in body weight occurred during the course of the study for both males (decrease) and females (increase) and were considered treatment related but body weights were no different than controls at study termination.

In the mammary gland of female mice, there was a clear increase in adenocarcinomas (incidence was 0, 1 in the two controls and 3, 3, 5, 5 in the treated gps). When combined with malignant adenoacanthomas (also called adenosquamous carcinoma; a variation of adenocarcinoma), the controls had 0, 1 and the treated gps had 3, 3, 6, 7 total malignant mammary tumors which is higher than the historical controls for any one group. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes.

In male mice, there was a statistically significant increase trend in hepatocellular carcinomas (1 and 6 in male controls, and 7, 8, 9, 11, in the treated gps) which was also significant in the pairwise test at the HD. For hepatocellular carcinomas in females, the results were 0 and 0 for controls and 0, 5, 1, 4 in the treated gps. There was a significant trend and pairwise difference at the 1200 mg/kg dose level when compared with pooled controls but not when compared with control 1 or control 2 separately. For combined adenomas and carcinomas, females showed a significant trend and pairwise difference at 1200 mg/kg when compared to pooled controls and control 2.

The maximum percent incidence in the historical controls for hepatic carcinoma controls was 17% for males and 4% for females (Sponsor excluded the first 1985 study because of the exceptionally high incidence). In this study, the percents were 1.4%, 8.6%, 10%, 11.4%, 12.9%, 15.7% for males and 0%, 0%, 0%, 7.1%, 1.4%, 5.7% for females.

There was a clear increase in combined foci of cellular alteration in livers of high dose males and possibly in females as well.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: **Study of fertility and early embryonic development to implantation in rats by oral administration, gavage.**

Key study findings: No effects on fertility

Study no.: 69S; U95-2214

Volume #, and page #: Electronic

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. (b) (4) GmbH, Biberach an der Riss, Germany

Date of study initiation: January 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9403;

Methods

Doses: 20, 80, 200 mg/kg

Species/strain: Chbb: (b) (4) (SPF) rats
 Number/sex/group: 24/sex/gp
 Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg
 Satellite groups used for toxicokinetics: none
 Study design: Males were dosed 28 days before mating and females 14 days before mating. Dosing continued without interruption until GD 6 in females. Males were dosed continuously until successful mating. Dams were sacrificed on GD 14-16 and subjected to cesarean section with an in situ macroscopic inspection.

Results

Mortality: none

Clinical signs: Some sedation in HD males. Sedation, prone posture, reduction of spontaneous activity and timidity were seen in HD females at the beginning of treatment. They were normal from the 4th day on.

Body weight: Some slight decrease in MD and HD females on GD 3 and 6 but same as controls on GD 14.

Food consumption: No significant changes

Toxicokinetics: not done

Necropsy: Females were normal

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): The normal duration of the estrus cycle for this strain of rat is 4-5 days. The duration of the estrus cycle was changed to shorter than 4 days in 4.2% of MD animals and longer than 5 days in 8.3 and 29.2% of MD and HD animals. The mean number of corpora lutea was significantly increased in the HD group.

Parameter (mean)	Control	5 mg/kg	15 mg/kg	100 mg/kg	Historical range [#]
Corpora lutea	15.6	16.3	15.9	17.8*	13.5-17.5

* significant difference (P<0.05), # = from vehicle controls and unaffected dosages

There were no effects on fertility (copulation index, fertility index, gestation index)

Parameter [%]	Control	20 mg/kg	80 mg/kg	200 mg/kg
Copulation index [#]	95.8	100	95.8	100
Fertility index [#]	95.8	95.8	95.8	100
Gestation index	95.6	100	95.6	100

[#] dams pregnant without sperm found in vaginal smear included

There were no effects on litter parameters (implantations, resorptions, viable fetuses)

Embryofetal development

Study title: Study for effects on embryo-fetal development in rats by oral administration, gavage

Key study findings: Maternal toxicity in MD and HD. Developmental abnormalities in the HD. Several different malformations in treated groups with no dose response. NoAEL considered to be 80 mg/kg (~15 X human exposure).

Study no.: 56S; U95-2254

Volume #, and page #:

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. ^{(b) (4)} GmbH, Biberach an der Riss, Germany

Date of study initiation: September, 1994

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9403;

Methods

Doses: 20, 80, 400 mg/kg

Species/strain: Chbb: ^{(b) (4)} (SPF) rats

Number/sex/group: 24 pregnant females/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg

Satellite groups used for toxicokinetics: 6/gp

Study design: Pregnant females were dosed from GD 7-16. Animals were sacrificed on GD 22.

Results

Mortality (dams): None

Clinical signs (dams): The number of pregnant rats/group were 20, 22, 18, and 22 in the control to high dose, respectively. An additional dam in the high dose group had total embryo resorptions. The clinical signs of the low dose group were similar to the control group. Within 2-3 hrs, MD females showed somnolence, sedation and timidity after the first treatment until the 9th treatment. In the HD group, there were severe clinical sign including somnolence, reduction of spontaneous activity, sedation, prone position, timidity, catalepsy, and others. After the 7th treatment there was slight sedation, somnolence and timidity and after the end of treatment, the animals behaved normally.

Body weight (dams):

There was moderate decrease in BW gain in the HD group and to a lesser extent, the MD group. Total body weight gains were 121, 118, 102, 73g control to HD, respectively.

