

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

022529Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

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

NDA: 22-529

Agency receipt date: 12/27/2011

Drug: Lorcaserin HCl

Sponsor: Arena Pharmaceuticals

Indication: obesity

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments:

In the first review of this NDA, the pharmacology/toxicology reviewer and supervisor recommended that lorcaserin not be approved for the proposed indication. This recommendation was based on the finding of drug-related tumors in a 2-year rat bioassay. The applicant has provided additional analyses of the data in the 2-year rat bioassay and other information addressing the risk of carcinogenicity in humans from lorcaserin exposure at clinical doses.

Discussion:

The first review of the 2-year rat carcinogenicity data concluded that the following tumors appeared drug-related in male rats: astrocytoma, hepatocellular adenoma and carcinoma combined, mammary adenocarcinoma and fibroadenoma combined, skin subcutis fibroma, skin squamous carcinoma, schwannoma, and thyroid follicular cell adenoma. Mammary adenocarcinomas and fibroadenomas appeared to be drug-related in female rats. Because of apparent difficulties in distinguishing adenocarcinomas and fibroadenomas in the initial report, CDER combined these tumors for analysis. This resulted in an inability to determine an exposure at which the mammary adenocarcinomas were not statistically significantly increased.

The applicant submitted a re-adjudication of the mammary tumors by a five member pathology working group. This group was able to distinguish fibroadenomas from adenocarcinomas. A reassessment of these tumors by CDER showed that the mammary fibroadenomas were still significantly elevated at all doses in female rats, but adenocarcinomas were only significantly elevated at the high dose of 100 mg/kg. This dose produces an AUC in rats that is 82 times higher than the human AUC.

Brain astrocytomas in male rats were increased in groups treated with the mid and high doses (30 and 100 mg/kg). Originally, an accurate estimate of brain exposure in humans was not available. The applicant subsequently submitted information on cerebrospinal fluid levels of lorcaserin from mice, rats, monkeys and humans. These data indicated that cerebrospinal fluid levels could be used to reasonably predict brain levels. Based on these data, the AUC in the rat brain at 30 mg/kg appears to be over 300 times higher than the likely AUC in human brain at the clinical dose.

The other tumors observed in male rats occurred at AUC values that were 5 to 55 times higher than the human AUC.

The applicant also provided some new pharmacology data examining the selectivity of lorcaserin for the 5HT2 receptors (A, B and C). These data show a greater selectivity of lorcaserin for the 5HT2C receptor than for 5HT2A and B. Drug levels achieved in humans appear unlikely to significantly activate the 5HT2A and B receptors. This suggests a reduced concern for cardiac valvulopathy because this toxicity is believed to be primarily associated with 5HT2B activation.

Conclusions:

It is noted that the increase in fibroadenoma in female rats occurred at all doses levels compared to control, although this is a common tumor in rats. Women taking lorcaserin may be at an increased risk for fibroadenoma.

The pharmacology/toxicology reviewer and supervisor have reviewed the nonclinical findings in detail in their respective reviews. They have concluded that the newly submitted information is adequate to support approval of lorcaserin for the indication listed above from a pharmacology/toxicology perspective.

I agree that the pharmacology/toxicology information are adequate to support approval of lorcaserin.

Although no additional nonclinical information is recommended before approval for use of lorcaserin in adults, the pharmacology/toxicology reviewer and supervisor have recommended that a juvenile animal study be conducted to support development of lorcaserin in pediatric patients. This seems to be a reasonable recommendation given the effects observed in nonclinical studies thus far (e.g., mammary effects).

I have provided comments on nonclinical portions of the labeling to the division.

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/s/

PAUL C BROWN
06/15/2012



**Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products**

NDA SECONDARY REVIEW MEMO

Date:	30 May 2012
NDA #	22-529, Complete Response Submission
Sponsor:	Arena Pharmaceuticals
Drug:	Lorcaserin
Primary Reviewer:	Fred Alavi, Ph.D.
Secondary Reviewer:	Todd Bourcier, Ph.D.

Arena Pharmaceuticals submitted a response to the CR letter issued by the Division in October 2010 for NDA 22529, the marketing application for lorcaserin HCl, proposed tradename Belviq, as a treatment for obesity. The Division's CR letter raised issues regarding the characterization and clinical relevance of the multiple tumor findings in rodents exposed to lorcaserin. Although not an issue in the CR letter, discrepant receptor pharmacology data in the NDA raised uncertainty regarding the receptor selectivity of lorcaserin, which is pertinent to interpretation of the valvulopathy data.

Dr. Fred Alavi (the primary pharm/tox reviewer) and I recommended against approval in the first review cycle. The CR letter issued by the Agency and prior reviews written by Dr. Alavi and myself in the first review cycle articulated our reasons for recommending against approval at that time. The data provided in the sponsor's resubmission sufficiently clarifies the rodent carcinogenicity data to enable re-assessment of clinical risk. As discussed below, based on the data provided to us by the sponsor, Dr. Alavi and I agree that the nonclinical data can now support marketing approval of lorcaserin.

Rodent carcinogenicity

Lorcaserin was identified as a non-genotoxic carcinogen that induces multiple tumor types in rats. Among the multiple tumor types observed, the occurrence of mammary and CNS tumors were identified in the CR letter as being of most concern because no safety margin was identified for the former and the safety margin was uncertain for the latter. Imbalanced diagnostic changes of benign and malignant mammary masses imparted little confidence that the tumor incidence data reported in the original NDA was reliable. Also, inadequate information regarding the tumorigenic mode of action was submitted in the first cycle, which is critical for evaluating human risk when safety margins are absent or uncertain.

Mammary Tumors

All mammary masses from female rats were re-adjudicated by a five-member pathology working group (PWG). The PWG apparently had little difficulty in distinguishing benign from malignant masses which is reflected in the high level (> 92%) of diagnostic consensus reached in the blinded slide evaluation. When asked at the 2012 Advisory Committee how so many diagnostic changes could have been made in the original study report, the PWG chairman Dr. Kenneth Schafer could not offer any insight. The re-adjudicated data show that lorcaserin increased the incidence, tumor onset and multiplicity, and lethality of mammary adenocarcinoma with a safety margin of 24-fold to the clinical dose. Lorcaserin also increased the incidence, tumor onset and multiplicity, and lethality of benign fibroadenoma at all doses without a safety margin (\leq 7-fold) to the clinical dose. Although not addressed by the PWG, numerical increases in mammary adenocarcinoma and fibroadenoma occurred in male rats, which is an uncommon finding, with a safety margin of 5x (tumors at 17x clinical dose).

How lorcaserin results in mammary tumors remains uncertain, and may eventually be found to include mechanisms beyond changes in prolactin as postulated by the sponsor. The mechanistic data weakly implicates prolactin as the intermediary factor. Specifically, it's questionable that the minimal changes in prolactin reported with lorcaserin are sufficient to result in the observed robust tumor response in female and male rats. Plausibility of this mode of action relies more on the fact that robust prolactin elevation is known to induce mammary tumors in rats, that the Sprague Dawley strain is very sensitive to prolactin, and that no pattern of change was observed for other hormones linked to mammary tumors. The role of prolactin in human breast tumors remains unsettled, but it is not a 'rodent-specific' phenomenon as stated by the sponsor^{1,2}. Of note, the prolactin response to lorcaserin in female rats is recapitulated in human subjects, with a small acute rise occurring after a single dose of drug and equivocal increases observed thereafter. Additional nonclinical studies that further pursue prolactin as the tumorigenic mechanism to the exclusion of alternative possibilities would likely not provide useful additional information. The relevance of the fibroadenoma response in rats at clinically relevant doses of lorcaserin remains uncertain but may reflect an increased risk of developing benign breast masses in patients chronically exposed to lorcaserin. This assessment will be appropriately conveyed in the carcinogenicity section of the drug label.

Astrocytoma

The additional clinical study conducted by the sponsor showed that the level of lorcaserin in the cerebrospinal fluid (CSF) of humans is much lower than anticipated based on studies in monkeys and rodents or from the physiochemical characteristics of lorcaserin, a highly soluble and permeable drug substance. Reasons for the unexpectedly low level of lorcaserin in human CSF were not addressed. Based on a relatively constant relationship of CSF to (total) brain levels of drug as measured in monkeys and rodents, it is estimated that exposure in human brain tissue is 1.7-fold higher than plasma drug levels. This data substantially changed the safety margin for brain neoplasms to 70-fold the clinical dose compared to 5- or 14-fold from the original NDA. Generally, a safety margin in excess of 25-fold the clinical dose for a rodent carcinogen would not be considered likely to reflect a relevant risk to humans. This re-analysis is predicated on the accuracy of the clinical data obtained from the nine subjects and on the reasonable

¹ Tworoger SS & Hankinson SE (2008) Prolactin and breast cancer etiology: an epidemiologic perspective. *J Mamm Gland Biol Neoplasia*. 13(1):41-53

² Harvey PW (2012) Prolactin is tumorigenic to human breast: dispelling the myth that prolactin-induced mammary tumors are rodent-specific. *J Appl Toxicol*. 32:1-9

presumption^{3,4} that the CSF/brain partitioning of lorcaserin is similar in rodents, monkeys, and humans.

Other tumor types

It is important to consider that lorcaserin also increased the incidence of benign subcutis fibroma, squamous carcinoma of the skin, and malignant schwannoma in male rats at the mid- and high doses. The increase in benign skin fibroma may have occurred at all doses if one considers the increase in incidence from 4.6% in control to 11% at the low dose as drug-related.

It is notable for a non-genotoxic compound to result in this array of tumor types affecting multiple tissues. Tumors of the peripheral nervous system and skin/subcutis are not shared by marketed centrally acting dopaminergic or serotonergic drugs or by current obesity medications. No studies or credible explanation was provided to address the spectrum of tumors induced by lorcaserin or the mechanism by which lorcaserin increased these tumors, so risk assessment must be based on the difference in exposure between rats and the clinical dose in humans. These tumors occurred at exposure 17-fold higher than the clinical dose, with a slim but perhaps tolerable safety margin of 5x (i.e., tumors were not observed in rats at exposure 5-fold higher than the clinical dose). Of some assurance, no drug-related tumors were observed in mice at a similar safety margin (5x-7x). The clinical relevance of this tumor response in rats remains uncertain and will be appropriately conveyed in the carcinogenicity section of the drug label.

Receptor Pharmacology

The sponsor's resubmission included additional studies intended to clarify discrepancies in the receptor potency data reported previously. The pharmacology data included in the original NDA made 'off-target' activation of 5HT2A and 2B either plausible or unlikely, depending on which dataset one considered, and had implications for potential adverse neurological and cardiac valve toxicity. Data in the resubmitted NDA could be considered the most reliable estimate of functional potency at each of the serotonin 5HT2 receptor subtypes based on the general concordance of receptor expression levels between relevant human tissues and the cells used in the *in vitro* potency assays. Clinically relevant drug concentrations of lorcaserin were shown to fall comfortably below the EC50 for activation of the 2A and 2B receptors. Most conservatively estimated, doses in excess of 40mg might be expected to result in activation of 2A in the CNS compartment, and doses in excess of 200mg might activate 2B in the peripheral compartment. It was questioned at the 2012 Advisory Committee meeting the degree to which one could rely on receptor selectivity data in predicting the risk of clinical valvulopathy. If one assumes, with notable justification^{5,6}, that activation of 5HT2B is a key event in increasing the risk of valvulopathy, then demonstrating that the plasma drug concentration falls considerably below a level predicted to result in activation of that receptor reduces the level of concern. This is supported by the observation that clinical drug levels (as a free fraction) of the known valvulopathogens (+) norfenfluramine and pergolide are twice as high as the EC50 for activation

³ Lin JH (2008) CSF as a surrogate for assessing CNS exposure: an industrial perspective. *Curr Drug Metab.* 9:46-59

⁴ Watson J et al (2009) Receptor occupancy and brain free fraction. *Drug Metab & Dispos.* 37(4):753-760.

⁵ Hutcheson JD et al (2011) Serotonin receptors and heart valve disease- it was meant 2B. *Pharmacology & Therapeutics.* 132:146-157.

⁶ Huang XP et al (2009) Parallel functional activity profiling reveals valvulopathogens are potent 5HT2B receptor agonists: implications for drug safety assessment. *Molec Pharmacol.* 76:710-722.

of 5HT2B⁷. Conversely, and there are limitations to this analysis, lorcaserin's potency at 5HT2B is similar to other 2B agonists (notably guanfacine and ropinirole) that are not known to be associated with clinical valvulopathy⁴. Unfortunately, limitations in the ability to screen for drug-induced valvulopathy in animals render *in vivo* assessments less than definitive, so short of clinical studies, 5HT2 receptor pharmacology data is currently the 'state of the art' for identifying potential valvulopathogens. Additional nonclinical studies are unlikely to provide insight relevant to neurological and cardiac risks of long-term use of lorcaserin that cannot be gained from well-designed clinical studies in the obese patient population.

Additional Regulatory Recommendations

The current nonclinical data are sufficient to support approval without additional nonclinical post-marketing studies. The tumor risk raised by the nonclinical data (specifically mammary masses but perhaps should extend to all malignancies) could be considered as an adverse event of special interest in subsequent long-term clinical studies, though modalities for monitoring may prove difficult.

The receptor pharmacology data supports the view but should not be interpreted as definitive evidence that the persistent clinical imbalance in FDA-defined valvulopathy reflects ascertainment bias in the studied patient population rather than a fenfluramine-type response. The published report that 5HT2B receptors are over-expressed in the heart of congestive heart failure patients⁸ implies that this and potentially other heart diseases may be associated with an as-yet uncharacterized increase in cardiac 5HT2B expression, and therefore may be more sensitive to activation by lorcaserin. Echocardiographic monitoring is prudent in clinical studies that enroll patients with underlying cardiovascular disease to define the risk of valvulopathy in this higher-risk population.

Consistent with other obesity drugs, lorcaserin should be contraindicated during pregnancy (category X) based on clinical considerations of weight loss in pregnant women. The reproductive toxicology studies did not identify a teratogenic hazard for lorcaserin.

A toxicology study in juvenile rats will be required to support long-term clinical studies in the preadolescent and adolescent pediatric populations. Endpoints specific to hormonal disruption, maturation, and neurological development should be emphasized.

⁷ FDA Advisory Committee Briefing Document (2010 EMDAC for lorcaserin)

⁸ Jaffe F et al (2009) Serotonin and angiotensin receptors in cardiac fibroblasts coregulate adrenergic-dependent cardiac hypertrophy. *Cir Res* 104:113-123.

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/s/

TODD M BOURCIER

05/30/2012

Concur with Dr Alavi; nonclinical data support AP

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 22-529
Supporting document/s: electronic NDA
Applicant's letter date: Dec 22, 2011
CDER stamp date: Dec 23-2011
Product: Lorcaserin HCl (BELVIQ[®], 10 mg BID)
Indication: Treatment of obesity
Applicant: Arena Pharmaceuticals
Review Division: DMEP
Reviewer: Fred Alavi, Ph.D.
Supervisor/Team Leader: Todd Bourcier, Ph.D.
Division Director: Mary Parks, MD
Project Manager: Patricia Madara

Disclaimer

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: Recommended for approval

1.1.2 Additional Non-Clinical Recommendations: Yes

The Division recommends a 3-month juvenile male and female rat study prior to initiation of the multiple dose pediatric study. The study should explore the effect of lorcaserin (at 1 to 20x the clinical dose) on sexual development and performance, neurological development, plasma prolactin and changes in mammary tissue differentiation in juvenile rats aged PND day 14.

1.1.3 Labeling- **Recommended Draft labeling** (subject to change)

8.1 Pregnancy

Pregnancy Category X.

Risk Summary

BELVIQ is contraindicated during pregnancy, because weight loss offers no potential benefit to a pregnant woman and may result in fetal harm. Maternal exposure to lorcaserin in late pregnancy in rats resulted in lower body weight in offspring which persisted to adulthood. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard of maternal weight loss to the fetus.

Clinical Considerations

A minimum weight gain, and no weight loss, is currently recommended for all pregnant women, including those who are already overweight or obese, due to the obligatory weight gain that occurs in maternal tissues during pregnancy.

Animal Data

Reproduction studies were performed in pregnant rats and rabbits that were administered lorcaserin during the period of embryofetal organogenesis. Plasma exposures up to 44 and 19 times human exposure in rats and rabbits, respectively, did not reveal evidence of teratogenicity or embryolethality with lorcaserin hydrochloride.

In a pre- and postnatal development study, maternal rats were dosed from gestation through post-natal day 21 at 5, 15, and 50mg/kg lorcaserin; pups were indirectly exposed in utero and throughout lactation. The highest dose (~44 times human exposure) resulted in stillborns and lower pup viability. All doses lowered pup body weight similarly at birth which persisted to adulthood; however, no developmental abnormalities were observed and reproductive performance was not affected at any dose.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

Mutagenesis

Lorcaserin hydrochloride was not mutagenic in an in vitro bacterial mutation assay (Ames test), was not clastogenic in an in vitro chromosome aberration assay in Chinese hamster ovary cells, and was not genotoxic in an in vivo micronucleus assay in rat bone marrow.

Carcinogenesis

The carcinogenic potential of lorcaserin hydrochloride was assessed in two-year carcinogenicity studies in mice and rats. CD-1 mice received doses of 5, 25 and 50 mg/kg/day. There were no treatment-related increases in the incidence of any tumor in mice at doses that produced plasma exposure in males and females of 8 and 4-times the daily human clinical dose, respectively.

In the rat carcinogenicity study, male and female Sprague-Dawley rats received 10, 30, and 100 mg/kg/day lorcaserin hydrochloride. In females, mammary adenocarcinoma increased at 100mg/kg, which was associated with plasma exposures that were 87-times the daily human clinical dose. The incidence of mammary fibroadenoma was increased in female rats at all doses with no safety margin to the clinical dose. These increases in adenocarcinomas and fibroadenomas may be associated with lorcaserin hydrochloride induced changes in prolactin homeostasis in rats. The relevance of the increased incidence of mammary adenocarcinomas and fibroadenomas in rats to humans is unknown.

In male rats, treatment-related neoplastic changes were observed in the subcutis (fibroadenoma, Schwannoma), the skin (squamous cell carcinoma), mammary gland (adenocarcinoma and fibroadenoma), and the brain (astrocytoma) at greater than or equal to 30 mg/kg (plasma exposure 17-times human clinical dose). At higher exposure, liver adenoma and thyroid follicular cell adenoma were increased. Human brain exposure (AUC_{24h,ss}) to lorcaserin at the clinical dose is estimated to be 70-fold lower than brain exposure in rats at the dose at which no increased incidence of astrocytomas was observed. These neoplastic findings in male rats are of unknown relevance to humans.

Impairment of Fertility

Potential effects on fertility were assessed in Sprague-Dawley rats in which males were dosed with lorcaserin hydrochloride for 4 weeks prior to and through the mating period, and females were dosed for 2 weeks prior to mating and through gestation day 7. Lorcaserin hydrochloride had no effects on fertility in rats at exposures up to 29 times the human clinical dose.

1.2 Brief Discussion of Nonclinical Findings

Lorcaserin is a serotonin receptor subtype 2C (5HT_{2C}) agonist designed to selectively bind to 5HT_{2C} to reduce appetite and body weight. The original lorcaserin NDA application was submitted to the FDA on Dec 22, 2009; therefore, this review covers only the data provided in the resubmission on Dec 23, 2011. The original lorcaserin application was not approved by the agency in part due to significant nonclinical safety signals. Lorcaserin resulted in a significant number of tumors in female (mammary tumors) and male (multi-type & -site) rats. Lorcaserin resulted in a significant number of mammary adenocarcinoma and fibroadenoma in female rats with substantial diagnostic uncertainty and brain astrocytoma in males (among other tumors) with an uncertain safety margin. The first advisory committee (AC) meeting was held on Sept 16, 2010 in part to discuss the 2-year rat carcinogenicity study findings. The committee voted 9 to 5 against approval of lorcaserin. The Agency issued a complete response letter on Oct 22, 2011, requesting additional data to resolve the safety signals observed in the rat study. The resubmission on Dec 23, 2011 aimed to resolve the outstanding nonclinical safety concerns. The resubmission included new receptor pharmacology studies, female rat mammary tumor re-adjudication by the pathology working group (PWG), mechanistic studies describing the role of prolactin in lorcaserin induced mammary tumors and lorcaserin CNS exposure in humans.

The carcinogenicity of lorcaserin was tested in 2-year lifetime exposure studies in CD-1 mice and SD rats. These studies were submitted with the original NDA in 2009. In the 2-year mouse study, lorcaserin resulted in no tumors in CD-1 mice at a maximum dose of 50 mg/kg (4 to 7x the clinical dose of 10 mg BID, based on AUC). The original study started at 100 mg/kg but was subsequently lowered due to increased mortality at this dose (8 to 13x the clinical dose AUC).

In the 2-year carcinogenicity study in SD rats, lorcaserin resulted in multiple tumors in both sexes. A tumor signal was identified early in the rat study that resulted in bimonthly updates requested by the FDA. The diagnosis of mammary tumors in the updates changed over time leading to the conclusion that there was significant diagnostic uncertainty by the CRO in distinguishing mammary adenocarcinoma from fibroadenoma. In the final NDA report, the incidence of mammary adenocarcinoma was increased numerically at 10 and 30 mg/kg and statistically at 100 mg/kg in female SD rats.

Tumors in Female SD Rats	Lorcaserin Dose, mg/kg			
	0	10 (LD)	30 (MD)	100 (HD)
Number of rats /treatment/sex	65	65	65	75
AUC Exposure Multiples	-	7x	24x	82x
Mammary adenocarcinoma	26	21	24	51**
Mammary, Fibroadenoma	24	54**	55**	51**
Lung metastases, secondary to adenocarcinoma	0	1	5	5

** p<0.01, * p<0.05

The incidence of benign mammary fibroadenoma increased significantly at all doses of lorcaserin in female SD rats with no safety margin.

In male rats, lorcaserin increased the incidence of multiple tumors but a primary concern was brain astrocytoma. The safety margin for brain tumors was not assessable due to location of tumor at the site of drug action (brain) and preferential partitioning of lorcaserin to the brain tissue.

Tumors in Male SD rats	Lorcaserin Dose, mg/kg			
	0	10 (LD)	30 (MD)	100 (HD)
Number of rats /treatment/sex	65	65	65	75
AUC Exposure Multiples	-	5x	17x	55x
Brain, astrocytoma #	1	0	4	8**
Mammary, Fibroadenoma	0	1	4	6*
Liver, adenoma	1	1	2	6*
Skin, Squamous cell, Carcinoma	0	0	4	5*
Skin, subcutis, fibroadenoma	3	7	11*	17**
Skin, subcutis, Schwannoma	0	0	1	5*
Thyroid, follicular adenoma	0	5*	4	8**

AUC exposure does not apply to brain astrocytoma due to preferential lorcaserin brain partitioning

** p<0.01, * p<0.05

The sponsor addressed the mammary tumor diagnostic uncertainty in the NDA resubmission (Dec 23, 2011) by establishing a pathology working group (PWG) for re-adjudication of mammary tumors. The safety margin for brain tumors was resolved by new clinical information on partitioning of lorcaserin to the CSF in obese human subjects. Brain lorcaserin exposure estimated from human CSF exposure significantly expanded the safety margin (70x the clinical dose) and thus mitigated the brain tumor safety signal observed in male rats.

Re-adjudication of Mammary Tumors in female SD rats

Mammary tumors in female rats were re-adjudicated in a blinded manner by five independent expert veterinary pathologists chosen by the sponsor. Each pathologist evaluated all available mammary tissue slides and discrepancies were resolved by consensus. The PWG members were able to easily distinguish malignant mammary adenocarcinoma (92.5%) from benign fibroadenoma (97.1%). The high degree of diagnostic certainty expressed by the PWG allowed their results to be considered definitive and final by the agency. With confidence in mammary tumor diagnosis, the incidence of mammary fibroadenoma and adenocarcinoma were analyzed independently.

The PWG identified lower incidence of adenocarcinoma and higher incidence of fibroadenoma in female rats. The numerically higher incidence of adenocarcinoma in the LD and MD female rats observed in the 2009 report was no longer apparent. The incidence of adenocarcinoma was increased statistically only at 100 mg/kg lorcaserin thus providing a 24 fold safety margin to the clinical dose of 10 mg BID based on AUC. With a reassuring 24-fold safety margin for adenocarcinoma, prolactin mode of action studies are not needed. The incidence of fibroadenoma

increased at all doses of lorcaserin with no safety margin (<7x the clinical dose AUC). Based on new mechanistic studies, the Agency considers the role of prolactin as the intermediary hormone in lorcaserin-induced fibroadenoma as plausible but not definitive.

Mammary tumors in female rats before and after re-adjudication by the PWG

Mammary tumors in female rats		Lorcaserin Dose, mg/kg			
		0	10	30	100
AUC Exposure Multiples		-	7x	24x	82x
Adenocarcinoma (Historical range 8.3-37%)	Original	28	34	35	60
	PWG	27	21	24	50 **
Fibroadenoma (Historical range 22-54%)	Original	20	47	53	51
	PWG	23	53 **	55 **	51 **

** p<0.0001

Lorcaserin induced-mammary tumors appeared to be more aggressive. Mammary adenocarcinomas occurred much earlier and at multiple sites at 100 mg/kg lorcaserin while mammary fibroadenomas had an earlier onset and occurred at multiple sites at all doses of lorcaserin compared to control female rats. One measure of the aggressiveness of the adenocarcinoma was the increased incidence of lung metastases in lorcaserin treated rats. The PWG concluded that the lung metastases in the LD female rats was similar to control and increased equivocally in the MD and HD. It should be noted that HD female rats died prematurely which may have reduced the time for mammary adenocarcinomas to metastasize to the lungs. There were no lung metastases in the control even though the incidence of lung adenocarcinoma in the control was equal to or greater than the LD and MD lorcaserin, suggesting that adenocarcinomas in control rats were not as aggressive as those in lorcaserin treated rats

Lung tumors in female rats		Lorcaserin Dose, mg/kg			
		0	10	30	100
Lung metastases, adenocarcinoma origin (Historical range 0-12.5%)	Original	0	2	7	6
	PWG	0	1 (5%)	5 (21%)	5 (10%)

Prolactin Mode of Action

The sponsor hypothesized that lorcaserin-induced mammary tumors were due to increased plasma prolactin. Since prolactin mechanistic studies reviewed in the original 2009 NDA failed to show an increase in prolactin in female rats, the sponsor carried out additional studies up to 3 months in male and female SD rats. In female rats, the highest dose of lorcaserin (100 mg/kg) increased plasma prolactin measured at 20 hrs post dose during the first 10 days of the 90-day study. Lower

doses (10 and 30 mg/kg) had no effect on plasma prolactin measured at 1 or 20 hr post dose. However, lorcaserin treated rats appeared to have spikes in prolactin that were more frequent than in control. The mean 90 day plasma prolactin and pituitary prolactin were also slightly but significantly increased, suggesting that lorcaserin indeed may have had a weak stimulatory effect on pituitary prolactin release. Since there was also a slight increase in mammary lobular differentiation, prolactin role in lorcaserin induced mammary fibroadenoma in female rats is indeed a plausible mechanism. The prolactin intervention studies demonstrated that lorcaserin-induced acute increases in prolactin were secondary to pituitary release. Overall, these studies appear to support a plausible prolactin role in lorcaserin-induced increase in mammary tumors in female rats, but fall short of providing definitive evidence.

Safety Margin for Brain Tumors Based on Human CNS Lorcaserin Exposure

The second tumor of most concern was the increased incidence of a rare brain tumor, astrocytoma in male rats. Since lorcaserin preferentially partitions to the brain in rats (25x plasma) and monkeys (10x the plasma), the safety margin could not be determined for a tumor at the site of drug action without some knowledge of partitioning of lorcaserin to the human brain. In the resubmission, the sponsor provided CSF lorcaserin exposure in humans under steady state conditions. With a relatively stable relationship between brain and CSF levels in mice, rats and monkeys, the brain lorcaserin AUC in obese humans was estimated to be about 1.73 µg.h/ml, significantly less than predicted. Human brain lorcaserin exposure was approximately 1.7x the plasma AUC (1.01 µg.h/ml at 10 mg BID), significantly less than the brain to plasma ratio observed in rats and monkeys. The lower than expected CNS partitioning of lorcaserin in humans significantly expanded the safety margin for brain tumors.

Brain tumors in male rat	Lorcaserin dose, mg/kg			
	0	10	30	100
Astrocytoma (Historical 0 to 5%)	1 (1.5%)	0	4 (6.2%)	8 ** (10.7%)
Brain AUC Exposure Multiples	-	70x	342x	1130x

** p<0.0001

The 70-fold safety margin to the no-effect dose of 10mg/kg lorcaserin for astrocytoma substantially mitigates the clinical risk of this safety finding.

In summary, data provided in the NDA resubmission has resolved the outstanding nonclinical tumor concerns indentified in the rat carcinogenicity study. There was a 24-fold safety margin for mammary adenocarcinoma and 70x safety margin for brain astrocytoma. Although mammary fibroadenoma occurred at all doses with no safety margin, the prolactin mechanistic studies appeared to provide a plausible though not definitive role for prolactin in lorcaserin-induced mammary fibroadenomas in female rats. There was a 5-fold safety margin for other tumors observed in male rats.

2 Drug Information

2.1 Drug: Belviq®

2.1.1 CAS Registry Number: 856681-05-5

2.1.2 Generic Name: Lorcaserin hydrochloride

2.1.3 Code Name: APD356 hemihydrate, AR226173 hydrochloride hemihydrate

2.1.4 Chemical Name:

(*R*)-8-Chloro-1-methyl-2,3,4,5 tetrahydro-1*H*-3-benzazepine hydrochloride hemihydrate

2.1.5 Molecular Formula/Molecular Weight: C₁₁H₁₅Cl₂N.5H₂O, MW (b) (4)

2.1.6 Structure:



2.1.7 Pharmacologic class: Serotonin receptor 2 C (5HT2C) agonist

2.2 Relevant IND/s, NDA/s: IND 69888 (Arena pharmaceuticals), (b) (4) NDA 20344 (dexfenfluramine)

2.3 Clinical Formulation: 10 mg lorcaserin hydrochloride tablets

2.3.1 Drug Formulation

Active ingredient: 10.4 mg of APD356 Hemihydrate (10.4 mg tablets)

Inactive ingredients: Silicified microcrystalline cellulose, hydroxypropyl cellulose, croscarmellose Na, magnesium stearate and (b) (4)

Component	Grade	Function	mg/tablet	%w/w
Core				
Lorcaserin HCl Hemihydrate	Arena	Drug substance	10.4 ^a	10.4
Silicified microcrystalline cellulose ^b	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Hydroxypropyl cellulose	NF			
Croscarmellose sodium	NF			
Magnesium stearate	NF			
(b) (4)	(b) (4)			
(b) (4)	USP			
			Total	

^a Equivalent to 10 mg lorcaserin HCl.

2.3.2 Comments on Novel Excipients: All the excipient are GRAS

2.3.3 Comments on Impurities/Degradants of Concern: There are several minor impurities in the product of which only the (b) (4) exceeded \geq (b) (4). The potential genotoxicity of the impurities were examined using MultiCASE software that evaluates for structural activity relationship of the impurities to the available database. Three of the 12 minor impurities (b) (4) and (b) (4) were found to have the potential for genotoxicity. Since the exposures to these impurities were less than the daily allowance of 1.5 $\mu\text{g}/\text{day}$ under the genotoxic impurities guidance (1.5 $\mu\text{g}/\text{day}$), no specific safety analysis is required and thus deemed to be safe. Residual solvents ((b) (4) (b) (4) in the drug substance were less than the accepted ICH limits.

2.4 Proposed Clinical Population and Dosing Regimen: (b) (4)

2.5 Regulatory Background

Lorcaserin IND was submitted to FDA on May 25, 2004 with some clinical experience in trials carried out in the UK. The rat and mouse carcinogenicity study protocols were submitted on Jan 25 of 2005 and May 23, 2006, respectively. Upon initiation of mouse carcinogenicity study, the unexpected rise in mortality within 16 days administration of 100 mg/kg of lorcaserin, the

sponsor requested dose adjustment to 50 mg/kg. Mid-way (63 weeks) through the rat carcinogenicity study the sponsor submitted a 15-day safety report on May 31, 2007 (#0047), showing a high incidence of mammary tumors in females and brain tumors in male and female rats. At the time of the submission, the sponsor was 8 months into the 2-year clinical study #3182. The Division recommended changes to the consent form to reflect the preliminary data describing higher than normal incidence of mammary tumors and brain tumors in the ongoing rat study. The Division requested bimonthly updates of mammary and brain tumor incidence as histopathology evaluation became available (page 148). In the 3rd bimonthly update on March 10, 2008 (WK 96) with all the HD females necropsied, there was an apparent dose-dependent increase in incidence of malignant mammary tumors (adenocarcinoma) in female rats at all doses. The division met with the sponsor to discuss the mode of action for mammary tumors and the possibility of a clinical hold. The Division allowed the ongoing phase 3 studies to continue since the data from other groups in the rat study were still missing, prolactin was a reasonable explanation of mode of action, and there were no mammary tumors in mice. The Division requested a draft report of the rat and mouse carcinogenicity studies as soon as possible and requested changes to the clinical protocol to include analysis of human serum prolactin. The bimonthly updates continued until the rat study was completed and draft report of the rat study was submitted (Feb 3, 2009). The original NDA was submitted in Dec 22, 2009 with advisory committee meeting held on Sept 16, 2010. The advisory committee voted 9 to 5 against approval of lorcaserin. The division issued complete response on Oct 22, 2010. The End-Of-Review meeting was held on Dec 15, 2010 where studies needed to resolve the nonclinical deficiencies listed in the CR letter were discussed. The sponsor resubmitted the NDA in Dec 23, 2011 with new data to mitigate the mammary tumor diagnostic uncertainty in female rats and uncertain safety margin for brain tumors in male rats. The second advisory committee meeting was held on May 10, 2012 to discuss in part the nonclinical safety concerns. The committee members voted 18 to 4 for approval with one abstention. The Division issued a CR letter on Oct 22, 2010 (Appendix C), listing nonclinical deficiencies that needed to be addressed. The resubmission on Dec 23, 2011 included new receptor potency and selectivity studies, re-adjudication of mammary tumors by PWG, prolactin mode of action studies and human brain lorcaserin exposure data to resolve brain tumor signal.

3 Studies Submitted in response to Complete Response letter:

Pharmacology Studies (reviewed by Dr. Bourcier, Appendix A)

Study#RP-11-001A: The Potency & Efficacy of Lorcaserin-Mediated Calcium and Inositol Phosphate Signaling at Human, Rat and Monkey 5-HT_{2A} Receptors in the Absence of Receptor Reserve Effects

Study#RP-11-001B: The Potency & Efficacy of Lorcaserin-Mediated Calcium and Inositol Phosphate Signaling at Human, Rat and Monkey 5-HT_{2B} Receptors in the Absence of Receptor Reserve Effects

Study#RP-11-001C: The Potency & Efficacy of Lorcaserin-Mediated Calcium and Inositol Phosphate Signaling at Human, Rat and Monkey 5-HT_{2C} Receptors in the Absence of Receptor Reserve Effects

Study#RP-11-002: An Analysis of Lorcaserin Mediated Signaling at the Human 5-HT_{2B} Receptor

Mammary Tumors and Prolactin Mechanistic Studies

Study # DBR-11-001: The Effect of Lorcaserin, Dexfenfluramine and DOM on Behavioral Signs Indicative of 5HT_{2C} and 5HT_{2A} Activation in the Male Rats

Study # DBR-11-002: Three Month Evaluation of Lorcaserin Effects on Prolactin Concentrations and Mammary Gland Histology in Female Sprague-Dawley Rats

Study # DBR-11-003: Effects of Prolactin Receptor Antagonism on Lorcaserin-mediated Changes in Mammary Glands of Female Sprague Dawley Rats

Study #DBR-11-004: Three month evaluation of Lorcaserin effects on prolactin concentrations and mammary gland histology in MALE Sprague Dawley rats

Study # DBR-11-025: Effect of Hypophysectomy on Lorcaserin-mediated Changes in Mammary Glands of Female Sprague Dawley Rats

Study # DBR-11-026: Effect of Bromocriptine on Lorcaserin-induced Increases in Plasma Prolactin

Readjudication of female rat mammary tumors in the 2-year rat carcinogenicity study

Study#TX05071: Fifth Amendment of the Final Report: A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Study#TX05071 (analysis): Time to Detection and Time to Death Analysis for Mammary Tumors in Female Rats

Study#TX11023: Tabular Listing of Tumor Incidence Before and After Re-Adjudication of Individual Slides Prepared From Mammary Gland, Lung, and Subcutaneous Masses from Female Rats in the Lorcaserin 2 Year Carcinogenicity Study
Rat

Study#TX11024: Historical Control Data for the Incidence of Mammary Adenocarcinoma Metastases to the Lung from Sprague Dawley Rats in a 2 Year Carcinogenicity Study

PK-Brain Exposure Studies

Study#MPR-11-001: Evaluation of the Potential Accumulation of APD356 (Lorcaserin) in the Female Rat Central Nervous System (CNS) after Multiple Daily Oral Dosing at 10 mg/kg

Study#MPR-11-007: Estimated Lorcaserin Brain Concentrations in Healthy Obese Human Volunteers and Associated CNS Safety Margin Determination for the Finding of Astrocytoma in the Rat Carcinogenicity Study

Study#MPR-11-017 (analysis): Estimated Lorcaserin Brain Concentrations in Humans using Updated *In-vitro* Functional Activity data at the 5-HT_{2C} Receptor – Brain Safety Margin Calculations

- 3.1 Studies Reviewed:** All studies in the resubmission (see list above)
- 3.2 Studies Not Reviewed:** Pilot nonclinical studies that were not submitted.
- 3.3 Previous Reviews Referenced:** Nonclinical studies reviewed in the original NDA submitted in 2009.

4 Pharmacology

4.1 Primary Pharmacology

Lorcaserin is a new molecular entity designed to selectively activate 5HT_{2C} serotonin receptors in the brain to suppress appetite and result in weight loss. Lorcaserin selectivity and potency has been extensively studied. In the resubmission, the sponsor provided additional receptor pharmacology studies to address the receptor reserve effect (not addressed previously) and potency data in the older studies. These studies reviewed by Dr. Bourcier are posted in the appendix A.

The 2011 data shows lorcaserin to be at least 3- to 5-fold less potent at all three 5HT₂ receptor subtypes than originally reported. Based on the new estimates of receptor potency, maximal concentrations of lorcaserin (free fraction) observed in human plasma and anticipated in human brain tissue is notably lower than the EC₅₀ for activation of 5HT_{2A} and 2B, while remaining above the EC₅₀ for activation of 5HT_{2C} *in vitro*. Plasma concentrations of lorcaserin at the therapeutic dose are therefore expected to remain within the selective range for activation of 5HT_{2C}.

Fold Selectivity of Lorcaserin for 5HT _{2C} receptor activation ¹		
Study data	vs. 5HT _{2A}	vs. 5HT _{2B}
2002/04	15x	90x
2009	8x	45x
2011	14x	61x

¹Fold selectivity determined by dividing the PI hydrolysis EC₅₀ value for 5HT_{2C} by the EC₅₀ value for 5HT_{2A} or 2B from the 2002/04, 2009, and 2011 studies.

The sponsor also demonstrated that based on functional activity, lorcaserin grouped with low-potency 5HT_{2B} agonists that are not known to be associated with clinical valvulopathy. By comparison, compounds known to cause clinical valvulopathy such as nordexfenfluramine and pergolide showed substantially higher 5HT_{2B} receptor potency in these assays.

Potency of lorcaserin in calcium release assays (Data from 2002/04 and 2011 studies)			
Study date	Lorcaserin, EC ₅₀ , nM		
	5HT _{2A}	5HT _{2B}	5HT _{2C}
2002/04	52	350	6
2011	948	1040	146

Potency of lorcaserin in inositol phosphate assays (Data from 2002/04, 2009, 2011 studies)			
Study date	Lorcaserin, EC ₅₀ , nM		
	5HT _{2A}	5HT _{2B}	5HT _{2C}
2002/04	133	811	9
2009	14	82	1.8
2011	553	2380	39

Based on the 2011 assays that eliminated receptor reserve, lorcaserin displayed partial agonist activity at 5HT_{2A} and partial to full agonist activity at 5HT_{2b} and 5HT_{2c}.

Efficacy data for lorcaserin in 5HT ₂ receptor activation assays ¹			
	5HT _{2A}	5HT _{2B}	5HT _{2C}
Percent activity vs. serotonin	25%	67- 151%	81 – 86%

¹Efficacy data from inositol phosphate and calcium release assays conducted in 2011

In summary, the 2011 receptor potency data provides supportive evidence that off-target activation of the 5HT_{2A} or 5HT_{2B} receptors is unlikely at the proposed clinical dose of lorcaserin (10mg bid). This appears to be consistent with neurological and cardiac assessments in animals, which did not identify major toxicities that would be anticipated if 5HT_{2A} and 5HT_{2B} were activated by lorcaserin. However, limitations in neurological assessments and the lack of validated models for drug-induced valvulopathy in animals preclude a definitive prediction that lorcaserin will be devoid of such toxicities should it be approved for marketing.

4.2 Secondary Pharmacology

Lorcaserin binding profile to other receptors, ion channels and transporters in the original NDA is posted to the Appendix B. Lorcaserin was very weak inhibitor of serotonin and dopamine transporter. The two transporters shift intra-synaptic junction levels of the respective neurotransmitters back inside the neurons, thus reducing the activation of the membrane receptors. Inhibition of the dopamine transporter would technically result in higher neuronal dopamine activity.

4.3 Safety Pharmacology

Safety pharmacology studies were reviewed in the first cycle, therefore only a brief summary is provided for reference. Lorcaserin induced a dose-dependent reduction in locomotor activity and increased periods of inactivity in animals. In the 28-day rat toxicology study there were no clinical signs of CNS toxicity at doses up to 50 mg/kg (>24X clinical dose, based on AUC). Some rats at 50 mg/kg were hypersensitive. An incidence of tremor was noted in one male rat at 50 mg/kg. As a CNS active

drug, lorcaserin has the potential to be addictive and cause psychological disturbances in humans; therefore the neurobehavioral effect of lorcaserin in humans was also closely monitored.

In the cardiovascular safety study, single oral doses of lorcaserin up to 100 mg/kg had no effect on cardiovascular parameters (MAP, HR, ECG, and QT) in monkeys (telemetry up to 20 hr post dose). When the action potential effect was examined in isolated Purkinje fiber assay, lorcaserin significantly prolonged action potential duration at 90% (ADP_{90}) in isolated canine Purkinje fiber assay at 30 μ M (6.96 μ g/ml vs. clinical of 85 ng/ml) but had no effect on ADP_{60} (3, 10 and 30 μ M). In hERG channel study, lorcaserin significantly inhibited hERG (I_{Kr}) current at all concentrations in a dose-dependent manner with IC_{50} =14 μ M (3.25 μ g/ml) but the potential clinical significance is minimal since the concentrations at which these findings were observed are several multiples higher than the anticipated plasma concentrations in humans (C_{max} of 85 ng/ml at 20 mg/kg). Therefore, lorcaserin is unlikely to prolong action potential in humans. A definitive human QT study in human subjects was conducted and reviewed by FDA. There was no apparent drug effect on QT in humans.

A recent review article McCann et al (278(8): 666-672, JAMA, 1977) discussed long-term use of fenfluramine on brain serotonin neurons, body weight, and pulmonary function in animals and humans. They reported that fenfluramine caused dose-related, long-lasting reductions in serotonin axonal markers in all the animal species tested at doses similar to doses used in humans. The primary human adverse findings were related to development of primary pulmonary hypertension. In animal studies where high doses of fenfluramine and dexfenfluramine were administered for longer than 2 weeks, degeneration of 5-HT nerve terminals throughout the forebrain of animals were reported. The degeneration of 5HT nerve terminals was characterized by depletion of tissue 5-HT, decreased 5-HT biosynthesis, and loss of 5-HT transporters. These effects are believed to be caused by accumulation of drug molecules into 5-HT nerve terminals and subsequent cytotoxic effects of these drugs or their metabolites. Although there is no data to suggest that lorcaserin may share the same features with fenfluramine, the potential for neurotoxicity exist since rat data found significant accumulation of lorcaserin (13 to 30 fold) in rat brain relative to plasma. It should be noted that no such findings of neuronal degeneration were reported in rats and it is not known if such damage occurs in humans or if there are clinical consequences. Other potential CNS effects of chronic use of 5HT drugs is a condition called serotonin syndrome, a potentially dangerous and fatal condition characterized by a hyperserotonergic state. Serotonergic syndrome which may occur when a combination of two or more serotonergic drugs are taken is relatively rare but could occur if lorcaserin is used in combination with another 5HT drug such as selective serotonin re-uptake inhibitors (SSRIs). The sudden death in some of the high dose animal toxicology studies reported with lorcaserin could have potentially been caused by a hyperserotonergic state.