Dose (mg/kg)	Mean of Body Weight Gain (g) relative to GD 7			
	GD 8	GD 12	GD 16	GD 21
control	1.7	24.1	45.3	121.3
20	0.6	18.2*↓	41.7↓	118.0
80	-3.7*↓	13.5*↓	31.5*↓	101.9*↓
400	-23.5*↓	-7.5*↓	10.0*↓	73.0*↓

* significant difference (P<0.05), ↓ decreased, GD = gestation day

▨ = administration period

Food consumption (dams):

There was a slight decrease in food consumption in the MD group and a moderate decrease at the HD.

Toxicokinetics:

Geometric means of $C_{max,ss}$ and AUC_{0-24h} and medians of $t_{max,ss}$ on GD 12 were

Dose [mg/kg/day]	N	$t_{max,ss}$ [h]	$C_{max,ss}$ [ng/ml]	AUC_{0-24h} [(ng/ml)×h]
20	6	1	1108	7131
80	6	1	3785	31592
400	6	2	8447	86643

Human AUC_{0-t} is 2080 ng.h/ml

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

One HD animal had complete resorption.

Parameter (mean)	Control	20 mg/kg	80 mg/kg	400 mg/kg	Historical range#
Fetal body weight [g]	5.5	5.5	5.2*	4.7*	4.8 - 5.6 g

* significant difference (P<0.05), # = from vehicle controls and unaffected dosages

Offspring (malformations, variations, etc.):

There was one runt (fetuses weighing less than 65% of the weighted control mean values) in the MD and 10 in the HD groups. Skeletal and visceral abnormalities were seen in controls and treated animals with equal distribution. In the HD group, there were a greater number of fetuses with reduced ossification of fore limbs and a greater number with lumbar ribs.

Malformations were seen only in the treated groups. They included frequent malformations like cleft or fused vertebrae (2 at MD, 2 at HD) or a single malformation (agnathia) at the LD. In the HD, there were 2 fetuses with anophthalmia and one with hydrocephalus, all within one litter.

Fetal findings (%)

Findings	Control	Dose mg/kg			Historical Data %
	G 0	20 G 1	80 G 2	400 G 3	
Runts			1 (0.40)	10 (3.29)	0.19
Variations:					
retinal fold	24 (17.91)	31 (20.26)	16 (13.44)	21 (14.19)	0.13*
dilated renal pelvis	6 (4.48)	4 (2.61)	1 (0.84)	-	0.84*
distance between A.carotis communis sinistra and A.subclavia sinistra enlarged	1 (0.75)	-	-	-	-
cervical ribs	2 (1.38)	4 (2.53)	-	1 (0.64)	1.74*
lumbar ribs	1 (0.69)	1 (0.63)	4 (3.12)	9 (5.77)	0.35*
delayed ossification of fore limbs	1 (0.69)	-	-	11 (7.05)	2.56*
delayed ossification of sternbrae	1 (0.69)	1 (0.63)	1 (0.78)	1 (0.64)	2.68*
delayed ossification of vertebral body	1 (0.69)	1 (0.63)	1 (0.78)	1 (0.64)	0.43 (ossification delay in general)*
split or displaced sternbrae	3 (2.07)	1 (0.63)	3 (2.34)	1 (0.64)	0.34*
rudimentary sternbrae	-	-	-	1 (0.64)	-
hypoplastic 13th rib	-	-	1 (0.78)	-	0.43*
short ribs	-	-	-	1 (0.64)	-
Malformations:					
dilated ventricle of telencephalon (hydrocephalus)	-	-	-	1 (0.33) ⁺	0.09*
agnathia	-	1 (0.32) ⁺	-	-	-
anophthalmia	-	-	-	1 (0.33) ⁺ 1 (0.67)	-
cleft vertebrae	-	-	2 (1.56)	1 (0.64)	0.11
fused vertebra	-	-	-	1 (0.74)	0.02

⁺ calculated for all living fetuses

* These data origin from an internal historical data set which is filed in the Teratology Laboratory at Dr. (b)(4) GmbH, Biberach

The complete resorption in one HD dam and the decreased fetal body weights may be attributable to the significant reduction in maternal body weight in the HD group.

The two fetuses in the high dose group with anophthalmia one of them also with hydrocephalus, occurred in the one litter.

Study title: Study for effects on embryo-fetal development in rabbits by oral administration, gavage

Key study findings: No teratology findings. Increased fetal resorptions at HD.

Study no.: 68S; U95-2267

Volume #, and page #:

Conducting laboratory and location: Dept. Experimental Pathology and Toxicology; Dr. (b) (4) GmbH, Biberach an der Riss, Germany

Date of study initiation: January, 1995

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS; batch 9402-P;

Methods

Doses: 20, 40, 80 mg/kg

Species/strain: Chbb:HM (SPF) rabbits

Number/sex/group: 21 pregnant females/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 5 ml/kg

Satellite groups used for toxicokinetics: 6/gp

Study design: Pregnant females were dosed from GD 6-18. Animals were sacrificed on GD 29.

Results

Mortality (dams): One high dose dam died on GD 21, cause of death not stated.