Abuse liability: As a CNS drug, serotonin receptor agonists including lorcaserin have the potential to activate $5HT_{2A}$ if drug concentrations are sufficiently high. Activation of $5HT_{2A}$ is known to result in psychological disturbances such as hallucinations. The potential CNS effect of lorcaserin was examined in a series of

studies in animals. In these studies, low doses of lorcaserin did not appear to result in adverse CNS effects, however since there was no reliable positive control in these studies and doses of lorcaserin were limited to 5 mg/kg, the absence of CNS behavioral effects is not reassuring. The crude assessment of behavioral effects of lorcaserin in the toxicology studies found no notable adverse effect. The sponsor provided additional new abuse potential studies in the 2011 resubmission. Dr. Katherine Bronson (CSS staff) reviewed these studies. Based on the abuse potential studies, CSS had recommended lorcaserin to be classified as a schedule IV drug.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Lorcaserin pharmacokinetic studies had been reviewed in the original NDA. Only a summary of the PK data and brain lorcaserin distribution studies are discussed in this section. Lorcaserin (APD356), prominently an R-enantiomer (>98% purity) is a highly bioavailable compound in animals (F: 93%, 51% and 37% in rats, monkeys and dogs respectively). Food had no effect on lorcaserin bioavailability in rats or humans. Animal studies found minimal chiral inversion. Lorcaserin exposure increased in a dose-proportional manner in oral pharmacokinetic studies. As a 5HT_{2C} receptor agonist, the appetite suppressant activity of lorcaserin occurs at the brain level and thus drug entry and accumulation in the brain are fundamental for its pharmacological effect. Brain distribution studies found preferential distribution of lorcaserin to the brain in mice (26 x the plasma), rats (13-35x the plasma) and monkeys (10x the plasma). The original NDA had no human brain exposure data. The new brain partitioning study in humans estimated the brain exposure based on the stable relationship observed in animals between CSF and brain levels. Using human CSF data, the brain lorcaserin exposure in humans was estimated to be 1.7x the plasma exposure.

Lorcaserin is metabolized to several prominent metabolites (7-hydroxy and N-hydroxy metabolites). The two prominent metabolites, namely M1 (lorcaserin sulfamate, APD244208) and M5 (N-carbamoyl glucuronide) are inactive. However, M2, a minor metabolite showed some agonistic potency to human 5HT_{2C} and 5HT_{2A} with 17 fold selectively to 5HT_{2B}. The remaining metabolites constituted a fraction of total in plasma. Several metabolites (M2, M3, M4 and M6) that were identified *in-vitro* were also found in some instances in urine and feces of the tested species. Since the human metabolites were also found in animals (rats, rabbits and cynomolgus monkey), the toxicology profile of the metabolites was of minimal concern. Lorcaserin reversibly increased hepatic microsomal enzymes (CYP1A, CYP2B, CYP1A1 and CYP2B1/2) in rats. It had no effect on CYP3A4 or CYP2C9. Lorcaserin minimally inhibited human CYP2D6 (2.9 μM), CYP2C19 (53 μM), CYP2C9 (61 μM), CYP1A2 (200 μM) and CYP3A4 (200 μM). Lorcaserin has moderate protein binding in humans (~74%), monkeys (~73%), rabbits (~62%), rats (~70%) and mice (~ 67%).

Distribution

Brain and plasma concentration profiles for lorcaserin after oral administration to male SD rats (single 10 mg/kg) were comparable with respect to $t^{1/2}$ (plasma 4.9 hr, brain 4.7 hr). The t_{max} for brain was 1 hr versus 0.25 hr to t_{max} for plasma concentrations in this study. At 24 hr post-dose, the ratio of lorcaserin concentrations in brain over plasma were 11.5 to 30 fold (μg lorcaserin per g brain/ μg lorcaserin per ml plasma) suggesting lorcaserin may have been actively transported across the blood brain barrier. Choroid plexus is known to be highly enriched in 5HT_{2C} receptors. Whether 5HT_{2C} at this site had some role in preferential partitioning of lorcaserin to the brain in animals is unknown.

Plasma ¹⁴C levels approached background by 120 hr after single oral dose of radiolabeled lorcaserin. In most tissues, the t_{max} for ¹⁴C-APD356 derived radioactivity was within 1 to 2 hr post-dose. As expected, ¹⁴C-APD356-derived radioactivity was highest in tissues associated with the drug pathway (gastrointestinal, stomach, small intestine and bladder). The lungs exhibited relatively high concentrations of ¹⁴C-APD356-derived radioactivity (t_{max} between 1 to 2 hrs). An earlier study had found brain exposure to be approximately 11 to 30 times greater than plasma. The ratio of ¹⁴C-lorcaserin in plasma to whole blood was between 1.5 and 1.6 through 24 hr post-dose, suggesting differential distribution to the plasma (75% to 80% of the total). In the in vitro human whole blood distribution study (200 ng/ml of lorcaserin), approximately 95% of lorcaserin was found in the plasma similar to the radioactivity study results in rats.

Brain tissue Distribution

In series of drug distribution studies, the brain and cerebrospinal fluid (CSF) levels of lorcaserin and its prominent metabolites (M1 and M5) were evaluated in rats, mice and monkeys. The original NDA did not contain any human CNS exposure data. Brain distribution studies found lorcaserin to preferentially partition to brain in male mice, rats and monkeys. Brain to plasma ratio for lorcaserin ranged from 10x in male monkeys to 24x in male SD rats. The brain distribution studies in male mice, rats and monkeys were reviewed in the original NDA. Since lorcaserin increased brain tumors in male rats at the site of pharmacological activity, the brain exposure in humans was needed for reliable determination of safety margin. In the resubmission, the sponsor provided clinical CSF and plasma exposure data in obese subjects to estimate brain lorcaserin levels. Brain exposure in humans was estimated from relatively consistent relationship between CSF and brain exposure across species. The resubmission also included brain exposure data in female rats. The review only covers the new brain distribution studies in the 2011 resubmission.

New Study MPR-11-001: Evaluation of the Potential Accumulation of APD356 (Lorcaserin) in the Female Rat Central Nervous System (CNS) after Multiple Daily Oral Dosing at 10 mg/kg (Aug 2, 2011)

In this new brain lorcaserin distribution study, female SD rats (n=6/time point, were treated with 10 mg lorcaserin (SPD356) for 14 days by oral gavage. The plasma, CSF and brain tissue levels of lorcaserin were determined on Day 1 and Day 14.

Study Design

Days of Dosing	Dose (mg/kg)	Volume (mL/kg)	Animals per Cohort Females
1	10	5	54
14	10	5	54

Lorcaserin appeared to be readily absorbed from the GI tract into the systemic circulation, reaching CSF and brain in 30 to 60 min. For prolactin study, the Division had recommended blood sample collection at close to brain Tmax (0.5 to 1 hr post dose) to achieve the highest drug effect on prolactin release from the pituitary and reduce gavage induced stress. PK parameters of lorcaserin in plasma, CSF and brain on Day 1 and Day 14 are shown in table below:

APD356 Pharmacokinetic Parameters after Oral Administration of APD356 to Female Sprague-Dawley Rats at 10 mg/kg/day for 14 Days

Parameter	APD356					
	Day 1			Day 14		
	Plasma	CSF	Brain	Plasma	CSF	Brain
t _{1/2} (h)	6.91	7.86	8.58	3.47	3.68	3.69
t _{max} (h)	0.250	0.250	0.500	0.500	0.500	1.00
C _{max} (µg/mL or g) ^a	0.793 (0.339)	0.107 (0.044)	7.40 (2.05)	0.709 (0.340)	0.199 (0.082)	11.4 (3.33)
AUC _{last} (h·µg/mL or g)	4.30	0.989	92.1	4.40	1.30	98.1
Accumulation Index (AUC _{last}) Day 14/Day 1	-	-	-	1.02	1.31	1.07
Tissue/Plasma Ratio (AUC _{last})	-	0.230	21.4	-	0.295	22.3

^a C_{max} values are mean (SD), n = 6 / time point

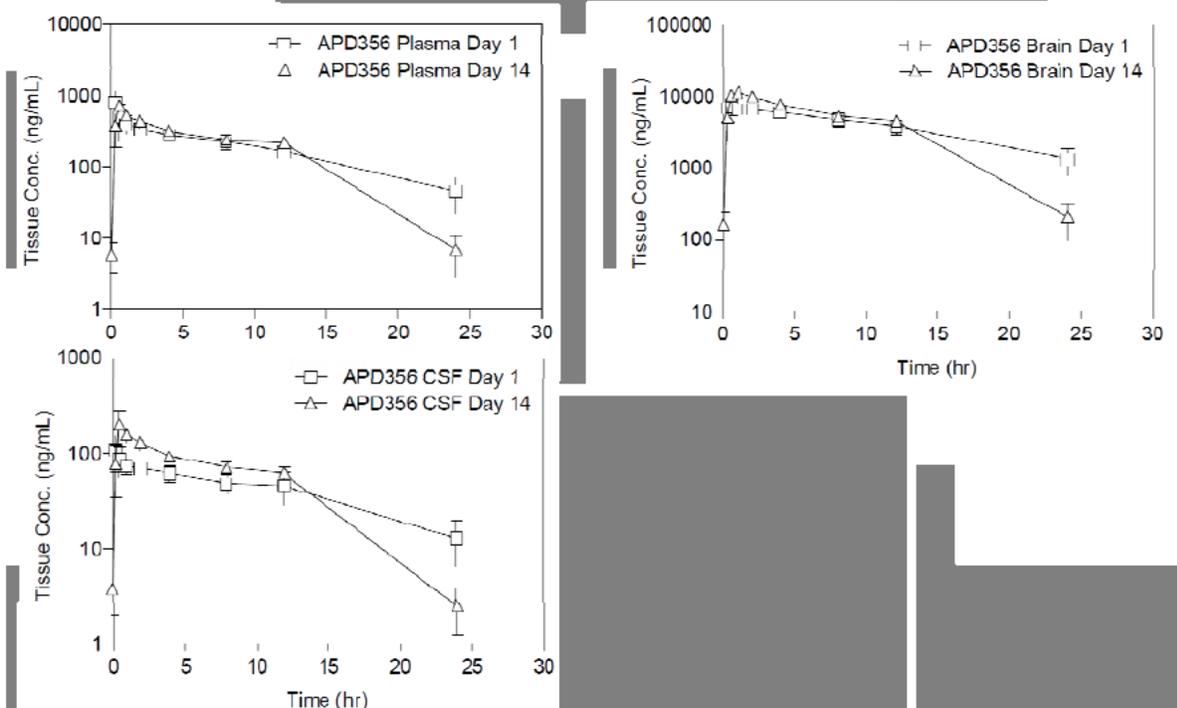
Plasma lorcaserin levels on Day 1 and Day 14 appeared to be similar; however, there appeared to be 30% lorcaserin accumulation in the CSF on Day 14 (1.3 µg.h/ml) relative to Day 1 (0.989 µg.h/ml).

The concentration time profile of lorcaserin on Day 1 and Day 14 for CSF was slightly different from the plasma and brain concentration time profile, marked by 30% greater exposure in CSF on Day 14 than on Day 1. The t_{1/2} after repeated administration was faster on Day 14 (3.5-3.7 hr) than on Day 1 (7-8.5 hrs).

Lorcaserin exposures in the CSF were 4.3 and 3.3 fold lower than plasma values on Day 1 and Day 14. Interestingly, CSF exposure was 93 and 75 fold lower than brain levels.

Best Possible Copy

APD356 Concentration vs. Time Curves after Oral Administration of APD356 at 10 mg/kg/day to Female Sprague-Dawley Rats for 14 Days – Day 1 vs. Day 14 (Mean ± SD, n = 6)



In spite of lower CSF levels, significant accumulation of lorcaserin occurred in the brain in female rats. The brain to plasma ratio of lorcaserin after a single dose (Day 1) was 21.4 suggesting that even with a single dose there is a significant accumulation of lorcaserin in the brain of female SD rats. The brain lorcaserin partitioning on Day 14 appeared to be similar to Day 1 partitioning.

Overall, oral administration of 10 mg/kg lorcaserin resulted in about 22 fold increase in brain exposure in female SD rats. Lorcaserin brain partitioning in male rats was about 24x the plasma at 10 mg/kg and 35 fold at 30 mg/kg. Brain to plasma exposure ratio in female rats was consistent with male rat data at 10 mg/kg suggesting that higher incidence of brain astrocytoma in male rats was not due to higher brain exposure in male rats. Put another way, the lower incidence of brain tumors in female rats was not due to lower drug exposure in the brain.

New Study MPR-11-007: Estimated Lorcaserin Brain Concentrations in Healthy Obese Human Volunteers and Associated CNS Safety Margin Determination for the Finding of Astrocytoma in the Rat Carcinogenicity Study.

Lorcaserin was associated with significant increase in number of astrocytoma in males in the 2-year rat carcinogenicity study. Since lorcaserin was shown to accumulate in the brain of mice (26 fold), rats (13 to 35 fold) and monkeys (~ 10 fold) relative to plasma, an accurate human brain exposure based on plasma levels could not be reliably estimated.

Therefore, in the CR letter, the agency requested information that would clarify the safety margin to the dose causing astrocytoma in rats. In discussions with the sponsor, it was agreed that analysis of CSF would be an adequate approach to estimating brain levels of drug based on the observation that CSF levels in animals have held a steady ratio to brain levels of lorcaserin.

Therefore, healthy obese patients were enrolled to receive 10 mg BID (20 mg/day, oral) lorcaserin for 7 Days. Plasma and CSF levels of lorcaserin were determined on Day1 and Day 7.

The CSF exposure in nine patients enrolled in study ranged from 5.29 to 11.8 ng.h/ml with 42% CV. The ratio of CSF/plasma lorcaserin in humans (0.0168) was 7 to 18 fold lower than CSF/plasma ratio in monkeys (0.112) and rats (0.225 to 0.3).

Human Lorcaserin CSF, Plasma, and CSF/Plasma Ratios after Oral Administration at 10 mg Twice Daily for 7 Days to Healthy Obese Subjects – APD356-022

Subject	001	003	004	005	006	007	008	009	011	Mean	SD	%CV
CSF												
C _{max,ss} (ng/mL)	0.669	1.01	1.97	0.656	1.22	0.507	1.05	0.983	0.520	0.954	0.457	48%
AUC _{12h,ss} (h·ng/mL)	6.53	11.4	16.9	6.41	11.8	5.12	9.90	10.4	5.29	9.31	3.87	42%
Plasma												
C _{max,ss} (ng/mL)	57.1	71.1	88.6	50.6	66.9	42.6	69.6	70.8	50.7	63.1	14.1	22%
AUC _{12h,ss} (h·ng/mL)	469	627	832	363	573	361	570	660	402	540	157	29%
CSF/Plasma												
C _{max,ss}	0.0117	0.0142	0.0222	0.0130	0.0182	0.0119	0.0151	0.0139	0.0103	0.0145	0.0037	26%
AUC _{17h,ss}	0.0139	0.0182	0.0203	0.0177	0.0206	0.0142	0.0174	0.0158	0.0132	0.0168	0.0027	16%

The table below lists the plasma, CSF and brain lorcaserin exposures across species. The brain to CSF ratio across animal species was relatively consistent ranging from 75 to 117. The ratio of brain to CSF was more consistent than brain to plasma or CSF to plasma. The AUC₀₋₁₂ for lorcaserin in different species are provided in table below:

Lorcaserin AUC_{last,ss} and CNS to Plasma Ratios for Rodents, Monkeys, and Humans

Species (gender)	Dose	AUC _{last,ss} (h·ng/mL)			AUC _{last,ss} Ratios		
		Plasma	CSF	Brain	CSF/Plasma	Brain/Plasma	Brain/CSF
Mice (male)	50 mg/kg	5050	1140	133,000	0.226	26.3	117
Rats (male)	10 mg/kg	2510	564	60,300	0.225	24.0	107
Rats (male)	30 mg/kg	7070	2130	247,000	0.301	34.9	116
Rats (female)	10 mg/kg	4400	1300	98,100	0.295	22.3	75
Monkeys (male)	10 mg/kg	7220	812	73,200	0.112	10.1	90
Mean (SD)							
Human (mixed)	10 mg bid	540 (157)	9.31 (3.87)	NA	0.0168 (0.0027)	1.70 (0.38)	101 (16)

Assuming that CSF to brain ratio in all species including humans is relatively similar, the brain lorcaserin AUC₀₋₂₄ in humans was estimated to be about 1.73 µg.h/ml. The brain exposure in humans was only 1.7x the plasma vs. those 24x in rats and 10x in monkeys. The lower ratio suggests relatively limited preferential brain distribution of lorcaserin in humans. The lower brain exposure in humans than initially predicted and those seen in animals, significantly expanded the safety margin for the brain tumors. Based on estimated human brain partition data, the safety margin for lorcaserin dose without brain tumor was 70x the clinical dose.

Species	Dose, mg/kg	Plasma, AUC _{0-24,ss} µg.h/ml	CSF AUC, µg.h/ml	Brain AUC, µg.h/ml	Safety margins based on brain AUC exposure (Animal/Human)
104-Week Rat Study	10	4.78	1.074	114.7	~ 70 (no brain tumors in male rats)
	30	16.9	5.09	591	~ 340 (Brain tumors in male rats)
Clinical Dose: 10 mg BID		1.02	0.00931	1.73	

5.2 Toxicokinetic table

Safety margin

Species	Daily Dose, (mg/kg)	lorcaserin AUC ₀₋₂₄ (µg.h/ml)	NOAEL, (mg/kg) M/F	Safety margins based on plasma AUC (Animal/Human)	
				male	Female
13-Week mouse Study	25	M:3.4 F:1.0		3	1
	50	M:7.6 F: 2.3	50/50	7	2
	250	M:34.8 F:9.2		34	9
	350	M:25 F:27		25	26
13-Week rat study	1	M:0.143 F:0.33	5/1	<1	<1
	5	M:0.75 F:1.71		<1	2
	50	M:16.6 F:32.5		16	32
	100	M:33.6 F:55.8		33	55
6-Month rat study	1	M:0.20 F:0.31		<1	<1
	5	M:1.19 F:2.87	5/5	1	3
	50	M:22.0 F:34.4		22	34
12-Month cynomolgus monkey study	2	M: 1.0 F: 0.6	2 / 2	1	0.6
	10	M: 7.9 F: 4.5		8	4
	50	M:43.6 F:31.4		43	31
	125	M: 50.9 F: 51		50	50
104-Week Mouse Carci Study	5	M:0.55 F:0.32		<1	<1
	25	M:3.9 F: 1.6		4	1
	50	M:7.5 F:3.7	50/50	7	4
104-Week Rat Study	10	M:4.78 F:6.7	5 / <7	5	7
	30	M:16.9 F:24.1		16.6	24
	100 ^b	M:55.9 F:83.8		55	82
Fertility and early embryonic development in rats	5	M:2.68 F: 4 ^a		3	4
	15	M:9.91 F:12 ^a	15/50	10	12
	50	M: 29.3 F:48.7 ^a		29	48
Oral Embryo-fetal development in rats	2	F:1.34			1
	10	F:7.99	10		8
	50	F:48.7			48
Oral Embryo-fetal development in rabbits	20	F: 0.155			<1
	60	F: 0.443	60		<1
	200	F:19.3			19
Pre- and postnatal development in rats	5	F:4 ^a	<5		4
	15	F:12 ^a			12
	50	F:48.7 ^a			48
Clinical Dose: lorcaserin, 10 mg BID		1.02			

^a The AUC value is derived from other existing similar studies.

Note: The lorcaserin AUC in the 2-year rat carcinogenicity study was collected from the TK animals treated with lorcaserin for 52 weeks. The lorcaserin AUC in the 52-week TK rats was inexplicably about 60% higher than the AUC in the 13-week toxicology and 28-day prolactin mechanistic studies (AUC 53 µg.hr/ml). Lorcaserin reaches steady state within 2 weeks of daily dosing, so AUC was expected to be similar across these studies.

6 General Toxicology

6.1 Single-Dose Toxicity

In the single dose studies (reviewed under the IND), 500 mg/kg of lorcaserin was considered the MTD in rats due to death at 1000 mg/kg. Salivation, penile erection, reduced activity, tremor in addition to decrease in BW and food intake were hallmarks of single dose lorcaserin in rats. Monkeys tolerated single doses up to 300 mg/kg of APD356. Similar to rats, penile erection was noted at doses \geq 10 mg/kg in monkeys. Penile erection, a pharmacological side effect of 5HTc activation, was seen in the 10-day monkey study at all dose levels (10, 100, 150 mg/kg). In addition to the decrease in activity (\geq 100 mg/kg), an increase in AST (1.8X) at doses greater than 10 mg/kg and ALT (1.4X) at 300 mg/kg were noted. The NOAEL and MTD were 10 and 100 mg/kg in the 10-day monkey study, respectively.

6.2 Repeat-Dose Toxicity

Multiple dose toxicology including reprotox studies were reviewed for the original NDA. In acute 10-day studies in rats, administration of lorcaserin doses up to 500 mg/kg resulted in salivation, increased sensitivity to touch (\geq 50 mg/kg), clonic convulsion (500 mg/kg), and decrease in BW and food intake. The increase in liver weight (\geq 50 mg/kg) was associated with hepatocellular vacuolation, likely due to a metabolic response. Although kidney weights were increased at all doses (20 to 23%) in rats, there was no evidence of macroscopic damage.

In the 6-month rat study (1, 5 and 50 mg/kg), there were no notable heart or brain lesions at doses up to 50 mg/kg, however significant increase in kidney and liver weight was seen at 50 mg/kg. Two of twenty female rats treated with 50 mg/kg lorcaserin had minimal mammary ductal hyperplasia. In the 12-month monkey study (2, 10, 50 and 125 mg/kg), there was a dose-dependent increase in incidence of renal tubular regeneration (minimal to moderate) at all doses of lorcaserin. They did not completely recover at the end of the short 4-week recovery period. There was no evidence of valvulopathy in any of the toxicology studies.

8 Carcinogenicity

Lorcaserin carcinogenicity was tested in CD-1 mice and SD rats. Both studies were reviewed in the review of the original NDA submitted in 2009. Brief summary of mouse study is provided for reference. The rat study was also reviewed earlier therefore the rat carcinogenicity study will examine the female rat mammary and lung tumor data re-adjudicated by the pathology working group.

Study title: A 2 year carcinogenicity study of APD356 (lorcaserin HCl) given by oral a gavage to CD-1 mice

Mouse (65/sex/group) study was initiated with 25, 50 and 100 mg/kg of lorcaserin. Due to excessive and unexpected mortality during the first 16 days of the study (22 total), lorcaserin doses were lowered to 5, 25 and 50 mg/kg (5 ml/kg) on Day 19 (concurrent with eCAC) and additional 10 mice /sex were added to the control and high dose groups (75/sex). Most of the deaths occurred in the first 2 days at 100 mg/kg (8F, 2M). The cause of death was not determined.

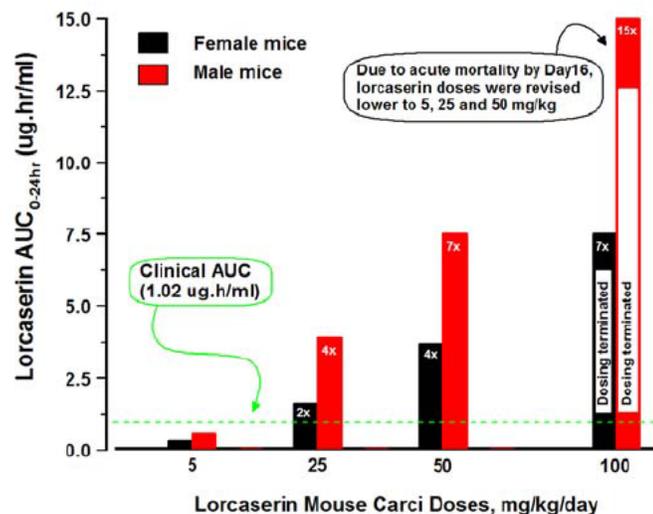
There was no treatment-related effect on survival rate after dose adjustment. All causes of death/moribundity during the course of the 2-year study were of the type typically seen in this type of study in mice. No clear or definitive treatment-related effects were noted on clinical findings (including masses), food consumption, or on macroscopic and microscopic evaluations during the study. Although possible slight treatment-related decreases in mean body weight were noted in males, primarily at 25 and 50 mg/kg, when compared to controls, there were no food consumption correlates and no similar effects in female animals, which showed slight increases at 50 mg/kg, making a relationship to treatment unclear.

The only safety concern in the study was the unexpected number of deaths after the initial dose of 100 mg/kg (7 to 15x the clinical dose) which remains unresolved.

Prior studies had shown minimal incidence of mortality in the first few days with lorcaserin (up to 250 mg/kg in the 13-week study) and nearly no deaths at doses up to 350 mg/kg in the 2-week study. Whether deaths were due to sensitivity of some mice to the sudden high lorcaserin exposure is not clear. It is highly possible that the remaining mice in the study

could have tolerated the 100 mg/kg since 50 mg/kg did not significantly impact the survival rate. The role of convulsion cannot be ruled out due to significant lorcaserin partitioning in the brain (25x greater than plasma).

In conclusion, once daily oral administration of APD356 to mice for 2 years at dose levels of 5, 25, and 50 mg/kg were well tolerated and APD356 did not produce any evidence of a carcinogenic effect in mice. The incidence of mammary adenocarcinoma were similar among groups in female mice (2/75, 1/65, 1/65 and 4/75 in control, 5, 25 and 50 mg/kg, respectively). There were no brain tumors in mice. Lorcaserin plasma exposure at top dose of 50 mg/kg in female and male mice was 4x and 7x the clinical dose AUC (10 mg BID, 1.02 $\mu\text{g}\cdot\text{h}/\text{ml}$, C_{max} 43.3 ng/ml).



Study title: A 2-Year carcinogenicity study of APD356 given by oral gavage to rats: Re-adjudication of mammary tumors in female rats by pathology working group (5th amendment to the final report)

Key findings of re-adjudication of female rat mammary tumors by PWG:

The mammary and lung tumor slides from female rats were re-adjudicated by 5 independent expert veterinary pathologists due to uncertainty in diagnosis of mammary adenocarcinoma from fibroadenoma in female rats by the CRO in the original study report.

The PWG re-adjudicated all the available mammary slides from female rats in a blinded manner. They easily and with high certainty distinguished mammary fibroadenoma from adenocarcinoma. The percent unanimous diagnostic certainty was 92% for adenocarcinoma and 97% for fibroadenoma. The PWG diagnosis of mammary tumors was considered definitive and final.

PWG lowered the incidence of adenocarcinoma in lorcaserin treated rats. The number of adenocarcinoma in the LD and MD were similar to control and only significantly increased at 100 mg/kg lorcaserin. The 24x safety margin relative to the clinical dose of 10 mg BID lowered concern of clinical risk for adenocarcinoma. The re-adjudication of fibroadenoma found significant increases at all doses of lorcaserin. No safety margin was identified (<7 fold clinical dose). Based on mechanistic studies, prolactin as the intermediary hormone in lorcaserin-induced fibroadenoma was considered plausible though not definitive.

Mammary and lung tumors incidence in female SD rats in the original report and after re-adjudication by the PWG		Lorcaserin Dose, mg/kg			
		0	10	30	100
AUC Exposure Multiples		-	7x	24x	82x
Mammary Adenocarcinoma (Historical range 8.3-37%)	Original	28	34	35	60
	PWG	27	21	24	50 **
Mammary Fibroadenoma (Historical range 22-54%)	Original	20	47	53	51
	PWG	23	53 **	55 **	51 **
Lung metastases from mammary adenocarcinoma (Historical range 0-12.5%)	Original	0	2	7	6
	PWG	0	1	5	5

The aggressiveness of the mammary adenocarcinoma at 100 mg/kg lorcaserin was marked by a significant increase in the tumor onset and multiplicity at this dose. Lorcaserin increased fibroadenoma tumor onset and multiplicity at all doses. Re-adjudication of lung tumors also found lower incidence of lung metastases originating from mammary adenocarcinoma. The incidence of lung metastases at 10 mg/kg was similar to control with equivocal increase at 30 and 100 mg/kg. Since HD female rats died early, premature deaths may have prevented adenocarcinomas metastasizing to the lungs.

Rat Carcinogenicity Study Background

Carcinogenicity of lorcaserin was tested after oral administration of 10, 30 and 100 mg/kg of lorcaserin to male and female SD rats. The study was carried out at (b) (4) labs. The specifics of the study protocol are shown in the table below:

Doses:	0, 10, 30 and 100 mg/kg (C, LD, MD & HD)
Frequency of dosing:	single oral dose/day
Dose volume:	5 ml/kg
Route of administration:	oral gavage
Formulation/Vehicle:	water
Basis of dose selection:	3-month rat tox study
Species/Strain:	CD@ [CrI:CD@ (SD)] rats
Number/Sex/Group:	65/sex/C, LD, MD and 75/sex/HD
Age:	6 weeks plus acclimation
Animal housing:	individually in stainless steel cages
Paradigm for dietary restriction:	ad lib food (Lab Diet #5002) and water
Dual control employed:	No
Interim sacrifice:	No (TK animals were sacrificed after 1 year)
Satellite groups:	TK rats (6, 15, 15 & 15 rats/sex for C, LD,MD and HD, respectively)

Mid-way through the rat study, a high incidence of brain and mammary tumors in SD rats was reported the agency. The high incidence of mortality and brain tumors in male rats and palpable mammary tumors in female rats early in the course of the study prompted the FDA to request formal bimonthly updates from the sponsor.

Mammary tumors are highly prevalent and develop spontaneously over the lifespan in aging female SD rats. Since rats have multiple (6 pairs) mammary glands distributed in pairs from axial to inguinal area on the abdominal surface, a single female rat may have one or multiple mammary tumors at necropsy. Both mammary adenocarcinoma and fibroadenoma can be fatal but for different reasons. Adenocarcinoma is a malignant tumor that can readily metastasize and cause early death depending on aggressiveness of the tumor. In contrast, fibroadenoma is a benign tumor that can eventually cause death of the animal by preventing normal activity and causing frequent skin lesions. Prolactin has been considered as the primary driving hormone of mammary tumors in SD rats. For example, a large number of antidopaminergic antipsychotic drugs are known to increase mammary tumors by robustly increasing plasma prolactin in rats.

In early discussions, the sponsor proposed that lorcaserin's effect on mammary tissue was likely mediated by prolactin, similar to the case with antipsychotic drugs. The hypothesis was reasonable but the agency requested mechanistic studies to demonstrate that prolactin indeed increases in a robust and persistent manner, as with the antipsychotic compounds. The Division's decision to continue with clinical studies was based on, a) carcinogenicity studies were ongoing and tumor incidence would change as more data was gathered, b) mammary tumors in rats could be mediated by a lorcaserin-induced increase in plasma prolactin, c) modified consent form reflected the ongoing but inconclusive rat findings.

Per FDA request, after the initial safety report on May 2007 (~46 WK in to the rat study), the sponsor began submitting formal bimonthly updates of mammary and brain tumors. These bimonthly updates were used in clinical risk assessment and the findings were periodically consulted with the executive carcinogenicity assessment committee (eCAC). With each update starting with WK 55, the number of deaths and the incidences of brain and mammary tumors increased in lorcaserin treated rats.

Mammary Adenocarcinoma Incidence in Female Rats from Bi-Monthly Updates (# positive / # examined)				
Data Update (Week)	Control	10 mg/kg	30 mg/kg	100 mg/kg
Week 55	0/1	2 / 4	5 / 7	13 / 15
Week 68	2 / 5	6/6	16 / 18	45 / 46
Week 88	16 / 28	27 / 38	36 / 45	72 / 74
Week 96	20 / 39	34 / 50	43 / 57	72 / 75
Week 104	30 / 65	35 / 65	35 / 65	63 / 75
Final update	29 / 65	35 / 65	36 / 65	62 / 75
Original NDA	28 / 65	34 / 65	35 / 65	60 / 75

Mammary Fibroadenoma Incidence in Female Rats from Bi-Monthly Updates (# positive / # examined)				
Data Update (Week)	Control	10 mg/kg/d	30 mg/kg/d	100 mg/kg/d
Week 88	4/28	16/38	24/45	35/74
Week 96	10 / 39	27 / 50	36 / 57	36 / 75
Week 104	20 / 65	47 / 65	60 / 65	53 / 75
Final update	20 / 65	48 / 65	56 / 65	51 / 75
Original NDA	20 / 65	47 / 65	53 / 65	45 / 75

With each subsequent update after week 96, the incidence of adenocarcinoma in the MD and HD females decreased. The incidence of adenocarcinoma increased in the control and stayed consistent in the low dose group over the same period. The incidence of fibroadenoma increased in all dose groups from week 96 to the final study report, though the numbers notably varied in the mid- and high dose groups. Although the incidences of mammary fibroadenoma and adenocarcinoma in the final report were dramatically different from the earlier reports, the incidences of brain astrocytoma in male rats were mainly unchanged. The uncertainty in accurately identifying mammary adenocarcinoma from fibroadenoma with no data to support the prolactin hypothesis and aggressiveness of adenocarcinoma (i.e. mammary tumor onset, multiplicity and lung metastases) became a major concern. The uncertainty in diagnosis of mammary tumors in female rats was discussed in the first advisory committee meeting (Sept 16, 2010). In the CR letter issued on Oct 22, 2010 (Appendix C), the unresolved rat carcinogenicity issues were communicated to the sponsor. The Division requested detailed diagnostic changes reported in the bimonthly updates and re-adjudication of all female rat mammary tissue slides by an independent group of veterinary pathologists.

The CR letter deficiencies were further discussed with the sponsor at the End of the Review meeting held on Dec 15, 2010. At the meeting, the sponsor informed the FDA that the contracting lab (CRO) had not kept records of the 8 bimonthly updates submitted to the FDA. The CRO considered the bimonthly updates as interim, even though the sponsor was aware that the agency was using the updates in making regulatory decisions. The interim data was replaced by the final peer reviewed diagnosis. As we will discuss soon, even the final peer review data was unreliable and inaccurate.

To resolve diagnostic uncertainty, the sponsor enlisted five independent expert veterinary pathologists (table below) to form a pathology working (PWG) group to diagnose all available female rat mammary and lung metastases slides in the 2-year rat carcinogenicity study.

Dr. K. A. Schafer (Vet Path Services, Inc., PWG Chairperson)
 Dr. P. H. Long (Vet Path Services, Inc.)
 Dr. R. E. Baumgartner (Vet Path Services, Inc.)
 Dr. D. Thake (Midwest ToxPath Sciences, Inc.)
 Dr. R. R. Maronpot (Maronpot Consulting, LLC)

The slides were blinded for animal ID, treatment and prior diagnosis. Each pathologist evaluated each slide independently before convening for consensus.

The PWG reached a high degree of consensus in accurately diagnosing mammary adenocarcinoma and fibroadenoma in the blinded assessment. The PWG stated that they were able to easily distinguish adenocarcinoma and fibroadenoma. The percent unanimous consensus in diagnosis was 92% for adenocarcinoma and 97% for fibroadenoma. Therefore, the Agency considered the results of the PWG as the definitive dataset for mammary tumor incidence in female rats for this study.

Degree of PWG Consensus for Neoplastic Lesions

Diagnosis	Number of consensus by 3/5 on PWG	Number of consensus by 4/5 on PWG	Number of consensus by 5/5 on PWG	% Unanimous Consensus
Adenocarcinoma	1	12	160	92.5%
Adenoma	2	2	15	78.9%
Fibroadenoma	7	14	715	97.1%

Since the rat carcinogenicity study was reviewed with the original NDA only the data that was altered by the PWG will be discussed.

Results of Female Rat Mammary Tumor Re-adjudication

Corrections and Error in Slide Submissions sent to PWG:

- Animal #3218 – The signed 12/30/10 PWG worksheet lists mass D/WIL. “WIL” was a transcription error when preparing the PWG worksheet from the processing records; it should have been transcribed as “ULC” which is an abbreviation for ulcer.
- Animal # 4201 – prior diagnosis of adenocarcinoma was for lymph node, which was not read by the PWG while they were at (b) (4). Therefore for thoroughness and completeness, the four lymph node slides were subsequently shipped to the PWG members in the same totally blinded fashion. This read is presented in the signed 5/5/11 PWG worksheet and the Sponsor was not informed of the results of this subsequent read until after the 5/5/11 signing of the PWG worksheets.
- Animal #4202 – This animal had a mass listed in the detailed clinical observations and the individual mass table but this mass was not called during gross necropsy. Therefore, for thoroughness and completeness, the wet tissues were pulled and the mass was located, processed to the slide stage, and shipped to the PWG (same shipment as the lymph node slides mentioned in the bullet point above) in the same totally blinded fashion. This read is also presented in the signed 5/5/11 PWG worksheet and the Sponsor was not informed of the results of this subsequent read until after the 5/5/11 signing of the PWG worksheets.
- Animal #4233 – Slide 15 for this animal was actually uterus (rather than mammary). This animal was diagnosed with adenocarcinoma by the PWG due to masses, so this issue does not affect the diagnosis for this animal.
- Animal #4212 – The cause of death entry was corrected for this animal in the (b) (4) tables (entry error by original pathologist for cause of death of this animal).
- Normal histology practices are to trim in trackable lesions unless the protocol section is deemed adequate for evaluation of the gross lesion. Protocol tissue sections were adequate for some trackable lesions and thus additional gross lesions did not need to be prepared in such instances (animal #4258 and animal #4274 for example).
- Animal #4231 – Labeling errors occurred for several slides that did not affect the PWG read. For this animal, slide 2 ended up being lymph node rather than lung, slide 15 ended up being lung, and 18-1 was supposed to be mass A but was mammary gland while 18-1 Dr. R-1 was mass A; this is because the blinding labels were applied incorrectly, resulting in this inconsistency among the slides, but this did not affect the blinded PWG read in any fashion, as all appropriate slides were provided to the PWG for evaluation. Also, slide 18-2 for animal #4230 had an incorrect blinding label (labeled as lung tissue for animal #4231 when it was actually the draining node for animal #4230), but should not even have been provided to the PWG because it was not a skin, mammary mass, or lung section; this did not affect the blinded PWG read in any fashion either, as all appropriate slides were provided to the PWG for evaluation for both these animals.
- Animal #2265 – Mass labels for this animal were revised about 6 months after its gross necropsy, but the reason for the change cannot be determined, as the Provantis edit sheet for the mass label only indicates the edit as a “format change” without a reason for the change articulated. The histopathology data were not changed to match and were used to generate the PWG worksheets; therefore the PWG mass

identifications are incorrect as both right and left axillary masses are listed as mass A. Because of this discrepancy, the mass correlates to the adenocarcinoma and fibroadenoma listings are not definitively clear. However, this animal was diagnosed with adenocarcinoma by the PWG, so this issue does not affect the diagnosis for this animal.

- The PWG generated subsequent revised signed/finalized PWG reports (revised reports were signed/finalized on June 28, 2011) relative to inclusion of various bullet point and other explanatory items added to their reports, as well as for revisions stemming from items such as the additional slide reads mentioned in the bullet points above (animal #4201 and animal #4202). In addition, the PWG then generated a second subsequent revised signed/finalized PWG report for the blinded report (second revised report was signed/finalized on July 21, 2011) relative to inclusion of minor corrections of an overall tally of the degree of PWG consensus. The PWG also then generated a second subsequent revised signed/finalized PWG report for the unblinded report (second revised report was signed/finalized on July 21, 2011) relative to correction of a P-value. All signed/finalized versions of the PWG reports are included in this document.

Mortality

- The most common cause of death in HD females was mammary tumor (68/75). Mammary tumor also caused death in control (15/65), LD (31/65) and MD (43/65) females. All probable cause of deaths in rats are listed below:

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Probable Cause of Death - FEMALE				
Cause of Death	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals	65	65	65	75
Summary of Animal Disposition				
died prior to euthanasia	0	0	0	1
euthanized <i>in extremis</i>	34	36	43	61
found dead	8	17	17	13
terminal necropsy	23	12	5	0
Cause of Death				
brain tumor	0	2	1	0
fibrosarcoma/fibroma	0	0	0	1
kidneys hydronephrosis, bilateral	1	0	0	0
kidneys nephropathy, chronic progressive	1	0	1	0
kidneys pyelonephritis, bilateral	0	1	0	1
leukemia	1	0	0	0
lymphoid tumor	0	2	0	0
mammary tumor	15	31	43	68
pituitary tumor	15	13	10	2
skin hemangiosarcoma	0	0	1	0
spleen sarcoma, undifferentiated	0	0	0	1
stomach, glandular sarcoma, undifferentiated	0	1	0	0
undetermined	9	3	2	2
uterus inflammation/necrosis	0	0	1	0
zygomatic gland tumor	0	0	1	0

The table below shows the number of deaths caused by adenocarcinoma, fibroadenoma and presumed adenocarcinoma and fibroadenoma. Female rats treated with 100 mg/kg lorcaserin were more likely to die from adenocarcinoma. When deaths due to presumed adenocarcinoma were considered, both 30 and 100

mg/kg treated rats were likely to have died from adenocarcinoma. Lorcaserin treated rats had significantly more mammary fibroadenoma than control.

Cause of Death Due to Mammary Tumors in female SD rats (amended)

Number of animals per group	65	65	65	75
Lorcaserin Dose, mg/kg	0	10	30	100
Due to fibroadenoma	3	18	25	21
Presumed to be due to fibroadenoma	6	27	38	43
Due to adenocarcinoma	9	4	5	25
Presumed to be due to adenocarcinoma	12	13	18	47
Due to adenocarcinoma and or fibro	3	9	13	22
Combined	15	31	43	68

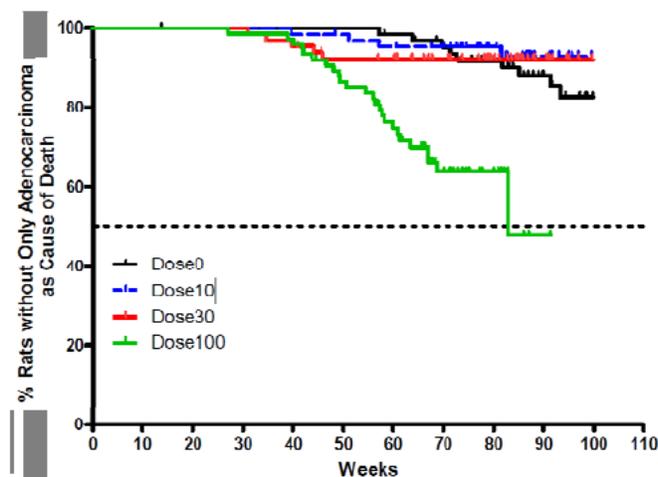
Analysis of deaths caused by adenocarcinoma

Oral administration of 100 mg/kg lorcaserin significantly increased mortality in female rats (premature deaths due to adenocarcinoma). HD female rats were 4.6x more likely to die of malignant adenocarcinoma than the control group.

Results for Time to Death due to Adenocarcinoma Specified Analysis

Treatment	Total	Number of Rats with Adenocarcinoma Specified as Cause of Death	Censored	Percent Censored
Placebo	65	9	56	86.15
Lorcaserin 10 mg/kg/day	65	4	61	93.85
Lorcaserin 30 mg/kg/day	65	5	60	92.31
Lorcaserin 100 mg/kg/day	75	25	50	66.67
	270	43	227	84.07

Treatment Comparisons	Hazard Ratio	95% Confidence Interval	p-value
Lorcaserin 10 mg/kg/day vs Placebo	0.49	(0.15, 1.61)	0.2406
Lorcaserin 30 mg/kg/day vs Placebo	0.72	(0.24, 2.16)	0.5548
Lorcaserin 100 mg/kg/day vs Placebo	4.63	(2.05, 10.44)	0.0002

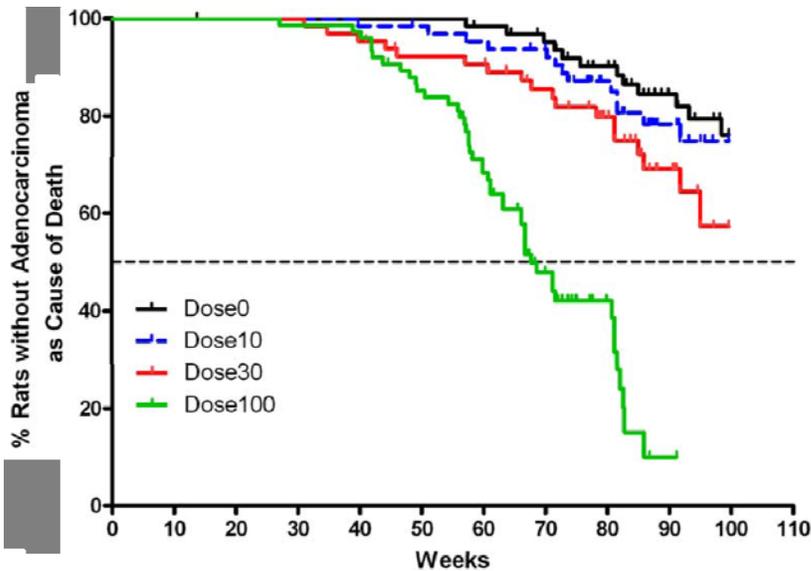


When deaths by “presumed to be due to adenocarcinoma” was considered, both 30 and 100 mg/kg lorcaseerin rats were more likely than control to increase hazard ratio. The hazard ratio for MD and HD lorcaseerin due to “presumed adenocarcinoma” were 2.2 and 10.2x the control, suggesting, that adenocarcinoma induced deaths at MD had also slightly had raised the trend.