Clinical signs (dams): The number of pregnant rabbits/group were 17, 17, 20 and 21 in control to high dose, respectively. Complete resorptions occurred in one MD dam and in 5 HD dams. There were abortions in one MD dam (2 fetuses) and one HD dam (5 fetuses).

Coprostasis (fecal impaction) was seen in 2/20 MD rabbits and in 14/21 HD rabbits.

I assume that the dams were culled to 17/group except for the HD which had only 15 pregnant dams remaining.

Body weight (dams):

Dose (mg/kg)	n	Mean of Body Weight Gain [g] relative to GD 6				
		GD 7	GD 8	GD 18	GD 21	GD 28
control	17	5.6	9.2	128.1	150.5	272.7
20	17	-5.1	-24.3	113.8	149.3	300.9
40	17	-27.7↓*	-55.7↓*	32.7↓*	84.2	238.5
80	15	-57.5↓*	-104.0↓*	-94.7↓*	-14.4↓*	180.1↓*

* significant difference (P<0.05) , ↓ decreased

Food consumption (dams):

FC was decreased during treatment in the MD and HD group.

Toxicokinetics:

Geometric means of $C_{max,ss}$ and AUC_{0-24h} and medians of $t_{max,ss}$ on GD 13/14:

Dose [mg/kg/day]	N	$t_{max,ss}$ [h]	$C_{max,ss}$ [ng/ml]	AUC_{0-24h} [(ng×h/ml)]
20	3	2	1286	7535
40	3	2	1821	15720
80	3	2	4852	53930

Human AUC_{0-t} is 2080 ng.h/ml

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Gestation index was 100% in controls and LD, 85% in MD and 71% in HD due to aborted fetuses in one MD and one HD dam, complete abortion in one HD dam and to complete resorptions in one MD and 5 HD dams

Parameter (mean)	Control	20 mg/kg	40 mg/kg	80 mg/kg	Historical range#
Corpora lutea	6.8	7.3	8.1*	8.0	7.5-9.1
Late resorptions	0.1	0.1	0.1	0.8*	0-0.1
Resorption rate	5.8	6.6	2.4	17.0*	4.6-14.4

* significant difference (P<0.05) , # = from vehicle controls

Offspring (malformations, variations, etc.):

Five fetuses of the HD group were classified as runts (less than 65% of control value).

Parameter (mean)	Control	20 mg/kg	40 mg/kg	80 mg/kg	Historical range#
Fetal body weight [g]	39.0	38.5	36.2*	35.9*	34.6-39.6

* significant difference (P<0.05) , # = from vehicle controls

There were no variations or malformations in the treated groups that were considered treatment related. None occurred more than once or at a significantly greater frequency than concurrent controls and all were within the historical control range.

Findings	Control	Dose mg/kg)			Historical Data* / %
		20	40	80	
Runts		-	-	5 (5.0%)	1.2
Variations:					
Flexure	2 (1.9%)	-	1 (0.8%)	-	0.9
Ventricular septal defect (VSD)	3 (2.8%)	3 (2.7%)	4 (3.1%)	2 (2.0%)	38.3
short 12th rib	-	1 (0.9%)	-	-	0.1
13th rib	-	1 (0.9%)	1 (0.8%)	-	0.4
Malformations:					
Hydrocephalus	-	-	1 (0.8%)	-	0.05
Synostosis of sternbrae	2 (1.9%)	-	-	1 (1.0%)	0.25

* These data origin from an internal historical data set which is filed in the Teratology Laboratory at Dr. (b) (4) GmbH, Biberach

Prenatal and postnatal development

Study title: **Study for effects on pre- and postnatal development including maternal function in rats by oral administration, gavage**

Key study findings: Decreased number of offspring, some developmental effects.

Study no.: 96B026; U97-2294

Volume #, and page #:

Conducting laboratory and location:

Date of study initiation: March, 1996

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 9403-P

Methods

Doses: 20, 80, 200 mg/kg

Species/strain: Chbb: ^{(b)(4)}(SPF) rats

Number/sex/group: 24 females/group

Route, formulation, volume, and infusion rate: oral gavage, 0.5% methocel E5, 10 ml/kg

Satellite groups used for toxicokinetics: none

Study design: Dosing began on GD 6 and continued until postpartum (pp) day 21

Results

F₀ in-life: Twenty control and low dose and 21 mid and high dose females became pregnant. One HD dam died after the first treatment. Slight treatment related sedation was seen in the LD on treatment day 1 and in the MD from treatment day 1 to 10. In the HD group there was slight to severe sedation and somnolence, reduction of spontaneous activity and occasional pilo-erection, chromorhinodacryorrhea and dyspnea. Gestation period was 22 days for the control group, 3 dams in the LD and MD had gestations of 23 days and 8/20 dams in the HD group had a gestation of 23 days.

Body weight gain of pregnant dams of all treated groups was dose dependently and significantly decreased during gestation. However, by lactation day (LD) 21, only the HD group had decreased body weight.