Results for Time to Death due to Mammary Adenocarcinoma Presumed Analysis

Treatment	Total	Number of Rats with		Percent Censored
		Adenocarcinoma Presumed to be Cause of Death		
Placebo	65	12	53	81.54
Lorcaseerin 10 mg/kg/day	65	13	52	80.00
Lorcaseerin 30 mg/kg/day	65	18	47	72.31
Lorcaseerin 100 mg/kg/day	75	47	28	37.33
	270	90	180	66.67

Treatment Comparisons	Hazard Ratio	95% Confidence Interval	p-value
Lorcaseerin 10 mg/kg/day vs Placebo	1.27	(0.58, 2.80)	0.5455
Lorcaseerin 30 mg/kg/day vs Placebo	2.18	(1.04, 4.54)	0.0386
Lorcaseerin 100 mg/kg/day vs Placebo	10.18	(5.18, 20.01)	<.0001



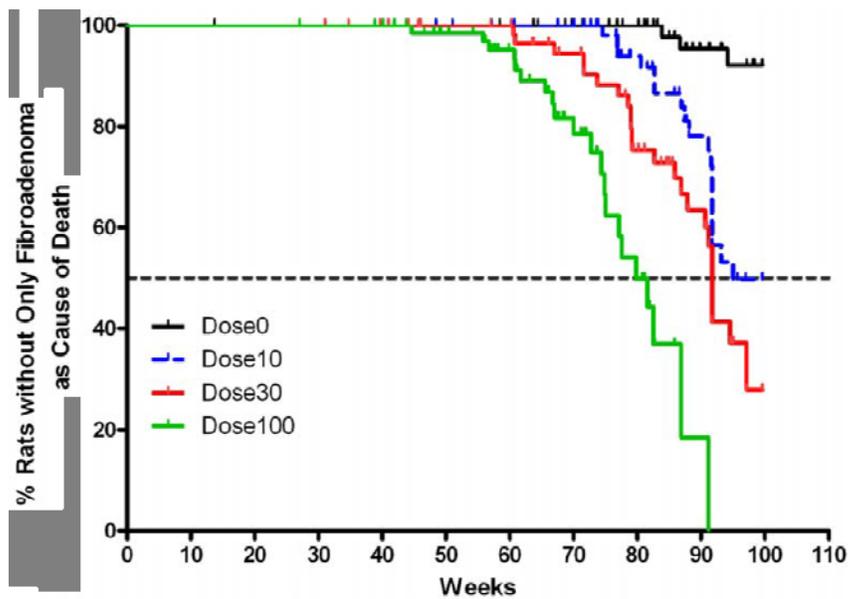
Analysis of deaths caused by fibroadenoma

Deaths caused by fibroadenoma were significantly increased at all doses of lorcaserin relative to control female rats. It should be noted that lorcaserin treated rats with fibroadenoma did not die quickly as seen with adenocarcinoma.

Results for Time to Death due to **Fibroadenoma** Specified Analysis

Treatment	Total	Number of Rats with Fibroadenoma Specified as Cause of Death	Censored	Percent Censored
Placebo	65	3	62	95.38
Lorcaserin 10 mg/kg/day	65	18	47	72.31
Lorcaserin 30 mg/kg/day	65	25	40	61.54
Lorcaserin 100 mg/kg/day	75	21	54	72.00
	270	67	203	75.19

Treatment Comparisons	Hazard Ratio	95% Confidence Interval	p-value
Lorcaserin 10 mg/kg/day vs Placebo	7.92	(2.33, 26.92)	0.0009
Lorcaserin 30 mg/kg/day vs Placebo	15.09	(4.54, 50.12)	<.0001
Lorcaserin 100 mg/kg/day vs Placebo	58.74	(16.53, 208.70)	<.0001



Deaths due to “presumed fibroadenoma” were also significant at all doses of lorcaserin relative to control.

Time of First onset (detection) of Mammary Tumors and multiplicity

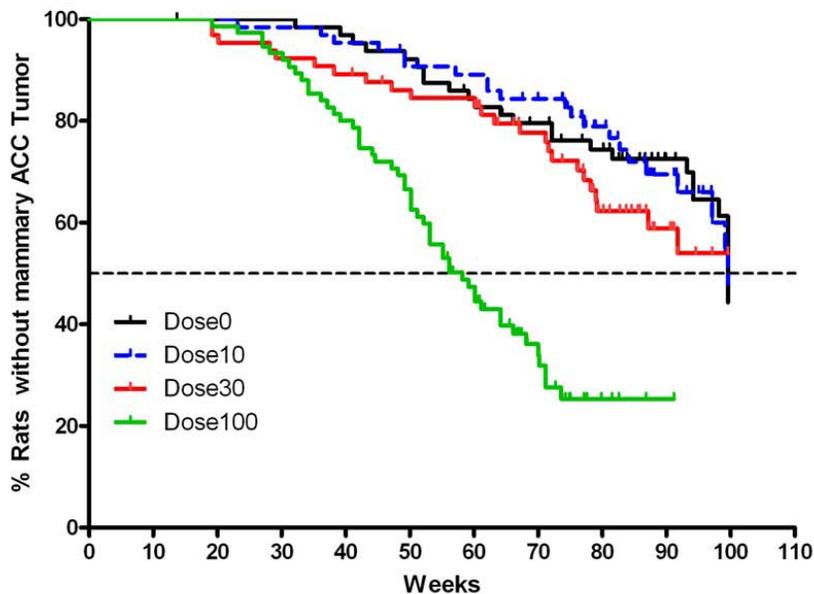
Mammary adenocarcinoma onset was significantly shorter in the HD lorcaserin rats than control. The first appearance of adenocarcinoma in the LD and MD was not different from control. HD lorcaserin was also associated with significant increase in adenocarcinoma tumor multiplicity suggesting that adenocarcinoma in these rats were more aggressive.

Results for Time to First Detection of Adenocarcinoma Analysis

Treatment	Total	Number of Rats with Adenocarcinoma	Censored	Percent Censored
Placebo	65	26	39	60.00
Lorcaserin 10 mg/kg/day	65	21	44	67.69
Lorcaserin 30 mg/kg/day	65	24	41	63.08
Lorcaserin 100 mg/kg/day	75	51	24	32.00
	270	122	148	54.81

Treatment Comparisons	Hazard Ratio	95% Confidence Interval	p-value
Lorcaserin 10 mg/kg/day vs Placebo	0.93	(0.52, 1.66)	0.7994
Lorcaserin 30 mg/kg/day vs Placebo	1.29	(0.74, 2.27)	0.3719
Lorcaserin 100 mg/kg/day vs Placebo	4.73	(2.82, 7.96)	<0.0001

Adenocarcinoma tumor onset in female rats



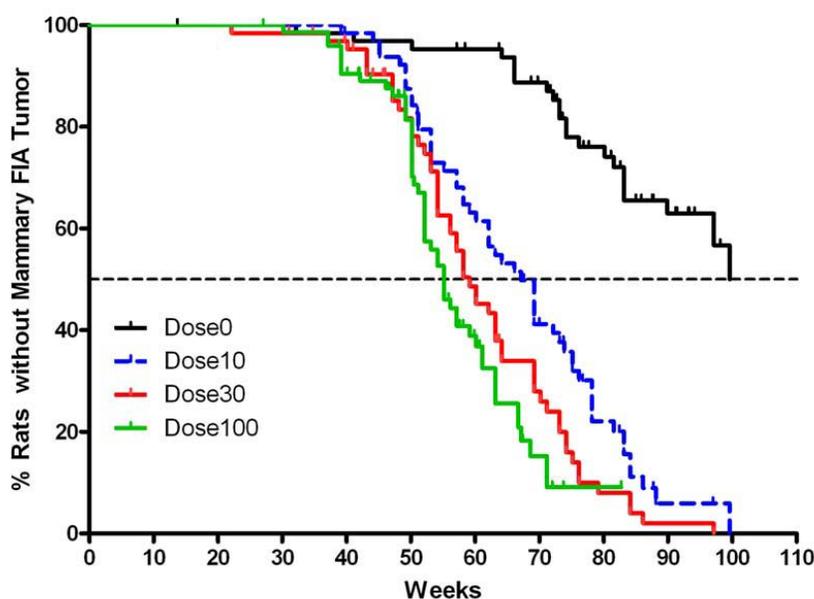
Mammary fibroadenoma tumor onset and multiplicity was significantly increased at all doses of lorcaserin relative to control.

Results for Time to First Detection of Fibroadenoma Analysis

Treatment	Total	Number of Rats with Fibroadenoma	Censored	Percent Censored
Placebo	65	24	41	63.08
Lorcaserin 10 mg/kg/day	65	54	11	16.92
Lorcaserin 30 mg/kg/day	65	55	10	15.38
Lorcaserin 100 mg/kg/day	75	51	24	32.00
	270	184	86	31.85

Treatment Comparisons	Hazard Ratio	95% Confidence Interval	p-value
Lorcaserin 10 mg/kg/day vs Placebo	5.28	(3.19, 8.76)	<0.0001
Lorcaserin 30 mg/kg/day vs Placebo	8.16	(4.88, 13.66)	<0.0001
Lorcaserin 100 mg/kg/day vs Placebo	11.49	(6.65, 19.87)	<0.0001

Fibroadenoma Tumor Onset in female rats



The sponsor stated that the significance of increased fibroadenoma multiplicity at LD and MD as compared to HD is unknown.

The significance of the greater incidence of mammary fibroadenomas and greater multiplicity in the 10 and 30 mg/kg/day groups as compared to the 100 mg/kg/day group is unknown, but may be related to the overall earlier mortality in the 100 mg/kg/day group.

Regions, size and the number of masses observed (number of times observed/number of animals affected) in female rats are listed in tables below:

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Mass Findings* - FEMALE
Weeks 2 to 100

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Alive at Start of Interval	65	65	55	75
Mass				
Abdominal region, Large >or=4 cm	0/0	22/2	0/0	12/1
Abdominal region, Mass > 10cm	0/0	5/1	0/0	0/0
Abdominal region, Medium 2-3.9 cm	5/1	15/4	25/2	4/1
Abdominal region, Small 1-1.9 cm	18/1	9/3	4/1	4/1
Abdominal region, Ulcerated, medium 2-3.9 cm	3/1	0/0	0/0	0/0
Abdominal region, Ulcerated, small 1-1.9 cm	2/1	0/0	0/0	0/0
Anogenital region, Large >or=4 cm	18/3	36/4	75/9	29/7
Anogenital region, Mass > 10cm	6/3	6/3	2/2	0/0
Anogenital region, Medium 2-3.9 cm	54/9	82/10	61/10	36/9
Anogenital region, Small 1-1.9 cm	20/5	24/7	36/8	53/10
Anogenital region, Ulcerated, large >or=4 cm	4/2	2/1	6/2	12/6
Anogenital region, Ulcerated, medium 2-3.9 cm	6/2	0/0	1/1	1/1
Anogenital region, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	1/1
Axillary region/left, Large >or=4 cm	25/3	62/10	124/13	99/17
Axillary region/left, Mass > 10cm	2/2	3/2	2/2	0/0
Axillary region/left, Medium 2-3.9 cm	57/6	154/21	153/33	129/33
Axillary region/left, Small 1-1.9 cm	57/6	104/21	171/34	157/39
Axillary region/left, Ulcerated, large >or=4 cm	0/0	7/2	2/2	18/10
Axillary region/left, Ulcerated, medium 2-3.9 cm	0/0	1/1	9/5	31/10
Axillary region/left, Ulcerated, small 1-1.9 cm	0/0	6/1	12/3	10/4
Axillary region/right, Large >or=4 cm	9/2	118/14	135/17	133/19
Axillary region/right, Mass > 10cm	0/0	5/2	3/2	0/0
Axillary region/right, Medium 2-3.9 cm	73/8	126/21	212/35	149/29
Axillary region/right, Small 1-1.9 cm	63/8	154/25	144/32	144/39
Axillary region/right, Ulcerated, large >or=4 cm	4/1	3/1	8/3	22/6
Axillary region/right, Ulcerated, medium 2-3.9 cm	5/3	8/2	2/2	20/9
Axillary region/right, Ulcerated, small 1-1.9 cm	2/1	9/1	0/0	9/3
Cervical region, Large >or=4 cm	20/2	3/1	19/2	23/5
Cervical region, Mass > 10cm	6/1	0/0	0/0	0/0
Cervical region, Medium 2-3.9 cm	7/2	59/6	41/6	22/7
Cervical region, Small 1-1.9 cm	3/2	36/7	14/5	10/6
Cervical region, Ulcerated, large >or=4 cm	1/1	0/0	2/1	1/1
Cervical region, Ulcerated, medium 2-3.9 cm	0/0	0/0	1/1	0/0
Dorsal surface, Large >or=4 cm	0/0	1/1	0/0	2/1
Dorsal surface, Medium 2-3.9 cm	11/1	0/0	0/0	1/1
Dorsal surface, Small 1-1.9 cm	21/1	0/0	0/0	3/1
Dorsal surface, Ulcerated, large >or=4 cm	0/0	1/1	0/0	2/1
Face, Small 1-1.9 cm	0/0	4/1	0/0	0/0
Face, Ulcerated, medium 2-3.9 cm	0/0	1/1	0/0	0/0
Face, Ulcerated, small 1-1.9 cm	0/0	5/1	0/0	0/0
Hind limb/left, Medium 2-3.9 cm	0/0	0/0	0/0	8/1
Hind limb/left, Small 1-1.9 cm	0/0	0/0	0/0	12/2
Hind limb/right, Small 1-1.9 cm	0/0	0/0	0/0	8/1
Inguinal region/left, Large >or=4 cm	4/1	96/15	162/17	116/13
Inguinal region/left, Mass > 10cm	1/1	11/5	11/5	1/1
Inguinal region/left, Medium 2-3.9 cm	58/4	210/22	104/18	70/18
Inguinal region/left, Small 1-1.9 cm	13/3	53/18	99/23	74/19
Inguinal region/left, Ulcerated, large >or=4 cm	0/0	2/2	6/3	3/2
Inguinal region/left, Ulcerated, medium 2-3.9 cm	0/0	0/0	2/1	2/2
Inguinal region/left, Ulcerated, small 1-1.9 cm	1/1	0/0	1/1	4/3
Inguinal region/right, Large >or=4 cm	20/4	96/11	81/12	99/16
Inguinal region/right, Mass > 10cm	2/1	5/3	1/1	0/0
Inguinal region/right, Medium 2-3.9 cm	37/5	159/21	94/21	109/22
Inguinal region/right, Small 1-1.9 cm	23/4	74/16	59/14	68/22
Inguinal region/right, Ulcerated, large >or=4 cm	5/2	1/1	5/3	16/7
Inguinal region/right, Ulcerated, medium 2-3.9 cm	1/1	1/1	3/2	2/2

*Number of times observed/Total number of animals affected

Summary of Mass Findings* - FEMALE
Weeks 2 to 100

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Mass				
Inguinal region/right, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	1/1
Shoulder/left, Large >or=4 cm	0/0	2/1	0/0	0/0
Shoulder/left, Medium 2-3.9 cm	4/1	11/1	0/0	0/0
Shoulder/left, Small 1-1.9 cm	12/1	13/1	4/1	0/0
Shoulder/left, Ulcerated, large >or=4 cm	0/0	0/0	0/0	2/1
Shoulder/left, Ulcerated, medium 2-3.9 cm	8/1	0/0	0/0	1/1
Shoulder/left, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	1/1
Shoulder/right, Large >or=4 cm	0/0	14/1	5/1	3/1
Shoulder/right, Mass > 10cm	0/0	0/0	0/0	1/1
Shoulder/right, Medium 2-3.9 cm	0/0	2/1	3/1	6/1
Shoulder/right, Small 1-1.9 cm	0/0	5/1	3/1	6/1
Shoulder/right, Ulcerated, large >or=4 cm	0/0	0/0	0/0	1/1
Thoracic region, BI Large Ulcerated >4cm	0/0	0/0	0/0	1/1
Thoracic region, Large >or=4 cm	4/1	1/1	30/4	14/3
Thoracic region, Mass > 10cm	1/1	0/0	0/0	0/0
Thoracic region, Medium 2-3.9 cm	13/2	4/1	34/7	9/3
Thoracic region, Small 1-1.9 cm	9/3	5/1	13/5	7/4
Thoracic region, Ulcerated, large >or=4 cm	0/0	0/0	0/0	2/1
Thoracic region, Ulcerated, medium 2-3.9 cm	0/0	0/0	1/1	4/2
Thoracic region, Ulcerated, small 1-1.9 cm	0/0	0/0	3/1	9/2
Ventral surface, Small 1-1.9 cm	1/1	0/0	0/0	0/0
Ventral surface, Ulcerated, small 1-1.9 cm	1/1	0/0	0/0	0/0

*Number of times observed/Total number of animals affected

Gross Pathology (amended report)

Summary of Macroscopic Observations - FEMALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	
Number of Animals Examined		42	23	53	12	60	5	75	0	
mammary gland										
enlarged	- severe	0	0	0	0	1	0	0	0	
swollen/thickened		7	7	12	1	10	2	13	0	
	- mild	7	3	6	1	2	0	3	0	
	- moderate	0	3	6	0	6	1	8	0	
thickened	- severe	0	1	0	0	2	1	2	0	
	- mild	0	0	0	0	0	0	2	0	
	- moderate	0	0	0	0	0	0	1	0	
pituitary gland										
cyst		0	0	0	0	3	0	1	0	
	- mild	0	0	0	0	2	0	0	0	
	- moderate	0	0	0	0	1	0	1	0	
enlarged		32	15	39	0	36	3	39	0	
	- minimal	1	2	2	1	2	1	1	0	
	- mild	6	5	11	2	11	2	23	0	
	- moderate	11	5	15	3	12	0	11	0	
	- severe	14	3	11	2	11	0	4	0	
skin										
abrasion/scab	- mild	1	0	0	0	1	0	0	0	
hair sparse		0	0	3	0	0	0	0	0	
	- mild	0	0	2	0	0	0	0	0	
	- moderate	0	0	1	0	0	0	0	0	
skin, subcutis										
abscess		1	0	0	0	2	0	3	0	
	- mild	1	0	0	0	1	0	1	0	
	- moderate	0	0	0	0	1	0	2	0	
skin, subcutis										
cyst		0	0	0	0	0	0	2	0	
	- mild	0	0	0	0	0	0	1	0	
	- severe	0	0	0	0	0	0	1	0	
edema	- mild	0	0	1	0	0	0	0	0	
mass	- present	39	21	143	36	210	15	222	0	
nodule	- mild	0	0	0	0	0	0	2	0	

DOS - Died or euthanized on study
SNC - Scheduled necropsy

Histopathology (amended)

(b) (6) performed the initial diagnosis of mammary tumors reported in the bimonthly update. (b) (6) from (b) (4) carried out the final diagnosis of the mammary tumors for the NDA. The external peer review pathologist for the original report was (b) (6). He peer reviewed all slides and all diagnoses from 10% of randomly selected control and HD male and female rats. (b) (6) was the peer review pathologist for toxicokinetic animal histopathology. He peer reviewed several tissues (testes, epididymides, mammary glands, pituitary gland, and brain) as well as all the neoplastic and hyperplastic from four randomly selected TK rats (rat #5104, 8115, 5202 and 8215).

The five pathologists in the PWG re-adjudicated all available female rat mammary slides for the 2011 resubmission. The slides were blinded for animal ID, prior diagnosis and treatment. The high degree of consensus and ease of diagnosis was accepted as final. The five independent veterinary pathologists in the PWG were Drs. Kenneth A Schafer, Robert R. Maronpot, Daryl Thaker, Roxanne E. Baumgartner and Philips H Long.

Female rat mammary tumor re-adjudication by PWG:

- The incidence of adenocarcinoma in the lorcaserin treated rats was significantly reduced by the PWG.
- The incidence of adenocarcinoma in the LD and MD became numerically equal to or less than control. Only the HD lorcaserin significantly increased the incidence of adenocarcinoma in female rats
- The number of fibroadenoma increased in all groups more consistently. The incidence of fibroadenoma was significantly increased at all doses of lorcaserin

Re-adjudicated incidence of mammary tumors compared to incidences reported in the original study report.

Re-Adjudicated Mammary Tumors in Female SD Rats		Lorcaserin dose, mg/kg			
		0	10	30	100
Number of female rats/group		65	65	65	75
Adenocarcinoma (historical 8.3 – 37%,)	Original study	28	34	35	60
	PWG	26	21	24	51*
Fibroadenoma (Historical 22-54%)	Original study	20	47	53	45
	PWG	24	54*	55*	51*
Adenoma	Original study	0	0	0	0
	PWG	1	2	5	4

*statistical significance by trend and pair-wise comparison

Female rat lung tumor re-adjudication by PWG:

The number of lung metastasis from adenocarcinoma in the lorcaserin treated rats was reduced. The PWG considered the incidence of lung metastases in the LD lorcaserin to be nearly similar to control and equivocally increased in the MD and HD. It should be noted that HD female rats died prematurely therefore, the impact of early deaths in preventing mammary metastases to spread to the lungs is unknown.

Re-adjudicated incidence of lung metastases compared to incidences reported in the original study report.

Lung tumors in female rats		Lorcaserin dose, mg/kg			
		0	10	30	100
Number of female rats/group		65	65	65	75
Adenocarcinoma		26	21	24	51
Lung metastases from primary mammary adenocarcinoma (Historical range 0-12.5%)	Original study	0	2	7	6
	PWG	0	1 (5%)	5 (21%)	5 (10%)

Summary of Neoplastic Lesions and Table of Tumor Bearing Animals - FEMALE

Tissue Diagnosis	Terminal											
	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
	No. with Tumor	Animal No.	Fate/Day	No. with Tumor	Animal No.	Fate/Day	No. with Tumor	Animal No.	Fate/Day	No. with Tumor	Animal No.	Fate/Day
lung	(65)			(65)			(65)			(75)		
adenocarcinoma, malignant, secondary	0			1	2224	E 425	5	3208 3237 3238 3240 3255	E 217 E 501 E 424 E 474 E 243	5	4206 4220 4238 4266 4270	D 393 E 272 E 601 E 442 E 380
carcinoma, bronchiolar alveolar, malignant, primary	0			0			1	3240	D 556	0		
carcinoma, malignant, secondary	0			3	2241 2249 2259	E 273 S 697 S 697	4	3202 3209 3244 3249	E 554 D 600 E 642 D 556	2	4234 4246	E 579 D 442

S - Scheduled necropsy
E - Euthanized *in extremis*
D - Died on Study
() - Total number examined

No. - Number

Incidence of Primary and Metastatic Mammary Adenocarcinomas in Female Sprague-Dawley Rats – PWG Re-adjudicated Findings				
	Lorcaserin dose (mg/kg/day)			
	0	10	30	100
Number of Females	65	65	65	75
Adenocarcinoma, primary tumor	26	21	24	51
Adenocarcinoma, secondary (lung) ^a	0	1	5	5
Lung metastases – as (%) of all rats	0%	1.5%	7.7%	6.7%
Lung metastases – as (%) of rats with primary adenocarcinomas	0%	5%	21%	10%

^a The PWG used “adenocarcinoma, malignant, secondary” to identify metastatic tumors of mammary origin.

Provided for Reference:

Updated incidences of tumors with statistical significance for trend and pair wise comparisons. Only mammary and lung tumors were re-adjudicated and adjusted.

Neoplastic tumors in female rats treated with lorcaserin ^a

(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in female rats		Lorcaserin dose, mg/kg				Dose Response
		0	10	30	100	
Brain	astrocytoma	0	2	0	1	NS
	mixed glioma	0	0	0	1	NS
	combined	0	2	0	2	
Lung	Adenocarcinoma in origin	0	1	5	5	NS
	Non mammary in origin	0	3	4	2	NS
Mammary	adenocarcinoma	26	21 NS	24 NS	51 p=0.0012	p=0.0005
	fibroadenoma	24	54 p<0.0001	55 p<0.0001	51 p=0.0003	p=0.0004
Skin,	fibrosarcoma, subcutis	0	0	0	2	p=0.067
Pituitary	adenoma, pars distalis, benign	50	46 (NS)	31 (p=0.001)	20 (p<0.0001)	p<0.0001
	carcinoma, pars distalis	0	2	0	0	NS
	combined	50	48	31	20	
Thyroid, adenoma follicular		2	2	4	3	p=0.03
Uterus with cervix, benign glandular polyp		0	0 NS	3 NS	3 NS	p=0.003

^a The statistical analysis and p values in the table were provided by the FDA statistician, Dr. Mathew Jackson.
NS = not significant

Tumors in male rats were not adjusted (original report)

Neoplastic tumors in male rats treated with lorcaserin ^a

(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in male rats		Lorcaserin dose, mg/kg				Dose Response
		0	10	30	100	
Brain	astrocytoma	1	0	4 ND	8 ^b p=0.0019	< 0.0001
	mixed glioma	0	0	0	1	NS
	reticularis	0	1	0	1	NS
	granular cell	0	0	1	1	NS
	oligodendroglioma	1	0	0	0	NS
	combined	2	1	5	20	
Liver	hepatocellular carcinoma	1	3	2	4	NS
	hepatocellular adenoma	1	1	2	6 p=0.030	p=0.033
	combined	2	4	4	10 p=0.0048	p=0.0012
Lung,	carcinosarcoma, sec.	0	1	1	0	NS
	fibrosarcoma, sec.	0	0	0	1	
	osteosarcoma, sec.	0	0	1	0	
	mesothelioma, sec.	1	1	0	0	
Mammary	adenocarcinoma,	0	0	2	2 NS	p=0.046
	fibroadenoma	0	1	4 NS	6 NS	p=0.0001
	combined	0	1	6 p=0.0131	8 p=0.0009	p< 0.0003
Pituitary, adenoma, pars distalis, benign		32	22 NS	22 NS	15 p=0.0003	p=0.0005
Skin, subcutis	benign fibroma	3	7 NS	11 p=0.017	17 p<0.0001	p<0.0001
	fibrosarcoma	1	0	0	0	
	combined	4	7	11	17	
Skin	squamous cell carcinoma,	0	0	4 NS	5 p=0.014	p=0.003
	papilloma	1	0	1	2	NS
	combined	1	0	5	7	
Schwannoma, all sites		0	0	2	9 p<0.0037	p<0.0001
Schwannoma, Subcutis alone			0	1	5 p=0.047	p=0.0053
Thyroid, follicular cell adenoma, benign		0	5 p=0.02	4 NS	8 p=0.0011	p=0.0035

^a the FDA statistician, Dr. Jackson, provided a Statistical analysis and p values in the table

^b One of the astrocytomas in the HD males was reclassified as infarct due to lymphocytic leukemia in an amendment to the NDA.

Brain Tumors (Astrocytoma) Safety Margin

Lorcaserin significantly increased brain astrocytoma in male rats in a dose-dependent manner (trend $p < 0.0001$). There was no significant increase in brain tumors in female rats. The incidence of brain astrocytoma was significantly increased in HD males (10.67%) and was numerically higher in the MD male (6.15%) vs. concurrent control (1%) and (b) (4) historical control data (0-5%). There were several cases of astrocytoma in the TK rats. Overall, out of 20 astrocytomas in rat carcinogenicity study (main and TK, male and female rats), 19 were in the lorcaserin treated rats suggesting that astrocytoma was clearly a lorcaserin related effect and that astrocytoma can appear within a year of lorcaserin treatment in rats.

Brain astrocytoma in SD rats		Lorcaserin dose, mg/kg			
		0	10	30	100
Safety margin			70	340	1303
Main Study Rats, astrocytoma	M	1/65	0/65	4/65	8/75
	F	0/65	2/65	0/65	1/75
Toxicokinetic Study Rats, astrocytoma	M	0/6	0/14	0/11	1/14
	F	0/5	0/14	1/14	2/10

Pituitary adenoma

The incidence of benign pituitary tumors of pars distalis (encompassing anterior pituitary) was significantly lower in the lorcaserin treated male (LD: 22/65, MD: 22/65, HD: 15/75) and female (LD: 46/65, MD: 31/65, HD: 20/75) than control male (32/65) and female rats (50/65). The incidence of benign pituitary tumors in the concurrent control males (49%) and females (77%) were within the (b) (4) historical data range for male (44 to 67%) and female SD rats (74 to 84.6%). In pairwise comparisons, the incidence was increased in males at 100 mg/kg and in females at ≥ 30 mg/kg. Pars distalis makes up the biggest section of the pituitary gland. It arises from Rathke's pouch surrounding pars nervosa from the brain. Pars distalis is under control of the brain and responsible for release of several hormones [prolactin (ϵ -cells), GH(α -cells), FSH(δ -cells), LH (δ -cells), TSH]. Prolactin released by the pars distalis of the pituitary gland is responsible for mammary gland development and milk secretion. Based on anatomical location and physiological function, a benign tumor of the pars distalis is likely to lead to excessive production of these hormones, particularly prolactin leading to mammary gland hyperplasia and eventually to mammary tumor. The high incidence of pituitary tumors in the control rats may explain the unusually higher incidence of mammary tumor in female rats (43%) and feminization of the control male rats (~50%). Most of the CNS active drugs that cause mammary tumors have been frequently associated with pituitary tumors. Interestingly, microscopic evaluation of the pituitary gland found significantly higher incidence of pituitary hyperplasia (particularly pars distalis) with increase in lorcaserin dose ($p < 0.05$ at 30 mg/kg) suggesting that a) premature deaths had prevented full development to tumor, b) perhaps lorcaserin prevented aggressive

growth of pituitary tumors from hyperplasia to adenoma which would support the decrease in prolactin in male rats.

Malignant Schwannomas

There was a lorcaserin-related increase in the incidence of malignant schwannomas (all sites combined) in male rats at 30 (2/65) and 100 (9/75) mg/kg. There was no schwannomas in the control or LD males (4.8x the clinical dose of 10 mg BID). The increase at the MD and HD is considered related to lorcaserin, thus providing a safety margin of 5x the clinical dose. Schwannomas across all locations (kidney, eyes thoracic and abdominal cavity, bone, skin subcutis) were characterized as small round neoplastic cells with unclear border. In at least 3 of the HD male rats their metastasis were seen in the lungs and thymus. Overall, the incidence of combined schwannomas in the HD males ($p=0.0037$) was above the (b)(4) historical control suggesting that schwannomas were drug-related.

Skin fibroma and Squamous cell carcinoma

Lorcaserin increased the incidence of benign skin fibroma in male rats in a dose-dependent manner (3/65C, 7/65 LD, 11/65 MD and 17/75 HD). Although the incidence of fibroma was statistically increased only in MD (16.9%) and HD (22.7%) male, the rate in the LD males (10.8%) was numerically higher than concurrent control (4.6%) and the (b)(4) historical control data (0 to 5%). There were only few incidences of fibroma in female rats (3LD and 1 MD). Lorcaserin also increased ($p > 0.05$) the incidence of skin squamous cell carcinoma in MD (4/65, 6.15%) and HD (5/75, 6.67%) male rats relative to corresponding control and (b)(4) historical control data (0 to 1.7%). Only two HD females had squamous cell carcinomas. The increase in skin fibroma and squamous carcinoma at the MD and HD are considered related to lorcaserin. The squamous cell carcinomas were observed in the anogenital/inguinal, head and neck area. The skin in these animals was visibly ulcerated. The increased incidence of fibroma at 10 mg/kg (4.8x the clinical dose, based on AUC) in male rats suggest that lorcaserin may have an off target peripheral tumorigenic effect at close to the clinical dose. Skin fibroma and squamous cell carcinomas occurred primarily in male rats. Whether shorter life span had played a role in female rats not developing these other tumors is not clear.

Liver and Thyroid tumors

Lorcaserin significantly increased the trend for hepatocellular adenoma in male rats (1/65, 2/65 and 6/75 in C, LD, MD and HD, respectively) but the increase was not significant in the pair wise comparisons relative to control. Similarly, higher incidence of hepatocellular carcinoma in male rats was not significant in the trend and pairwise comparisons (1, 3, 3 and 4 in C, LD, MD and HD).

Since the incidences of both adenoma and carcinoma in HD (adenoma 8% and carcinoma 5.3%) males were greater than the (b)(4) historical data for adenoma (0-5.7%) and carcinoma (0 to 1.7%) suggests that liver tumors in males at 100 mg/kg were likely drug metabolism related. With no significant increase in pair wise comparison and high exposure multiples ($> 55x$ the clinical dose of 10 BID on AUC basis), the potential risk to humans is deemed minimal.

The trend for incidence of thyroid follicular cell adenoma was significant in lorcaserin treated males. The incidence in the C, LD, MD and HD male rats were 0/65, 5/65, 4/65 and 8/75, respectively. Although the trend was positive only the incidence in

the HD (10.6%) was statistically significant. The (b) (4) historical control for follicular cell adenoma in male rats ranged from 0 to 11.7% (\bar{x} =2.2%). The incidence of follicular cell adenoma was within the historical range but it should be noted that historical range is skewed by one group at 11.7% with remaining between 0 and 3%. The trend for this tumor was not significant in female rats.

Lorcaserin induced UGT and cytochrome P450 enzymes and resulted in a greater degree of hepatocellular hypertrophy in males than in females. Thyroid follicular cell tumors often accompany hepatic tumors that arise from induction of drug-metabolizing enzymes, a likely consequence of increased triiodothyronine turnover by the liver with secondary chronic thyroid follicular cell stimulation. Induction of drug-metabolizing enzymes in the liver of human subjects is not known to lead to hepatic carcinogenesis, as typified by clinical experience with phenobarbital. With a reasonable safety margin to non-tumorigenic exposure and, more significantly, a recognized mechanism of tumorigenesis, the potential risk of hepatic and thyroid tumors to humans is considered minimal.

Non-neoplastic findings in male rats:

Microscopic evaluation of the tissues in the control and lorcaserin treated male rats were not changed. The most affected target organs in males are listed below.

- Adrenal gland, cortical angioectasis/cystic degeneration
- Brain, minimal gliosis and mineralization
- Bone, marrow atrophy and granulocytic hyperplasia
- Epididymal, vacuolation
- Liver, centrilobular hypertrophy, vacuolation, focal cystic degeneration,
- Lung, alveolar histiocytosis and lipidosis or cholesterol cleft
- Pituitary gland hyperplasia of pars distalis
- Skeletal muscle atrophy and degeneration
- Spinal cord degeneration
- Spleen and mesenteric lymph node lymphoid depletion
- Skin ulceration
- Thyroid, follicular cell hyperplasia and hypertrophy, follicular cysts

Non-neoplastic findings in female rats:

Notable microscopic finding (hypertrophy, hyperplasia and vacuolation) in female rats have been reviewed earlier and have not changed.

- Liver, centrilobular hypertrophy, cellular alterations and necrosis
- Bone, femur and sternum hyperostosis
- Bone marrow (femur and sternum), atrophy and granulocytic hyperplasia
- Pituitary, hyperplasia/hypertrophy
- Lung, alveolar lipidosis and histiocytosis
- Tibiofemoral cartilage joint degeneration/necrosis
- Lymph nodes, hyperplasia
- Extramedullary hematopoiesis in adrenal glands, liver, spleen
- Vaginal atrophy

Re-adjudication of mammary and lung secondary tumors reduced the number of adenocarcinoma and increased incidence of fibroadenoma in lorcaserin and control

rats in the resubmission. Therefore only the microscopic observation of the mammary and lung tissues shown in tables below. The microscopic evaluation of the remaining tissues can be found in the original 2009 NDA review.

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE

Tissue	Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
			DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
	Number of Animals Examined		42	23	53	12	60	5	75	0
	lung		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
	adenocarcinoma, malignant, secondary		0	0	1	0	5	0	5	0
	carcinoma, bronchiolar alveolar, malignant, primary		0	0	0	0	1	0	0	0
	carcinoma, malignant, secondary		0	0	1	2	4	0	2	0
	cholesterol crystals		4	3	2	0	3	1	2	0
	- minimal		2	2	2	0	3	1	1	0
	- mild		2	1	0	0	0	0	1	0
	hemangiosarcoma, malignant, multicentric		0	0	0	0	1	0	0	0
	hemorrhage		0	0	0	0	1	0	0	0
	histiocytosis, alveolar		7	5	15	5	28	3	47	0
	- minimal		6	6	11	4	17	3	22	0
	- mild		1	0	4	1	10	0	24	0
	- moderate		0	0	0	0	1	0	4	0
	lipidosis, alveolar		0	0	0	0	3	0	24	0
	- mild		0	0	0	0	2	0	14	0
	- moderate		0	0	0	0	1	0	6	0
	- severe		0	0	0	0	0	0	4	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE
Terminal

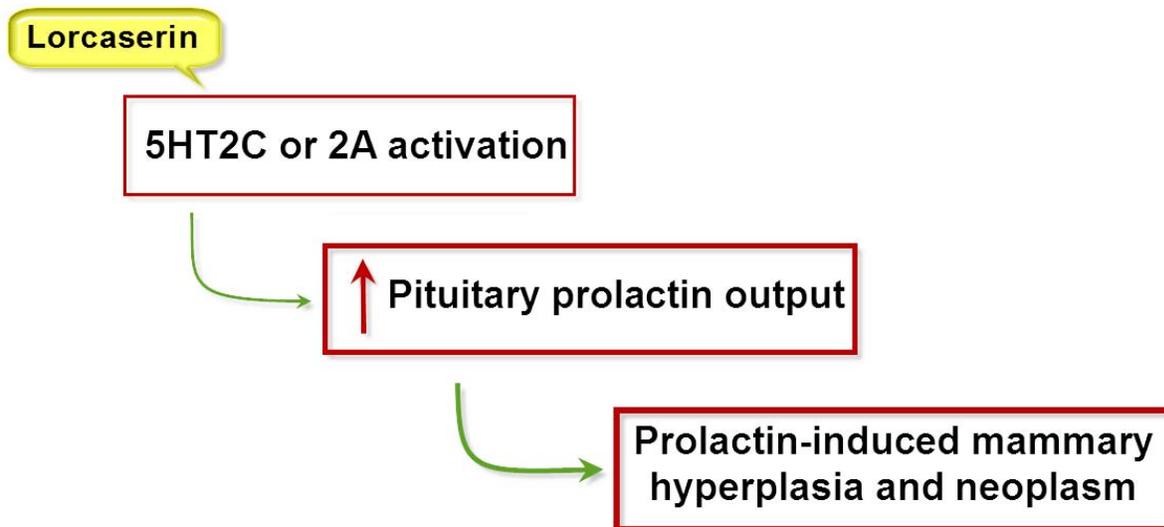
Tissue	Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
			DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
	Number of Animals Examined		42	23	53	12	60	5	75	0
	mammary gland		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
	adenocarcinoma, malignant, primary		16	10	16	5	24	0	51	0
	adenoma, benign, primary		1	0	2	0	4	1	4	0
	carcinosarcoma, malignant, primary		0	0	0	0	0	0	1	0
	fibroadenoma, benign, primary		16	8	42	12	50	5	51	0
	galactocoele		8	10	10	4	2	4	1	0
	- minimal		0	2	2	2	1	1	0	0
	- mild		8	8	8	2	1	3	1	0
	- moderate		0	0	0	1	0	0	0	0
	hemorrhage		12	12	10	4	17	5	26	0
	hyperplasia, lobular		1	5	2	1	0	3	2	0
	- minimal		9	6	15	3	13	0	19	0
	- mild		2	1	2	0	4	2	5	0
	- moderate		14	4	16	1	26	0	22	0
	hyperplasia, lobular with atypia		5	3	8	0	8	0	10	0
	- mild		9	1	8	1	18	0	12	0
	- moderate		3	3	2	0	1	0	0	0
	within normal limits									

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

10 Mechanistic Studies

New Mechanistic Studies in the 2011 Resubmission Designed To Explore the Role of Prolactin in Lorcaserin Induced Mammary Neoplasia in Rats

The sponsor has proposed the lorcaserin-related mammary tumors were likely due to activation of 5HT_{2C}, which is reported to increase pituitary prolactin output, which is associated to induction of mammary hyperplasia and neoplasms in rodents.



Anti-dopaminergic antipsychotic drugs are known to result in a robust and sustained increase in plasma prolactin in rodents and to some extent in humans. Chronic administration of antidopaminergic drugs are known to result in mammary tumors in rodents via this mechanism. The relevance of this MOA to human risk is unresolved, but rodents are clearly susceptible to this MOA. In the original NDA, the sponsor performed series of prolactin mechanistic studies to support the prolactin hypothesis. However, the studies (discussed in at the first advisory committee meeting) failed to show a dose dependent increase in prolactin in intact female SD rats. An increase in prolactin was only observed in one study after a single dose of lorcaserin in male SD rats.

In the post NDA review meeting, the sponsor cited improved methodology would enable detection of prolactin signal. New studies were designed to measure plasma and pituitary prolactin in female and male SD rats treated with lorcaserin up to 3 months. Mammary tissue preneoplastic changes were evaluated at specific time points. The resubmission also included three prolactin intervention studies in female SD rats.

Study Title: Three-Month Evaluation of Lorcaserin Effects on Prolactin Concentrations and Mammary Gland Histology in Female Sprague-Dawley Rats (DBR-11-002)

Non GLP Study: Initiated June 1, Completed on Oct 27, Finalized on Dec 14, 2011

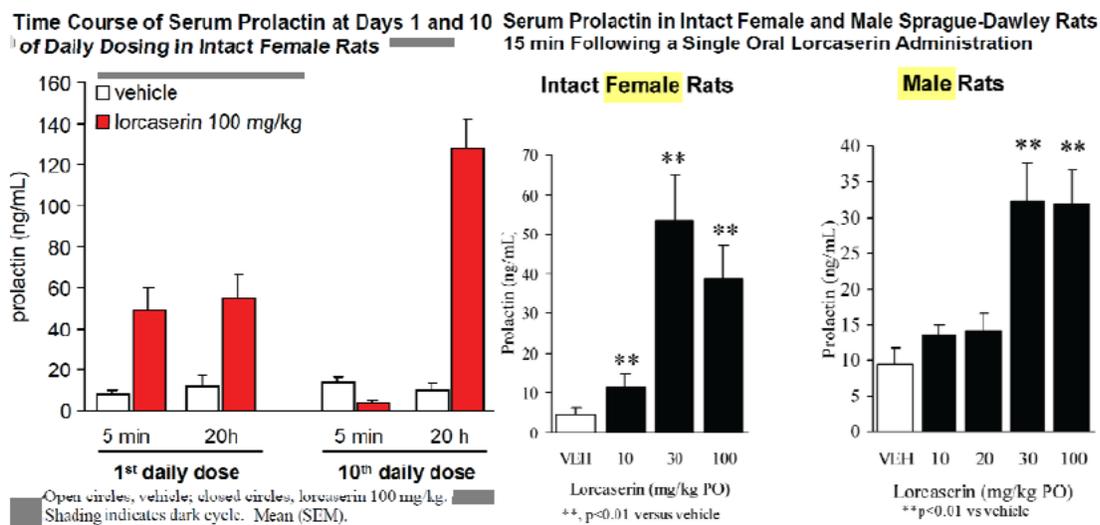
Key Findings:

- Lorcaserin had no effect on 1 hr post dose plasma prolactin levels. Lorcaserin dose of 100 mg/kg significantly increased 20 hr plasma prolactin levels during the first 10 days (Day 1, 7 and 10). Prolactin levels were unchanged after 10 and 30 mg/kg lorcaserin.
- The positive control, perphenazine (5 mg/kg) significantly and robustly increased 1 and 20 hr post dose plasma prolactin levels throughout the study. Plasma prolactin levels at 1 hr post dose were up to 80x higher than 20 hr post dose prolactin levels (in spite of significant elevation at 20 hr post dose) corresponding to perphenazine T_{max}.
- Lorcaserin doses increased pituitary prolactin levels (28, 63 and 91) while perphenazine significantly reduced pituitary prolactin levels on Day 28 and 91.
- Mammary gland epithelial prolactin levels were increased by perphenazine but not by lorcaserin.
- Dr. Russo had performed the whole mount histopath evaluation of the mammary tissues in unblinded manner. There were no changes in the number of terminal ducts (TD) with lorcaserin treatment on Day 28 and only a slight decrease (78 vs. 88 in control, p<0.05) was seen on Day 91. Perphenazine significantly decreased TD on both Day 28 and 91 (24.4-21 vs. 88 in control) relative to corresponding controls.
- There was no lorcaserin related change in total lobular structures (TLS) on Day 28, however, they were increased on Day 91 at 10 mg/kg and 100 mg/kg. In contrast, perphenazine significantly increased TLS on Day 28 and Day 91. According to Dr. Russo, the pattern of reduced terminal duct and increase in lobular structures in the whole mount preparation observed in lorcaserin rats was consistent with increased prolactin.
- Analysis of individual animal plasma prolactin levels and the incidence of terminal duct found no direct relationship between the prolactin and terminal duct. Furthermore, tissues identified with suspicious pathology did not match the animal number and treatment in most instances. There was a major error in assigning the right treatment to the right animal during unblinding.
- (b) (4) analysis of mammary tissues (blinded) found no significant increase in incidence of lobular hyperplasia on Day 28 or Day 91 at 30 and 100 mg/kg lorcaserin. In contrast, perphenazine significantly increased lobular hyperplasia on Day 28 and 91 (mild to marked). Lobular hyperplasia was observed in 100% of the perphenazine treated rats. Small increase in secretory product on Day 28 in lorcaserin rats was not clear since there was no increase on Day 90.
- Analysis of PCNA immunohistochemical staining of the mammary gland on Day 28 and 91 found that lorcaserin increased the number of positive nuclei at 10 mg/kg on Day 91 and at 30 mg/kg on Day 28. There was no change at 100 mg/kg. In contrast, perphenazine increased PCNA score consistent with histologically-verified mammary tissue proliferation.
- Lorcaserin had no effect on reproductive hormones known to be associated with promotion of mammary tumors, such as estradiol, progesterone, and LH.
- Plasma concentrations of perphenazine was greatest at 1 hr corresponding to maximal increase in plasma prolactin. In contrast, the lorcaserin T_{max} (~ 1 hr) did not correspond to maximal rise in plasma prolactin (20h post-dose).