Dose [mg/kg]	Mean of body weight gain (relative to GD 6) during gestation [g]				
	GD 7	GD 10	GD 17	GD 20	GD 21
control	4.4	15.6	62.9	109.4	127.5
20	-0.3*↓	8.6*↓	47.7*↓	84.8*↓	100.0*↓
80	-1.0*↓	6.1*↓	38.5*↓	73.7*↓	88.4*↓
200	-11.0*↓	1.0*↓	25.4*↓	55.9*↓	69.6*↓

* significant difference (P<0.05), ↓ decreased, GD=gestation day

Dose [mg/kg]	Mean of body weight gain (relative to LD 1) during lactation [g]			
	LD 4	LD 7	LD 14	LD 21
control	10.6	16.2	39.1	42.9
20	5.8	14.0	36.2	47.6
80	5.3	11.1	30.5*↓	44.0
200	0.8*↓	4.2*↓	23.0*↓	25.7*↓

* significant difference (P<0.05), ↓ decreased, LD=lactation day

F₀ necropsy:

No macroscopic changes were seen in the dams at weaning. All litter parameters were comparable in control, LD and MD groups. The HD group had significantly reduced numbers of implantations and newborns, due primarily to postimplantation loss.

Parameter (mean; range)	Control	20 mg/kg	80 mg/kg	200 mg/kg	Historical range#
Implantations	15.8	14.3	14.6	12.5*↓	14.3; 13.5-15.0
Number of offspring	14.1	12.4	12.7	10.8*↓	12.8; 12.8-12.9
Postimplantation loss [%]	10.7	13.3	12.8	16.6	12.2; 10.0-14.0

* significant difference (P<0.05), # = from vehicle controls

At birth anophthalmia was seen in two pups in the high dose group, exceeding the historical control range for the strain. No variations were seen in controls or treated groups.

In the 200 mg/kg dose group viability rate was decreased to 49.3% and weaning rate to 85.5%. In the 80 mg/kg dose group the effect on viability was 82%. All offspring survived after weaning.

At delivery the body weights of the F₁ pups from the 80 and 200 mg/kg groups were significantly lower than the control and below the mean historical control mean. Body weight gain of these groups remained significantly decreased during lactation.

F₁ physical development:

Dose [mg/kg]	Mean of body weight [g]	Mean of body weight gain (relative to LD 1) [g]			
	LD 1	LD 4	LD 7	LD 14	LD 21
Control	5.9	1.4	6.4	22.0	38.1
20	5.9	1.2	6.0	21.0	36.6
80	5.6*↓	0.7*↓	4.7*↓	18.0*↓	32.6*↓
200	5.3*↓	0.1*↓	2.6*↓	14.4*↓	27.7*↓

* significant difference (P<0.05), ↓ decreased, LD=lactation day

Time points for incisor eruption, growth of fur, opening of the ear canals, opening of the eyes, correct running, descent of the testes and vaginal opening were similar for controls, LD and MD. In the HD, growth of fur, opening of the ear canals and vaginal opening were significantly delayed for one day in 10/72 (fur), 12/72 (ear) and 5/21 (vagina) pups or more than one day in 6/72, 3/72 and 3/21 pups.

Pupillary reflex, air-righting reflex and hearing were not affected by treatment.

F₁ behavioral evaluation:

Biel Water T-maze Test: Results of the learning approach in wk 6 and test of memory function in wk 7 were similar in all groups.

Test on motility (Actiframe): Time dependent activity was similar between all groups. In controls, locomotor activity could be seen at 4 pm due to switch off of room light. The reaction in the MD and HD groups was significantly decreased (less total activity). In the LD, only the females were affected.

F₁ reproduction:

No treatment related effects were seen. Copulation and fertility indices were not affected by treatment and there were no abortions, resorptions of entire litters or intercurrent deaths. Mean numbers of viable fetuses, resorption rate and preimplantation loss were comparable between treated and control groups.

2.6.6.7 Local tolerance

Not done

2.6.6.8 Special toxicology studies

Study title: **34-week oral (diet) toxicokinetic study in the mouse**

Key study findings: no increase in serum prolactin levels

Study no.: 06R074

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim, Pharm, Ridgefield, CT

Date of study initiation: Sept, 2006

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, lot 05071202M, purity not stated

Methods

Doses: 10, 80, 200, 1000 (males) 1200 (females) mg/kg

Species/strain: CD-1 mice

Number/sex/group or time point (main study): 35/sex/gp

Route, formulation, volume, and infusion rate: oral in diet

Satellite groups used for toxicokinetics or recovery: none

Results:

Mortality: none drug related

Clinical signs: none drug related

Body weights: At the end of the study, HD males weighted about 15% less than controls. There were non-significant decreases in BW in the other male groups as well. No effects on BW in females.

Toxicokinetics:

Summary Table 1 Mean toxicokinetic parameters of flibanserin after dietary administration to mice

Parameter	Week	Gender	10 mg/kg	80 mg/kg	200 mg/kg	1000 mg/kg	1200 mg/kg
C(max)	3	m	39.1	176	376	1430	-
[ng/mL]	3	f	47.6	192	313	-	1710
AUC(0-24h)	3	m	591	3150	6020	16300	-
[ng·h/mL]	3	f	671	2370	5680	-	20800
C(1000)	3	m	18.5	115	217	628	-
[ng/mL]	3	f	18.6	66.1	188	-	477
C(0800)^a	13	m	18.0	207	247	787	-
	13	f	16.2	101	197	-	558
[ng/mL]	26	m	23.0	150	233	748	-
	26	f	16.5	88.6	311	-	764

^a actual collection between 0800 and 1050 hours

In humans taking flibanserin 100 mg, the AUC_{0-ss} is 2080 ng.h/ml.