Background:

As part of the CR letter (issued on Oct 22, 2010), the sponsor had proposed several nonclinical studies to explore the role of prolactin in lorcaserin-induced mammary tumors in female rats SD rats. The 3-month non-GLP study was carried out by the sponsor to show that chronic administration of lorcaserin will result in persistent and robust increase in prolactin similar to positive control, perphenazine. The Division reviewed the protocol for the study. The timing of the blood collection was originally set at 15 min post dose which the Division rejected as likely to be prone to stress and low drug exposure. The Division recommended at least 45 to 60 min post dose blood collection, to permit attainment of lorcaserin brain Tmax in rats. Based on pilot studies, the sponsor chose both 1 and 20 hrs post dose blood sample collection for plasma prolactin analysis in female SD rats^{1,2,3,4}. According to the sponsor lorcaserin raised plasma prolactin levels at 1 hr after single dose and at 20 hrs after multiple doses in females SD rats. The rise in prolactin was not age dependent. These studies were not provided, therefore it is not clear what other time points blood samples were collected. Perphenazine is an anti-D2 receptor antipsychotic agent (clinical dose: 4 to 8 mg TID).

The sponsor had conducted preliminary studies to determine the time to maximal rise in plasma prolactin in lorcaserin treated male and female SD rats. The peak time for male and female SD rats were 15 and 60 min post dose, respectively. The peak prolactin response occurred at 30 mg/kg in males and females. In the 10-day repeat dose study, prolactin was highest at 20-hr post dose on Day 10. In spite of that prolactin was increased on Day 1 at 20 hr post dose in the 3-month study.



¹ Arena Pharmaceuticals, Inc. Study Report [DBR-11-020]: Increased Plasma Prolactin after a Single Administration of Lorcaserin to Intact Female Rats (On file at Arena).

² Arena Pharmaceuticals, Inc. Study Report [DBR-11-022]: Time course of Plasma Prolactin Concentrations after Acute and Repeated Administration of Lorcaserin to the Female Rat (On file at Arena).

³ Arena Pharmaceuticals, Inc. Study Report [DBR-11-023]: Female but not Male Rats Develop a 20-hour Post-dose Peak in Plasma Prolactin after Repeated Lorcaserin Administration (On file at Arena).

⁴ Arena Pharmaceuticals, Inc. Study Report [DBR-11-024]: Lorcaserin-stimulated Increases in Plasma Prolactin are not Age Dependent in the Female Rat (On file at Arena).

Hypothesis: “The sponsor had hypothesized that lorcaserin treatment in 3-month or less will cause histological changes in mammary tissue which is associated with altered levels of prolactin in the pituitary, mammary gland, or plasma that is predictive of mammary tumor growth.”

Objective: The study was designed to evaluate plasma and tissue prolactin, mammary tissue histology (H&E and PCNA stain), reproductive hormone levels at different time intervals in female SD rats treated with lorcaserin and perphenazine for 3 months.

Methods: In this non-GLP but pivotal 3-month mechanistic study (DBR-11-002), 6-7 week old female SD rats from [REDACTED] (b)(4) were individually housed with ad lib access to food and water and acclimated for a week prior to the study. At the time of drug dosing, rats were 8-9 weeks old (56 to 63 days old mature females, 190 to 250 g). Based on literature, the window of sensitivity to prolactin is at puberty, between Day 30 and 50. The rats were treated with lorcaserin (0, 10, 30 and 100 mg/kg in water) or positive control, perphenazine (5 mg/kg in acidified water). Treatment arms were as follows: water (vehicle control), LD lorcaserin (10 mg/kg), MD lorcaserin (30 mg/kg), HD lorcaserin (100 mg/kg) and positive control, perphenazine (5 mg/kg). Rats were gavaged approximately 3 hrs after lights on (light cycle: 10:45 AM to 10:45 PM) which is approximately 2 PM. The study had 5 treatment arms run in parallel with data collection on Days 1, 7, 28, 60 and 90. More rats were used on Day 28 and 90 for histological examination of the mammary tissue samples.

Treatment Group Assignments [REDACTED]

Treatment Arm	Number of Animals Per Treatment Group				
	Vehicle	Lorcaserin 10 mg/kg	Lorcaserin 30 mg/kg	Lorcaserin 100 mg/kg	Perphenazine 5 mg/kg
1 day	12	12	12	12	12
7 day	12	12	12	12	12
28 day	60	60	60	60	60
60 day	12	12	12	12	12
90 day	60	60	60	60	60

Data Collection, time and day in the study

- Blood collections for prolactin: Interim blood samples for plasma prolactin analysis were collected from tip of the tail at 1 and approximately 20 hrs post dose corresponding to 3 PM and 10 AM, respectively. Pilot studies by the sponsor had found 1 and 20 hr post dose to be the best time points to collect blood while minimizing the effect of estrus- and circadian related effects on prolactin. In the preliminary single dose studies, lorcaserin was shown to produce maximal increase in prolactin at 15 min in males and 60 min in female SD rats.
- Terminal trunk blood samples were collected after decapitation (i.e. Day 7, 28 and 90). Plasma prolactin levels were measured by standard sandwich ELISA (rat prolactin ALPCO Immunoassay DEV 9966, Salem, NH).

- Plasma hormone levels: estradiol, LH and progesterone levels at 20-h post dose on Day 1, 7, 28, 60 and 90 in control and 100 mg/kg lorcasein rats
- Mammary tissues were collected at terminal sacrifice on Days 7, 28 and 91. Right side mammary glands were harvested on Days 28 and 90 for whole mounts (mammary tissue analysis). Left side was collected on Days 7, 28 and 90. Day 7 samples were used for mammary prolactin only while Day 28 and 90 samples were used for histological analysis and mammary prolactin content. Two independent labs (Dr Russo's lab (b) (4) evaluated mammary tissue histology. Analysis of the samples by Dr Russo's lab was not blinded while those at (b) (4) were carried out in blinded manner.

-**Dr Russo lab** examined the whole mount examination of the mammary tissue for tumorigenesis with special emphasis on the following structures:

- Percentage of structures with lobules of types 1, 2, and 3 and/or the degree of differentiation.
- Alteration in the ductal pattern of branching.
- Presence of ductal hyperplasia and/or lobular hyperplasia.
- Detection of ductal carcinoma *in situ* or invasive tumors.
- Detection of tumor masses e.g. fibroadenomas.

(b) (4) carried out standard histopath of H&E stained mammary tissue slides, PCNA (proliferating cell nuclear antigen, measure of DNA synthesis and repair) and mammary prolactin content (immunohistochemistry). For Day 28, half of the randomly selected tissues from perphenazine treated rats but none of the LD lorcasein (10 mg/kg) were analyzed. For Day 90, again half of the randomly selected perphenazine group and all the remaining groups were analyzed. It should be noted that rats have a total of 12 mammary glands (6 located in anterior thoracic, 2 abdominal area and 4 inguinal). The higher incidence of mammary tumors in rats is also likely to be due to higher number of mammary glands in rats. Both cats and dogs with 8 to 10 mammary glands have high incidence of mammary tumors. The incidence of mammary tumors in dogs (8 to 10 glands) is 3 times that in humans. The most common tumor in rats is the mammary tumor with majority being fibroadenoma⁵ (adenocarcinoma incidence is less than 10%)⁶. The incidence of breast cancer in women in US is about 120/100000 females (1.2/1000) or 0.12%.

- Pituitary tissue prolactin content was analyzed at 20 hr post dose on Day 7, 28, 60 and 90. The sponsor used methods developed by Haggi et al 1981. Briefly, frozen tissues were placed in extraction buffer. The homogenate was cleaned by centrifugation and prolactin content was measured by ELISA.

⁵ Greenacre C (2004). "Spontaneous tumors of small mammals". *Vet Clin North Am Exot Anim Pract*, 7 (3): 627–651 2004

⁶ Hillyer, Elizabeth V.; Quesenberry, Katherin E. (1997). *Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery* (1st ed.). W.B. Saunders Company

Statistical Analysis:

The non-normally distributed data such as plasma, pituitary prolactin levels were analyzed by nonparametric methods (ranked before ANOVA). Plasma prolactin levels were also categorized into low (<100 ng/ml) or high (>100 ng/ml) before analysis using Fisher's Exact Test for contrast to the control group. The normally distributed data such as mammary prolactin and PCNA immunohistochemistry were analyzed by standard one-way ANOVA. If significant, pairwise comparisons/contrasts were made between the lorcasein and the vehicle control. The sponsor describes the primary and secondary endpoints of the analysis of the collected data below:

Primary endpoints:

- Difference in mean concentration of circulating prolactin at 20 hours after dose administration on days 1, 7, 28, 60, and 90 to rats with perphenazine 5 mg/kg/day (positive control) or 10, 30, or 100 mg/kg/day lorcasein, compared to rats treated with vehicle (negative control).
- Proportion of rats with circulating prolactin concentrations greater than 100 ng/mL in the lorcasein and perphenazine groups compared with vehicle control groups, at each study collection day.

Secondary endpoints:

- Mammary histology scores (derived from whole mount analysis, H&E analysis, and PCNA analysis) and mammary prolactin content (immunohistochemical analysis) after 28 and 90 days of treatment with perphenazine 5 mg/kg/day (positive control) or 10, 30 or 100 mg/kg/day lorcasein, compared to rats treated with vehicle (negative control).
- Pituitary tissue prolactin content approximately 20 hours after dose administration on days 7, 28, 60, and 90 to rats with perphenazine 5 mg/kg/day (positive control) or 10, 30, or 100 mg/kg/day lorcasein, compared to rats treated with vehicle (negative control).
- Concentrations of circulating estradiol, luteinizing hormone, and progesterone approximately 20 hours after dose administration on days 1, 7, 28, 60, and 90 to rats with lorcasein (100 mg/kg/day) compared to rats treated with vehicle.

Results

Mortality: There were 10 deaths out of 780 rats in the study (1 from perphenazine, 2 at 30 mg/kg lorcasein and 7 at 100 mg/kg lorcasein). There were no deaths in the control animals. Two of the deaths in the high dose lorcasein were due to seizure (WK 1 and 13). The most common clinical signs were labored breathing in the first two weeks in the HD lorcasein rats (100 mg/kg).

On-Study Animal Mortality

Rat number	Treatment and Dose (mg/kg)	Study Arm	Study Day	Comments
3	Lorcaserin 30	28 day	7	Found dead; no cause found
33	Lorcaserin 30	28 day	9	Died within 5 min of dosing
124	Lorcaserin 100	28 day	9	Found dead subsequent to severe dyspnea and wasting
189	Lorcaserin 100	28 day	9	Found dead; dyspnea prior to death
245	Perphenazine	28 day	18	Found dead; no cause found
304	Lorcaserin 100	90 day	7	Died within 5 min of dosing subsequent to seizure
519	Lorcaserin 100	90 day	87	Found dead
554	Lorcaserin 100	90 day	28	Found dead; no cause found
559	Lorcaserin 100	90 day	62	Found dead subsequent to severe dyspnea and wasting; mechanical damage to esophagus
730	Lorcaserin 100	60 day	6	Died within 5 min of dosing subsequent to seizure

Original data can be found in Arena notebook (b) (4) A000140-02.

Body weight

- The 30 and 100 mg/kg lorcaserin reduced body weight of female rats by 7.3% and 13% at the end of the 3-month study, respectively. There was no meaningful change in BW at 10 mg/kg. The decrease in BW reached a plateau within 3 to 4 weeks and maintained until terminal sacrifice.
- Perphenazine (5 mg/kg) treatment resulted in significant weight loss (8%), similar to MD lorcaserin.

Summary of Body Weights

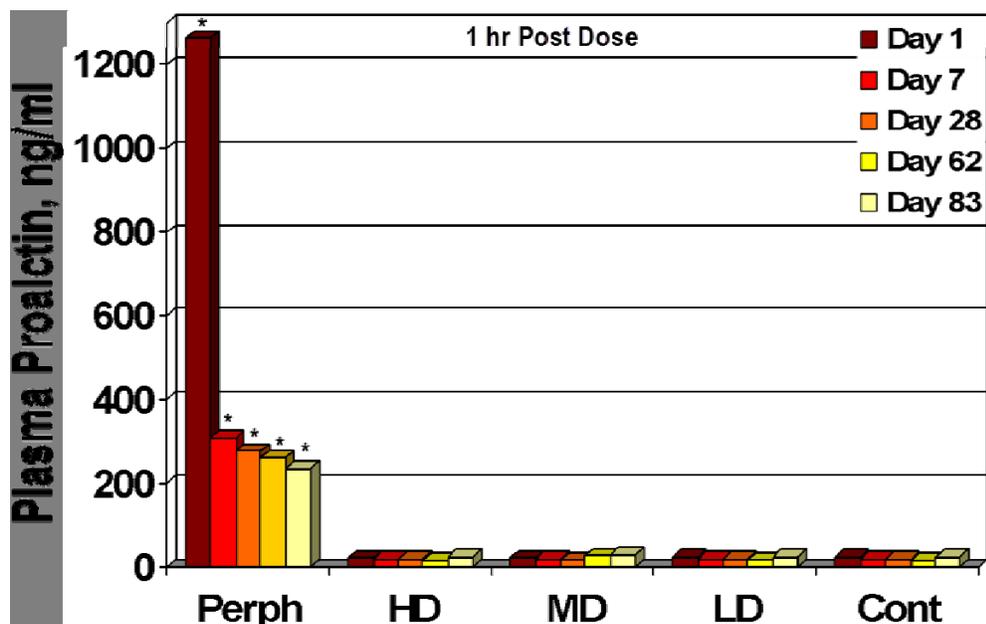
Study Arm	Treatment and Dose (mg/kg)	Body Weights (g) ^a												
		Study Week												
		1	2	3	4	5	6	7	8	9	10	11	12	13
28 day	Vehicle	227.7	236.3	251.7	266.0	ND								
		± 1.4	± 1.6	± 1.8	± 2.3									
	Lorcaserin 10	215.7	230.0	245.9	260.9	ND								
		+ 1.1	+ 1.2	+ 1.3	+ 1.6									
	Lorcaserin 30	208.5	220.3	235.5	249.8	ND								
		± 1.2	± 1.3	± 1.4	± 1.7									
60 day	Lorcaserin 100	206.1	215.1	228.1	239.0	ND								
		± 1.3	± 1.4	± 1.4	± 1.7									
	Perphenazine	220.5	239.9	247.7	260.1	ND								
		± 1.4	± 1.4	± 1.5	± 2.0									
	Vehicle	235.1	248.8	260.8	271.5	281.0	291.5	300.8	307.1	309.0	ND	ND	ND	ND
		± 1.9	± 2.2	± 2.6	± 2.5	± 2.7	± 3.0	± 3.1	± 3.4	± 5.6				
90 day	Lorcaserin 10	226.3	240.7	253.1	263.1	273.9	283.6	294.3	300.0	301.6	ND	ND	ND	ND
		+ 1.6	+ 1.8	+ 1.9	+ 2.2	+ 2.2	+ 2.6	+ 2.9	+ 5.4					
	Lorcaserin 30	215.8	229.4	241.7	252.6	262.6	273.3	281.4	286.8	285.9	ND	ND	ND	ND
		± 1.5	± 1.8	± 1.8	± 2.1	± 2.5	± 2.6	± 2.6	± 2.5	± 4.3				
	Lorcaserin 100	208.0	216.3	224.2	234.7	242.9	250.8	258.2	263.3	264.9	ND	ND	ND	ND
		± 2.4	± 3.1	± 3.6	± 3.0	± 3.0	± 3.1	± 2.9	± 3.1	± 5.9				
90 day	Perphenazine	224.0	245.3	247.0	255.8	261.5	266.7	273.0	278.6	280.4	ND	ND	ND	ND
		+ 3.1	+ 2.8	+ 2.6	+ 2.9	+ 3.0	+ 3.1	+ 3.7	+ 3.0	+ 5.9				
	Vehicle	221.3	236.5	250.2	264.7	275.4	287.8	289.7	297.6	303.6	307.3	311.6	315.8	319.7
		± 1.3	± 1.5	± 1.7	± 1.9	± 2.0	± 2.1	± 2.1	± 2.2	± 2.3	± 2.4	± 2.5	± 2.6	± 3.0
	Lorcaserin 10	214.4	231.4	247.1	262.7	274.9	283.4	291.7	298.2	305.9	307.7	312.4	315.8	319.7
		+ 1.1	+ 1.2	+ 1.5	+ 1.6	+ 1.7	+ 1.8	+ 1.9	+ 2.1	+ 2.2	+ 2.2	+ 2.3	+ 2.3	+ 2.6
90 day	Lorcaserin 30	206.8	220.3	235.5	247.6	259.1	266.3	272.4	277.9	282.4	286.0	290.4	293.8	296.4
		+ 0.9	+ 1.1	+ 1.2	+ 1.3	+ 1.4	+ 1.4	+ 1.5	+ 1.5	+ 1.6	+ 1.7	+ 1.8	+ 1.9	+ 2.2
	Lorcaserin 100	203.7	214.9	225.9	235.6	243.8	249.8	255.3	259.4	263.9	268.4	272.9	275.0	278.8
		+ 1.0	+ 1.2	+ 1.3	+ 1.2	+ 1.3	+ 1.3	+ 1.3	+ 1.3	+ 1.5	+ 1.5	+ 1.6	+ 1.7	+ 1.9
	Perphenazine	212.0	233.0	241.3	252.3	260.8	267.4	272.2	279.5	283.1	285.5	288.3	291.2	294.0
		+ 1.5	+ 1.4	+ 1.5	+ 1.5	+ 1.5	+ 1.6	+ 1.7	+ 1.7	+ 1.8	+ 1.8	+ 1.8	+ 1.8	+ 2.0

^a Mean ± SEM. Original data can be found in Arena notebook (b) (4) A000140-02.

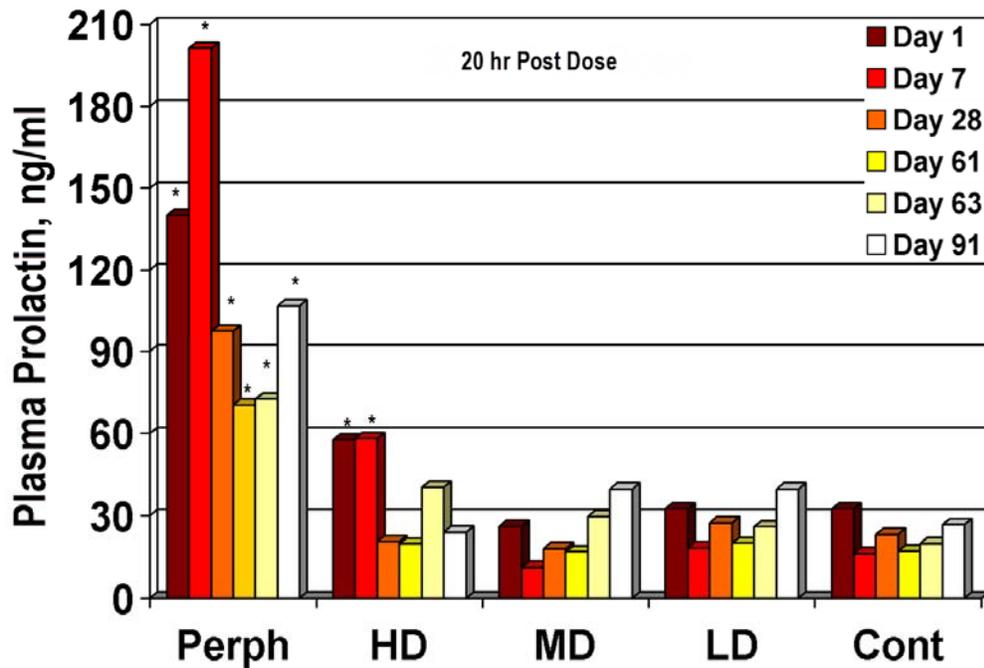
Plasma Prolactin

- Plasma prolactin levels at 1 hr post dose were not significantly increased by lorcaserin (10, 30 or 100 mg/kg) at any time point during the 90 day study.
- Plasma prolactin levels at 20 hr post dose were significantly increased only at 100 mg/kg lorcaserin during the first 10 days of the 90-day study.
- Lorcaserin doses of 10 and 30 mg/kg had no effect on plasma prolactin at any time point on any day in female rats.
- The mean prolactin levels over the 90 days however were increased at 10 and 100 mg/kg lorcaserin. There were more spikes in prolactin in lorcaserin treated rats than control.
- Positive control, perphenazine treatment (5 mg/kg) consistently and robustly and significantly increased plasma prolactin levels in female rats at 1 hr and 20 hr post dose on every data collection day (Days 7, 28 and 90) in the study, suggesting that positive control was working as expected.
- Plasma prolactin levels in the vehicle control rats tended to be slightly higher (up to 2x) at 20 hr post gavage than 1 hr post gavage. In contrast, prolactin levels in positive control were dramatically higher (up to 80x) at 1 hr post dose vs. 20 hr post dose.
- The number of rats with prolactin levels exceeding 100 ng/ml in the rats treated with 0, 10, 30 and 100 mg/kg lorcaserin and perphenazine were 0/60, 1/60, 1/60, 7/60 and 49/60 rats respectively.
- The number of rats with prolactin levels exceeding 40 ng/ml in the rats treated with 0, 10, 30 and 100 mg/kg lorcaserin and perphenazine were 1/60, 4/60, 5/60, 16/60 and 52/60 rats respectively.

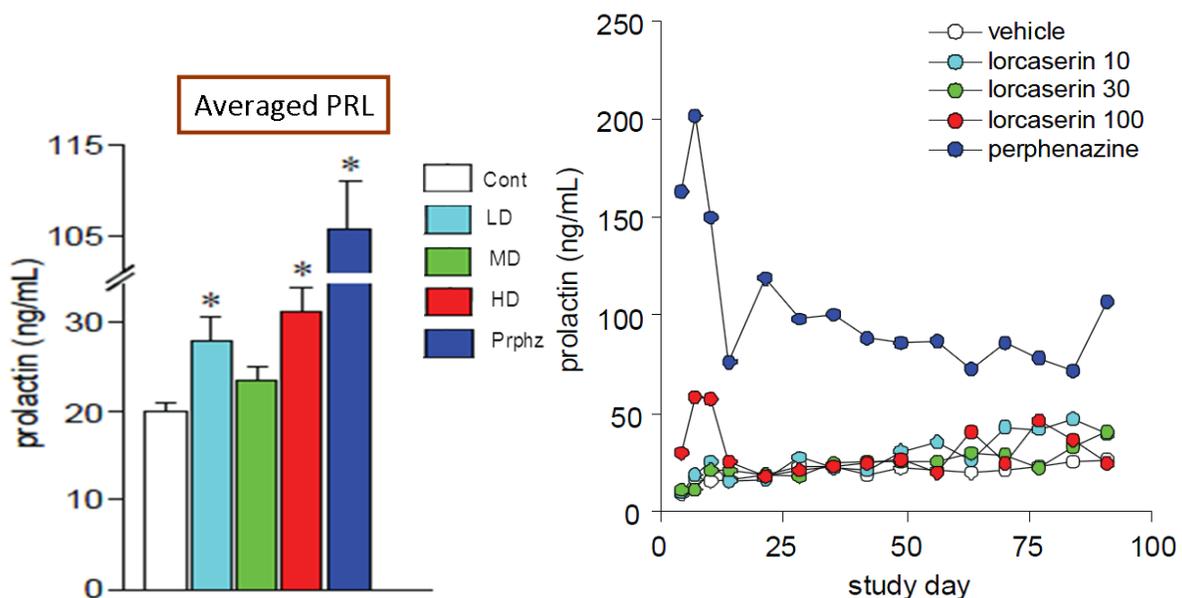
Plasma prolactin levels at 1 hr post dose in female rats treated with lorcaserin or perphenazine on Day 1, 7, 28, 60 and 90.