Prolactin levels - means

Prolactin ng/ml	control	10 mg/kg	80 mg/kg	200 mg/kg	1000/1200 mg/kg
wk 14	129 ± 58*	128 ± 107	134 ± 42	115 ± 55	177 ± 104
wk 34	106 ± 153	33 ± 31	146 ± 152	103 ± 66	104 ± 77

* ± SD

Prolactin levels (ng/ml), week 14, individual data, summary statistics and t-tests

Animal	Control	Low-Dose	Low-Mid-Dose	High-Mid-Dose	High-Dose
1	136.65	77.93	93.94	70.27	163.47
2	179.06	142.06	160.03	200.57	185.50
3	169.86	315.66	169.87	102.10	114.38
4	185.08	156.46	180.49	175.00	140.99
5	105.97	4.24	155.71	54.58	126.31
6	19.53	177.52	93.57	121.59	101.86
7	107.31	19.75	81.15	79.38	404.58
N	7	7	7	7	7
Mean	129.066	127.660	133.537	114.784	176.727
SD	58.257	106.628	42.087	54.851	104.468
Geom. Mean	107.951	69.869	127.407	104.120	158.790
Geom. SD	2.200	4.546	1.403	1.612	1.584
p value	-	0.3363	0.7124	0.9359	0.3930

Prolactin levels (ng/ml), week 34, individual data, summary statistics and t-tests

Animal	Control	Low-Dose	Low-Mid-Dose	High-Mid-Dose	High-Dose
1	51.47	21.94	18.84	80.63	238.68
2	3.85	20.19	161.12	141.14	111.34
3	51.75	11.25	147.24	199.12	50.52
4	444.08	12.50	17.50	16.00	155.58
5	100.53	48.02	186.28	128.04	33.89
6	87.98	97.23	39.61	53.91	115.12
7	5.36	19.75	451.20		20.97
N	7	7	7	6	7
Mean	106.431	32.983	145.970	103.140	103.729
SD	153.368	30.839	152.206	66.030	77.078
Geom. Mean	41.608	24.784	82.512	79.384	77.694
Geom. SD	5.357	2.148	3.510	2.479	2.404
p value	-	0.4077	0.2760	0.3225	0.3196

Analyzed by ANOVA followed by Dunnett's test. Sponsor also did statistical tests on the geometric means. There were no significant effects.

In female mice given flibanserin, there were no effects on prolactin levels at either 14 or 34 wks at doses up to 1200 mg/kg or approximately 10 times the human exposure.

As quoted by sponsor

Prolactin levels measured in Drug Weeks 14 and 34 did not show any evidence of drug-related differences as compared to vehicle Control animals, nor were any dose-related changes observed. In Drug Week 34, estrus stage as evaluated by vaginal smear showed no correlation to prolactin levels or flibanserin dose level.

Summary: TK study with flibanserin gave exposure multiples in female mice of approximately 0.3, 1, 3 and 10 times the human exposure in humans taking 100 mg. The doses had no effect on plasma prolactin levels and cannot explain the increase in mammary tumors seen in mice given flibanserin

Study title: Four week oral (gavage) immunotoxicity study in female rats

Key study findings:

Study no.: 04B105

Volume #, and page #: Electronic

Conducting laboratory and location: Boehringer Ingelheim Pharma, Biberach/Riss, Germany

Date of study initiation: May, 2004

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BIMT 17 BS, batch 99111078M, 99.4% pure

Formulation/vehicle: 0.5% methylcellulose

Methods

Doses: 0, 20, 100, 250 mg/kg/day

Study design: Flibanserin was administered daily for at least 28 days by oral gavage to groups of 10 female Chbb. (b)(4) rats (gps 1-4). A fifth group of 10 females was gavaged with 25 mg/kg cyclosporin A. An additional 10 animals received either 0 or 250 mg/kg flibanserin for 28 days and were kept for a 4 week recovery period.

Group No.	Daily dose of BIMT 17 BS [mg/kg]	Females	
		Main study	Recovery
1	0 (vehicle)	151-160	161-170
2	20	251-260	-
3	100	351-360	-
4	Day 1: 400 Day 2- 28: 250	451-460	461-470
5	Cyclosporin A 25 mg/kg	551-560	-

High dose was reduced due to unexpected severe catalepsy in individual animals and inter-individual aggressiveness in all animals at 400 mg/kg, the HD was reduced from day 2 on to 250 mg/kg. Neither the catalepsy nor the aggressiveness had been seen in other studies at 400 mg/kg. Apparently, the aggressiveness was due to a combination of flibanserin treatment and group housing. On day three, animals were housed in individual cages.

Results:

Semiquantitative comparison of cell markers in Cyclosporin-A treated animals versus control

Cell marker	CD 2	CD 3	CD 8	CD 45RA
Lymphocyte subset	T-subset	T-subset	T-subset	B-subset
Thymus				
Cortex	unchanged	unchanged	unchanged	unchanged
Medulla	reduced	reduced	reduced	reduced*
Spleen				
Follicle	unchanged	unchanged	unchanged	unchanged
PALS	reduced	reduced	reduced	reduced*
Marginal zone	unchanged	unchanged	unchanged	unchanged
Red pulp	unchanged	unchanged	unchanged	unchanged
Lymph node, mesenterial				
Follicle	unchanged	unchanged	unchanged	unchanged
Paracortex	reduced	reduced	reduced	reduced*
Sinusoidal lymphocytes	unchanged	unchanged	unchanged	unchanged

unchanged: Similar or equal to Control / No noteworthy difference when compared to Control

reduced: Reduced volume of the respective anatomical zone as a consequence of the reduced number of positive staining lymphocytes. Note: the remaining lymphocytes may stain positive.

reduced* Note: there are generally only a few or no B-lymphocytes within the respective anatomical zone.

Results of flow cytometry of peripheral blood cells (means)

Blood, spleen and thymus cells were placed into flow cytometry tubes containing combinations of antibodies to rat leukocyte cell surface proteins. These antibodies had been conjugated to different fluorochrome dyes to allow detection and discrimination of the different cell populations.

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.97	99.98	99.95	99.99*	99.96	100.00	100.00
T-lymphocytes (CD45 ⁺ CD3 ⁺ cells) (% of leukocytes)	44.15	43.75	42.64	42.32	28.36*	50.26	48.16
T-helper-lymphocytes (CD3 ⁺ CD4 ⁺ cells) (% of T-lymphocytes)	83.80	83.84	82.87	82.12	80.41	80.70	79.58
Double positive T-lymphocytes (CD3 ⁺ CD4 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	1.54	0.70*	1.00	1.01	0.66*	2.16	2.83
Cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	16.86	15.79	17.34	18.01	18.90	20.60	22.28
B-lymphocytes (CD45 ⁺ CD45RA ⁺ cells) (% of leukocytes)	45.60	45.93	47.30	46.47	58.73*	35.78	33.54
Monocytes (CD3 ⁺ CD4 ⁺ cells) (% of analysed cells)	3.59	3.15	2.81*	2.88*	6.14*	9.04	11.58
Total Natural Killer Cells (CD45 ⁺ CD161 ⁺ cells) (% of leukocytes)	4.80	4.11	3.97	4.16	5.36	5.51	6.47
Ratio of T-lymphocytes (CD3 ⁺) to B-lymphocytes (CD45RA ⁺) (calculated)	1.00	1.04	0.96	0.96	0.50*	1.46	1.51
Ratio of T-helper-lymphocytes (CD3 ⁺ CD4 ⁺) to cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺) (calculated)	5.22	5.58	5.15	4.72	4.53	4.08	3.63

* Statistically significant differences (p<0.05) versus Control

Cyclosporine A treatment tended to decrease T-cells and increase B-cells in peripheral blood. There was an isolated effect of flibanserin treatment to decrease monocytes in the MD and HD.

Results of flow cytometry of spleen cells (immune cell activity)

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.97	99.98	99.99*	99.99*	99.98*	100.00	100.00
T-lymphocytes (CD45 ⁺ CD3 ⁺ cells) (% of leukocytes)	44.67	44.01	45.11	44.73	37.95	45.47	45.58
T-helper-lymphocytes (CD3 ⁺ CD4 ⁺ cells) (% of T-lymphocytes)	67.05	67.66	66.83	68.68	60.48	67.77	73.15*
Double positive T-lymphocytes (CD3 ⁺ CD4 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	0.87	0.95	0.91	1.07	0.87	1.06	1.23
Cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺ cells) (% of T-lymphocytes)	28.38	28.92	30.98	28.88	35.69*	29.52	24.31
B-lymphocytes (CD45 ⁺ CD45RA ⁺ cells) (% of leukocytes)	44.62	43.45	44.10	43.76	46.60	27.17	31.37*
Monocytes (CD3 ⁺ CD4 ⁺ cells) (% of analysed cells)	4.18	5.30	4.92	5.77	6.93*	12.81	12.26
Total Natural Killer Cells (CD45 ⁺ CD161 ⁺ cells) (% of leukocytes)	6.50	6.45	6.75	6.43	8.29	16.03	11.94*
Ratio of T-lymphocytes (CD3 ⁺) to B-lymphocytes (CD45RA ⁺) (calculated)	1.06	1.02	1.11	1.14	0.84	1.73	1.49
Ratio of T-helper-lymphocytes (CD3 ⁺ CD4 ⁺) to cytotoxic T-lymphocytes (CD3 ⁺ CD8 ⁺) (calculated)	2.54	2.43	2.41	2.53	1.72*	2.38	3.14

* Statistically significant differences (p<0.05) versus Control

Cyclosporin administration resulted in a decrease of both the ratio of T to B lymphocytes and the ratio of T-helper to cytotoxic T-lymphocytes. There was a relative increase in both monocytes and natural killer cells in these animals.

Results of flow cytometry of thymus cells

Parameter	Daily dose [mg/kg]						
	Main Study					Recovery	
	BIMT 17 BS				Cyclosporine A	BIMT 17 BS	
	0 (Control)	20	100	400/250	25	0 (Control)	400/250
Number of thymus cells ($\times 10^{-7}$)	27.10	25.57	30.87	30.80	23.59	25.35	30.88
Leukocytes (CD45LCA ⁺ cells) (% of analysed cells)	99.51	99.73	99.82	99.30	99.80	99.56	99.64
CD3 ⁺ thymus cells (% of thymus cells)	21.76	18.14	20.91	18.61	6.97*	14.39	20.13
CD4 ⁺ thymus cells (% of analysed cells)	95.81	95.29	94.69*	94.94	95.46	96.66	96.92
CD8 ⁺ thymus cells (% of analysed cells)	91.93	92.59	91.79	92.60	98.45*	95.83	97.06
CD4 ⁺ CD8 ⁻ thymus cells (% of analysed cells)	6.79	5.96	6.66	5.97	0.72*	3.34	2.48
CD4 ⁺ CD8 ⁺ thymus cells (% of analysed cells)	3.03	3.39	3.90*	3.77*	3.81	2.60	2.72
CD4 ⁺ CD8 ⁺ thymus cells (% of analysed cells)	88.90	89.20	87.90	88.82	94.64*	93.23	94.35
CD4 ⁺ CD8 ⁻ thymus cells (% of analysed cells)	1.28	1.45	1.55	1.44	0.83*	0.83	0.46*
CD3 ⁺ CD4 ⁺ CD8 ⁻ thymus cells (% of CD3 ⁺ cells)	26.50	28.59	28.93	27.52	3.23*	19.03	9.37
CD3 ⁺ CD4 ⁺ CD8 ⁺ thymus cells (% of CD3 ⁺ cells)	7.03	5.91	7.48	8.18	5.30	5.93	4.87
CD3 ⁺ CD4 ⁺ CD8 ⁺ thymus cells (% of CD3 ⁺ cells)	63.35	62.72	60.99	61.49	89.57*	73.44	85.08*
CD3 ⁺ CD4 ⁺ CD8 ⁻ thymus cells (% of CD3 ⁺ cells)	3.12	2.79	2.60	2.81	1.90*	1.61	0.69*
Ratio of CD4 ⁺ to CD8 ⁺ thymus cells (calculated)	1.04	1.03*	1.03	1.03	0.97*	1.01	1.00*

* Statistically significant differences ($p < 0.05$) versus Control

Cyclosporine treatment reduced the number of isolated thymocytes and shifted the thymocyte subsets. There was a reduction in more mature CD3⁺ and CD4⁺CD8⁻ thymocytes whereas the relative number of immature double positive CD4⁺CD8⁺ was increased. As a consequence, the relative number of mature CD3⁺CD4⁺CD8⁻ cells was markedly reduced. There was a small reduction in mature CD3⁺CD4⁺CD8⁺ thymocytes and the relative number of CD3⁺CD4⁺CD8⁺ thymocytes increased.

Natural Killer Cell activity of spleen cells

Daily Dose [mg/kg]	Mean Specific Release [%] at various effector to target cell ratios					
	ratio 200:1	ratio 100:1	ratio 50:1	ratio 25:1	ratio 12:1	ratio 6:1
Main study 0 (Control)	9.2	6.5	4.9	3.1	1.6	1.2
Main study 20 BIMT 17 BS	11.6	8.3	6.1	3.7	2.0	1.3
Main study 100 BIMT 17 BS	14.3*	10.3*	7.1	4.5	2.4	1.5
Main study 400/250 BIMT 17 BS	14.6*	10.5*	7.4	4.2	2.0	1.2
Main study 25 Cyclosporine A	25.7*	18.9*	12.7*	7.6*	4.1	2.6
Recovery: 0 (Control)	27.3	22.2	15.7	9.7	5.5	3.0
Recovery: 400/250 BIMT 17 BS	36.7*	30.3	20.9	12.6	7.0	4.3

* Statistically significant differences ($p < 0.05$) versus Control

In the main study, animals treated with flibanserin had an increased killing of YAC-1 target cells compared to controls at both 100 and 250 mg/kg. At the higher dose, this increase was still seen after the recovery period. Treatment with Cyclosporin-A resulted in a pronounced increase in spleen cell NKC activity along with an increased phenotypic presence of NKC's in the spleen.

The sponsor believes that the increase activity with HD flibanserin was due to the abnormally low control activity level. Hard to say although the NKC activity of the control for the recovery group was considerably higher. However, if control activity was higher, then the Cyclosporine A activity would not have shown an increase.

I'm no expert on immunotoxicity studies but it seems clear that cyclosporin A had activity in altering the numbers of T and B cells in blood, spleen and thymus. In contrast, flibanserin treatment clearly had no effects on immune cell numbers or ratios. However, flibanserin treatment at both 100 and 250 mg/kg did increase natural killer cell activity. Whether this was due to abnormally low activity in controls is not clear.

Overall, it seems that the effect of flibanserin administration on immune activity is minimal at most.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Flibanserin is a 5-HT_{1A} agonist and 5-HT_{2A} antagonist that is intended to treat women with hypoactive sexual desire disorder (HSSD). In studies in mice, chronic flibanserin administration decreased serotonin levels in the median prefrontal cortex and the nucleus accumbens shell while increasing norepinephrine levels. Effects on dopamine appear mixed.

Flibanserin had a number of neurological effects, some severe at high doses but not unexpected for a centrally acting drug. In rats, flibanserin produced mild elevations of

blood pressure and heart rate but had no effect on the ECG rhythm or waveform morphology. There were no respiratory effects in rats.

Flibanserin is metabolized extensively but is considered the active moiety since most of the metabolites are pharmacologically inactive and only two active metabolites cross the blood brain barrier, and neither are near the concentration of flibanserin.

In chronic toxicity studies in rats and dogs, flibanserin produced clear clinical signs of discomfort at high doses (exposures 28 times higher in rats and 100 times higher in dogs than human exposure). In dogs there were dose-related ophthalmologic and histopathologic changes of the eyes characterized by discrete, focal, smoky opacity or by focal thickening of the corneal epithelium. This effect was also seen in a shorter, 13 week study in dogs but was not seen in any study in rats.

Also in dogs, flibanserin produced fatty changes in the liver of males and females after 26 and 52 weeks. This effect seemed both dose and duration related with an increase in severity and numbers of dogs affected. Hepatocellular fatty changes were also seen in the 13 and 26 week rat toxicity studies.

There was an increase in fatty accumulation in the myocardium of dogs of both sexes without an increase in severity or number of dogs affected from 26 to 52 weeks. This fat deposition occurred in both the MD and HD with the no effect level being 3 mg/kg or approximately 0.6 times the human exposure. The mechanism for the fat deposition is unknown but it did not produce measurable changes in cardiac electrical patterns measured throughout the study. Significantly, there was no increase in cardiac fat deposition in rats at any dose. There were fatty changes in the livers of both rats and dogs.

Because of the ophthalmologic findings in dogs, the Sponsor was asked to include ophthalmologic endpoints in a clinical trial. In A 24 week, randomized, double blind placebo controlled trial with an open-label extension, exams were performed at screening and end of treatment and included the best corrected distance visual acuity, tonometry (intraocular pressure measurement) and with pupils dilated, slit lamp evaluation of the anterior segment, including the cornea and lens. There were no increased findings or corneal abnormalities with flibanserin treatment.

Flibanserin was not mutagenic in vitro in bacteria or Chinese hamster ovary cells but was positive in the in vitro human lymphocyte assay. It was negative in the in vivo rat micronucleus assay and the Comet assay for DNA damage. Based on the weight of evidence, I consider flibanserin to be non-genotoxic.

When administered orally to mice and rats for two years, flibanserin increased the incidence of liver tumors. In male mice there was a significant increase in hepatocellular carcinomas. In females, there was an increase in the incidence of hepatocellular carcinomas and combined carcinomas and adenomas in the three higher dosed groups that was significant pairwise and for trend at the high dose group. In rats, there was a modest increase in the incidence of hepatocellular carcinomas in males but not in females

(significant for trend against one control group only). Flibanserin induced centrilobular hepatocellular hypertrophy probably by inducing hepatic cytochrome P-450 enzymes. Sponsor believes that flibanserin is acting like phenobarbital in promoting liver tumors. Because flibanserin is an enzyme inducer in rodents and is essentially non-genotoxic, this seems like a reasonable explanation.

In the mammary gland of female mice, there was a clear dose-related increase in adenocarcinomas and malignant adenoacanthomas. There was also a statistically significant increase in the incidence of metastasis of the primary tumor to lung and bronchial lymph nodes. This effect was not seen in rats.

In reproduction studies in rats and rabbits, flibanserin administration did affect the duration of the estrus cycle in rats but had no effect on female fertility as measured by copulation index, fertility index or gestation index. When given during the period of organogenesis, flibanserin produced some visceral and skeletal variations in rats at the 400 mg/kg dose (approximately 42 times human exposure) and there were different malformations in pups from all treated groups without a discernable pattern. There were resorptions in rabbits treated with 80 mg/kg (26 times human exposure) but there was no increase in fetal variations or malformations. In a pre-and postnatal development study in rats, flibanserin produced maternal toxicity in all groups with a significant reduction in number of implantations and newborns from dams treated with the HD (200 mg/kg or roughly 30 times human exposure). Dams treated with the HD showed poor maternal care leading to failure to thrive and high mortality. There were some development delays in these pups but fertility and the F₂ generation seemed unaffected.

In general, flibanserin administration produced no clear signal for adverse effects on fetal development. Although almost all malformations occurred in fetuses from treated rats they affected different organ systems without an obvious common mechanism. In both the embryofetal and the prenatal and postnatal development study, flibanserin administration resulted in pups with anophthalmia. In the rat embryofetal development study, two pups from one litter from the HD had anophthalmia and in the postnatal study, there were two affected fetuses from the high dose group (drug blood levels not determined but probably around 30 times human exposure extrapolating roughly from the rat embryofetal development toxicokinetic data). There was one fetus with hydrocephalus in both treated rats (42 times human exposure) and rabbits (8 times human exposure). Considering the different types of malformations, lack of dose response and the fairly high drug exposure levels in which they occurred, the animal data do not indicate to me that the offspring are at significant risk if flibanserin is inadvertently taken by a pregnant woman.

In special toxicology studies, flibanserin increased natural killer cell activity but had no other effects on the rat immune system and did not seem to produce self-administration behavior in rats.

In general, flibanserin produced few significant toxicological effects in rats or dogs. In reproduction studies, flibanserin did not affect fertility and was not obviously teratogenic.

In carcinogenicity studies, flibanserin produced malignant mammary tumors in mice but not rats. I recommend that flibanserin be contraindicated in women with an increased risk of breast cancer and that women taking flibanserin be followed post-approval, if possible.

Unresolved toxicology issues (if any): none

Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-22526

ORIG-1

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FLIBANSERIN

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ALEXANDER W JORDAN
07/07/2010