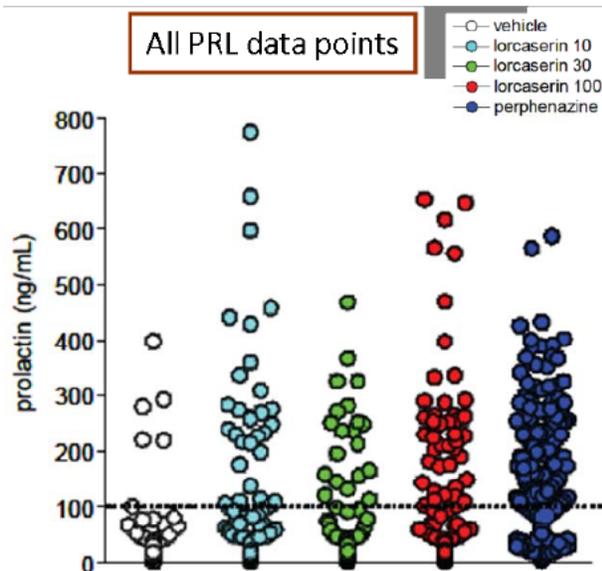


Plasma prolactin levels at 20 hr post dose in female rats treated with lorcaserin or perphenazine on Day 1, 7, 28, 60 and 90.



Averaging all plasma prolactin levels sampled during the 90-day study in each treatment group found mean prolactin in the LD and HD lorcaserin to be significantly higher than control prolactin levels (28-30 ng/ml vs. 20 ng/ml in control). Plotting all data points over time also showed occasional rise in plasma prolactin levels in lorcaserin treated rats. Lorcaserin treated rats appeared to have more frequent spikes in plasma prolactin than control rats.





The plasma prolactin levels measured in female rats treated with 10, 30 and 100 mg/kg lorcaserin or 5 mg/kg perphenazine (positive control) at different time points during the 3-month study are provided for reference.

Mean Plasma Prolactin Concentrations at 1 h Post-Dose during 90 Days of Treatment

Treatment Day	Study Arm	Treatment Group and Dose (mg/kg)	Plasma Prolactin Concentration (ng/mL) ^a
1	1 day	Vehicle	23.2 ± 1.3
		Lorcaserin 10 mg/kg	22.6 ± 1.9
		Lorcaserin 30 mg/kg	20.4 ± 0.1
		Lorcaserin 100 mg/kg	21.5 ± 1.0
		Perphenazine	1264.7 ± 227.2
7	7 day	Vehicle	18.8 ± 0.4
		Lorcaserin 10 mg/kg	19.2 ± 0.8
		Lorcaserin 30 mg/kg	18.8 ± 0.4
		Lorcaserin 100 mg/kg	19.2 ± 1.0
		Perphenazine	308.3 ± 64.5
28	28 day	Vehicle	18.1 ± 1.6
		Lorcaserin 10 mg/kg	17.9 ± 0.9
		Lorcaserin 30 mg/kg	16.9 ± 0.9
		Lorcaserin 100 mg/kg	18.0 ± 1.5
		Perphenazine	274.9 ± 34.1
62	60 day	Vehicle	13.1 ± 1.0
		Lorcaserin 10 mg/kg	17.2 ± 2.7
		Lorcaserin 30 mg/kg	24.8 ± 8.3
		Lorcaserin 100 mg/kg	13.0 ± 0.8
		Perphenazine	261.4 ± 30.8
83	90 day	Vehicle	24.3 ± 1.8
		Lorcaserin 10 mg/kg	23.6 ± 1.0
		Lorcaserin 30 mg/kg	28.6 ± 3.0
		Lorcaserin 100 mg/kg	23.5 ± 2.5
		Perphenazine	234.3 ± 20.1

^a Mean ± SEM. Original data can be found in Arena notebook (b) (4), 000140-02.

Mean Plasma Prolactin Concentrations at 20 h Post-Dose during 91 Days of Treatment

Study Arm	Treatment and Dose (mg/kg)	Plasma Prolactin Concentration at 20 h after Dosing (ng/mL)														
		Study day														
		4	7	10	14	21	28	35	42	49	56	63	70	77	84	91
28 day	Vehicle	11.0	10.9	17.8	19.4	18.9	14.1	ND								
	Perphenazine 5	174.3 ^b	197.4 ^b	142.5 ^b	77.2 ^b	97.2 ^b	88.7 ^b	ND								
	Lorcaserin 100	54.9 ^a	58.5 ^b	47.8	17.7	15.0	17.7	ND								
	Lorcaserin 30	11.4	9.8	17.4	11.3	18.0	20.1	ND								
	Lorcaserin 10	9.5	10.7	11.4	11.0	17.0	18.5	ND								
90 day	Vehicle	8.7	16.2	15.9	16.4	18.3	23.2	23.2	19.2	22.3	21.3	19.8	20.7	22.7	25.5	26.7
	Perphenazine 5	163.5 ^b	201.2 ^b	149.6 ^b	76.3 ^b	118.6 ^b	97.7 ^b	99.9 ^b	87.7 ^b	86.3 ^b	86.8 ^b	72.5 ^b	86.0 ^b	78.5 ^b	71.4 ^b	107.0 ^b
	Lorcaserin 100	29.7	58.1 ^b	57.3 ^b	25.2	17.3	20.6	23.1	24.4	26.3	20.1	40.3	23.8	45.9	36.1	24.0
	Lorcaserin 30	10.8	11.2	21.4	20.5	18.2	18.0	23.8	25.6	25.2	24.8	29.8	28.9 ^a	22.5	32.8	41.0
	Lorcaserin 10	12.5	18.3	24.8	15.3	16.9	27.4	21.9	21.2	31.2	34.8	26.0	42.1 ^b	41.1	46.6	39.7 ^b

^a P < 0.05 versus vehicle control ^b P < 0.01 versus vehicle control

Plasma Prolactin Levels at 20 h Post-Dose during 91 Days of Treatment

Treatment Day	Study Arm	Treatment Group and Dose (mg/kg)	Plasma Prolactin Concentration ^a	Animals with Prolactin > 100 ng/mL (% of Total)
1	1 day	Vehicle	32.6 ± 3.8	0.0
		Lorcaserin 10	32.6 ± 6.4	9.1
		Lorcaserin 30	26.1 ± 1.2	0.0
		Lorcaserin 100	57.6 ± 13.4 ^b	16.7
		Perphenazine 5	140.0 ± 40.4 ^c	50.0 ^b
7	7 day	Vehicle	19.1 ± 1.8	0.0
		Lorcaserin 10	19.4 ± 1.9	0.0
		Lorcaserin 30	42.3 ± 25.2	8.3
		Lorcaserin 100	101.9 ± 42.7 ^b	33.3
		Perphenazine 5	120.3 ± 23.5 ^c	50.0 ^b
	28 day	Vehicle	10.9 ± 1.1	0.0
		Lorcaserin 10	10.7 ± 1.7	1.7
		Lorcaserin 30	9.8 ± 1.0	0.0
		Lorcaserin 100	58.5 ± 15.4 ^c	16.7 ^c
		Perphenazine 5	197.4 ± 16.0 ^c	73.3 ^c
90 day	Vehicle	16.2 ± 3.6	1.7	
	Lorcaserin 10	18.3 ± 7.2	1.7	
	Lorcaserin 30	11.2 ± 0.7 ^c	0.0	
	Lorcaserin 100	58.1 ± 14.2	16.9 ^c	
	Perphenazine 5	201.2 ± 16.6 ^c	70.0 ^c	
28	28 day	Vehicle	14.1 ± 0.6	0.0
		Lorcaserin 10	18.5 ± 2.7	1.7
		Lorcaserin 30	20.1 ± 3.2	3.4
		Lorcaserin 100	17.7 ± 3.0	1.7
		Perphenazine 5	88.7 ± 8.5 ^c	39.0 ^c
	90 day	Vehicle	23.2 ± 6.4	1.7
		Lorcaserin 10	27.4 ± 6.8	5.0
		Lorcaserin 30	18.0 ± 1.5	0.0
		Lorcaserin 100	20.6 ± 3.8	1.7
		Perphenazine 5	97.7 ± 10.8 ^c	35.0 ^c
61	60 day	Vehicle	17.1 ± 2.5	0.0
		Lorcaserin 10	19.9 ± 5.2	0.0
		Lorcaserin 30	16.9 ± 0.9	0.0
		Lorcaserin 100	19.7 ± 4.1	0.0
		Perphenazine 5	70.3 ± 7.1 ^c	0.0
63	90 day	Vehicle	19.8 ± 0.7	0.0
		Lorcaserin 10	26.0 ± 4.5	3.3
		Lorcaserin 30	29.8 ± 6.4	3.3
		Lorcaserin 100	40.3 ± 10.4	7.0
		Perphenazine 5	72.5 ± 6.2 ^c	21.7 ^c
91	90 day	Vehicle	26.7 ± 4.5	1.7
		Lorcaserin 10	39.7 ± 8.1 ^c	6.7
		Lorcaserin 30	41.0 ± 10.1	5.0
		Lorcaserin 100	24.0 ± 2.1	1.8
		Perphenazine 5	107.0 ± 7.3 ^c	45.0 ^c

^a Mean ± SEM ^b P < 0.05 ^c P < 0.01

Frequency of Animals with High Plasma Prolactin Concentrations at 20 h Post-Dose during 91 Days of Treatment

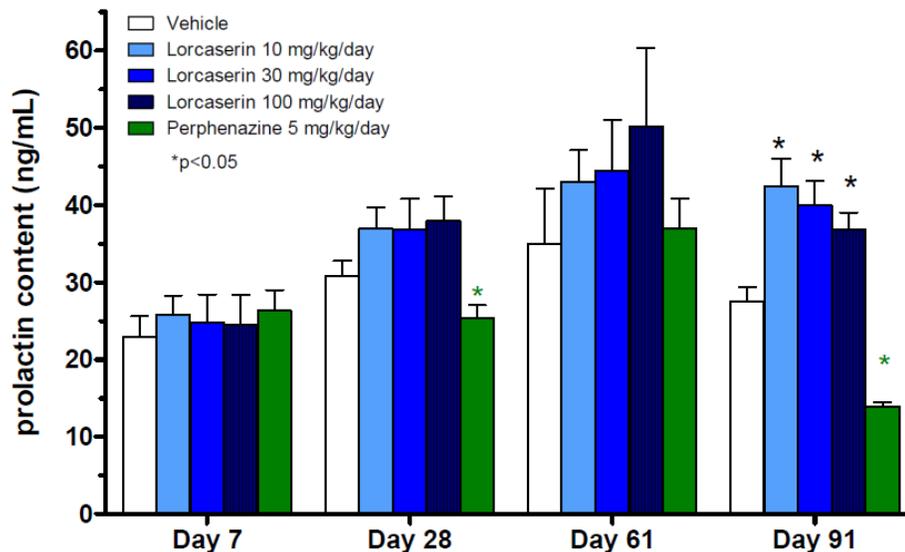
Study Arm	Treatment Group and Dose (mg/kg)	Animals with Prolactin Concentration ≥ 100 ng/mL at 20 h after Dosing (%)														
		Study Day														
		4	7	10	14	21	28	35	42	49	56	63	70	77	84	91
28 day	Vehicle	0.0	0.0	1.7	1.7	0.0	0.0	ND								
	Perphenazine 5	56.7 ^b	73.3 ^b	63.3 ^b	28.3 ^b	37.3 ^b	39.0 ^b	ND								
	Lorcaserin 100	13.3 ^a	16.7 ^b	10.3	1.7	0.0	1.7	ND								
	Lorcaserin 30	1.7	0.0	1.7	1.7	1.7	3.4	ND								
	Lorcaserin 10	0.0	1.7	0.0	0.0	0.0	1.7	ND								
90 day	Vehicle	0.0	1.7	0.0	0.0	0.0	1.7	1.7	0.0	0.0	1.7	0.0	1.7	0.0	0.0	1.7
	Perphenazine 5	63.3 ^b	70.0 ^b	63.3 ^b	26.7 ^b	53.3 ^b	35.0 ^b	43.3 ^b	33.3 ^b	35.0 ^b	30.0 ^b	21.7 ^b	28.3 ^b	21.7 ^b	23.3 ^b	45.0 ^b
	Lorcaserin 100	8.3	16.0 ^b	15.3 ^b	3.4	0.0	1.7	1.7	1.7	3.4	0.0	7.0	3.5	7.0	3.5	1.8
	Lorcaserin 30	0.0	0.0	1.7	1.7	0.0	0.0	3.3	5.0	1.7	1.7	3.3	5.0	1.7	3.3	5.0
	Lorcaserin 10	3.3	1.7	1.7	0.0	0.0	5.0	1.7	1.7	3.3	1.7	3.3	8.3	3.3	6.7	6.7

ND = Not Determined. ^a P < 0.05 versus vehicle control ^b P < 0.01 versus vehicle control

Pituitary Prolactin

- Pituitary prolactin content tended to be higher in lorcaserin treated rats, reaching statistical significance on Day 91. Accumulation of pituitary prolactin might reflect prevention of release from pituitary into plasma over time. As a weak dopamine transporter inhibitor (IC₅₀ 33 μ M, EC₅₀ 23 μ M), lorcaserin might increase levels of dopamine, which is known to block prolactin release from the pituitary. Although the IC₅₀ for transport inhibition is very high, brain accumulation of lorcaserin in rats (> 35x) might be sufficient to produce an effect with repeated administration.
- In contrast, perphenazine, a dopamine receptor antagonist, resulted in a profound increase in initial plasma prolactin levels. Over time, the persistent stimulation of pituitary by perphenazine may have resulted in depletion of pituitary prolactin by Day 90.

Pituitary Prolactin Content in Female Sprague-Dawley Rats

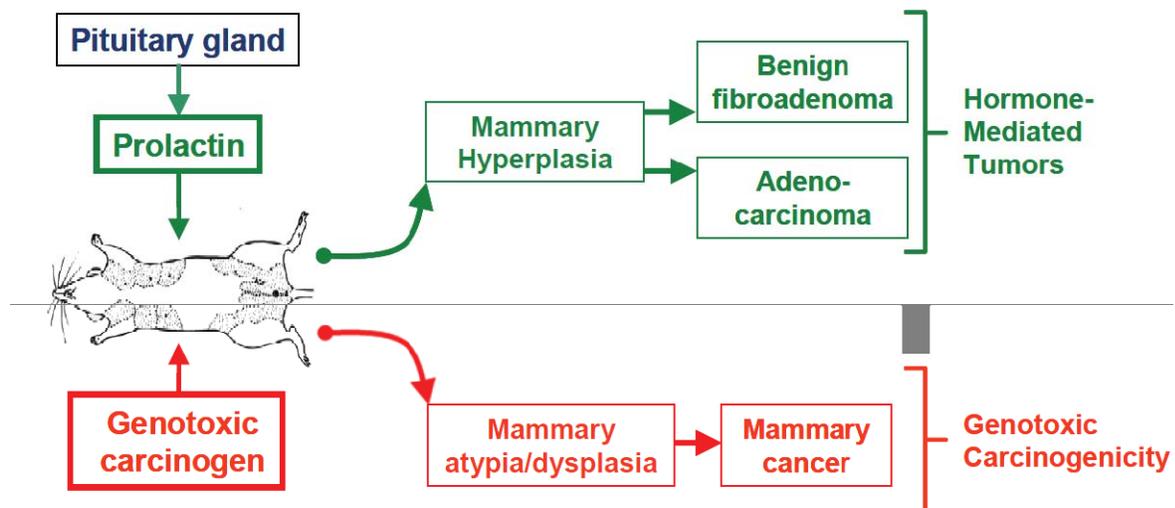


Summary of Microscopic Observations - FEMALE

Tissue	Severity	Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Observation		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
pituitary gland		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, pars distalis, benign, primary		31	19	36	10	28	3	20	0
carcinoma, pars distalis, malignant, primary		0	0	2	0	0	0	0	0
hypertrophy/hyperplasia		10	4	14	2	27	2	46	0
- minimal		3	3	2	1	4	1	15	0
- mild		5	1	11	1	19	1	28	0
- moderate		2	0	1	0	4	0	3	0

In normal aging SD rats and rats treated with antidopaminergic drugs, the mammary tumors are associated with increased incidence of pituitary tumors. The process is believed to begin with stimulated pituitary prolactin release to mammary hyperplasia and eventually to mammary and pituitary tumors. In the case of lorcaserin, the dose dependent increase in mammary fibroadenoma was associated with a dose-related decrease in pituitary tumors and a dose-related increase in pituitary hypertrophy/hyperplasia. There were more pituitary tumors in the control group than in lorcaserin-treated rats. It is not clear why lorcaserin did not increase pituitary tumors similar to antidopaminergic drugs. As a highly brain partitioning drug in rats, lorcaserin levels may have been high enough to inhibit dopamine transport resulting in pituitary hypertrophy but not tumors.

Mammary Tumors in Rats have Two Main Causes Distinguishable by Microscopic Changes that Precede Tumor Formation



The sponsor stated that mammary tumors develop under hormonal influence (i.e. prolactin, estradiol) where mammary tissue differentiates and, if excessive, leads to mammary hyperplasia and hormone-related tumor development. This is stated to be different than a genotoxic carcinogen which results in mammary atypia/dysplasia.

Plasma Reproductive Hormones

- There were no changes in progesterone, estradiol and LH on Day 7, 28, 61 or 91 in lorcaserin treated rats relative to the control.
- A slight increase in progesterone and decrease in luteinizing hormone was seen in 100 mg/kg lorcaserin group on Day 1.

Plasma Levels of Progesterone, Estradiol, and LH during 91 Days of Treatment

Treatment Day	Treatment and Dose (mg/kg)	Progesterone (ng/mL) ^a	Estradiol (pg/mL) ^a	LH (pg/mL) ^a
1	Vehicle	15.9	201.3	468.9
	Lorcaserin 100 mg/kg	32.8 ^b	199.4	180.8 ^c
7	Vehicle	15.5	239.0	853.4
	Lorcaserin 100 mg/kg	24.6	227.8	658.0
28	Vehicle	12.6	280.2	687.7
	Lorcaserin 100 mg/kg	11.6	250.7	611.0
61	Vehicle	22.4	241.9	809.3
	Lorcaserin 100 mg/kg	10.4	226.6	703.2
91	Vehicle	19.8	321.1	845.6
	Lorcaserin 100 mg/kg	16.2	278.8 ^c	695.7 ^c

^a Median; ^bP < 0.01 versus vehicle control; ^cP < 0.05 versus vehicle control.

Plasma Lorcaserin and Perphenazine Concentrations

- Plasma concentrations of lorcaserin increased with dose. Lorcaserin concentrations were higher at 1 hr than 21 hr post dose, thus greatest effect on plasma prolactin should have occurred at 1 hr post dose similar to perphenazine.
- Low levels of lorcaserin were detected in some of the control and perphenazine-treated rats suggesting cross contamination of sample or drug delivery.
- Exposure to perphenazine also was highest at 1 hr (approximately 4 to 6 fold higher than levels at 21 hr post dose) corresponding to sharp rise in plasma prolactin levels at 1 hr post dose.
- Interestingly, nine of the perphenazine rats on Day 28 had high levels of lorcaserin, suggesting a dosing error. These rats may have also received 100 mg/kg lorcaserin in addition to perphenazine or lorcaserin and perphenazine doses may have been administered to wrong animals. Whether occasional spikes in prolactin levels in the LD and HD lorcaserin rats were due to accidental administration of perphenazine is not clear.

Plasma Levels of Lorcaserin and Perphenazine at 1 h and 21 h after the 28th and 91st Treatments

Treatment	Dose (mg/kg/day)	Experimental Day	Post-Dose Sampling Time (hr)	Drug Plasma Concentration (ng/mL)
Lorcaserin	10	28	1	473 ± 130
	30	28	1	1300 ± 350
	100	28	1	4070 ± 906
	10	28	21	18.5 ± 8.9
	30	28	21	229 ± 138
	100	28	21	2790 ± 846
Perphenazine	5	28	1	77.1 ± 42.9
	5	28	21	13.2 ± 9.5
Lorcaserin	10	91	1	618 ± 179
	30	91	1	1750 ± 365
	100	91	1	4610 ± 876
	10	91	21	34.7 ± 23.4
	30	91	21	357 ± 169
	100	91	21	3380 ± 845
Perphenazine	5	91	1	99.1 ± 60.1
	5	91	21	27 ± 21.1

Mammary tissue samples from Day 28 (60 rats /group) were sent by the sponsor to Dr. Russo's lab located at Fox Chase Cancer Center (FCCC), Philadelphia, PA for whole mount histomorphological analysis. Tissue samples were sent to Dr. Russo presumably in blinded manner. The report was completed and signed by Dr. Russo on Aug 31, 2011. The objective was to determine the percentage of undifferentiated terminal duct (TD) and terminal end buds (TEB) in the whole mount preparations. Adenocarcinoma is thought to arise from undifferentiated terminal end buds (TEB) in rats (Dr. Russo).

Based on Dr. Russo's publications, mammary tumors that develop under hormonal influence (i.e. prolactin, estradiol) have a different characteristic. Mammary tissue is more vulnerable to these hormones when undergoing differentiation from TEB to lob 1, 2 and 3.

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Whole Mount Mammary Tissue Findings on Day 28:

- Lorcaserin doses up to 100 mg/kg had no significant impact on the terminal duct (TD) or terminal end bud (TEB) in female rats at Day 28.
- Perphenazine (5 mg/kg) induced a significant decrease in terminal duct structures (TD and TEB) suggesting that perphenazine was producing a **differentiation** effect on mammary terminal duct structures. Differentiation is thought to be associated with tumor development over time in rats and humans (⁷Dr. Russo 1998).
- Lorcaserin doses up to 100 mg/kg had no significant effect on the lobular structures type 1. Perphenazine induced a significant decrease of lobular type 1.
- HD Lorcaserin (100 mg/kg) induced significant changes in the number of lobules type 2 (8.4 vs. 1.8 in control) indicating that lorcaserin exerts a stimulatory hormonal effect on lobular development according to Dr Russo. Perphenazine also significantly increased Lobule 2 (84.6 vs. 1.8 in control). Perphenazine also had modified lobules type 3
- Whole mount evaluation found no evidence of intraductal carcinoma, adenocarcinoma or fibroadenoma.
- According to the sponsor, when selected suspicious areas were examined, couple of animals in each lorcaserin treatment level had periductal and interlobular fibrosis. Dr. Russo emphasizes that these provide further

⁷ Russo and Russo, Mammary Gland Neoplasia in Long-Term Rodent Studies (review). 938-967, Vol 104 (9), Sept 1996, Environmental Health Perspectives

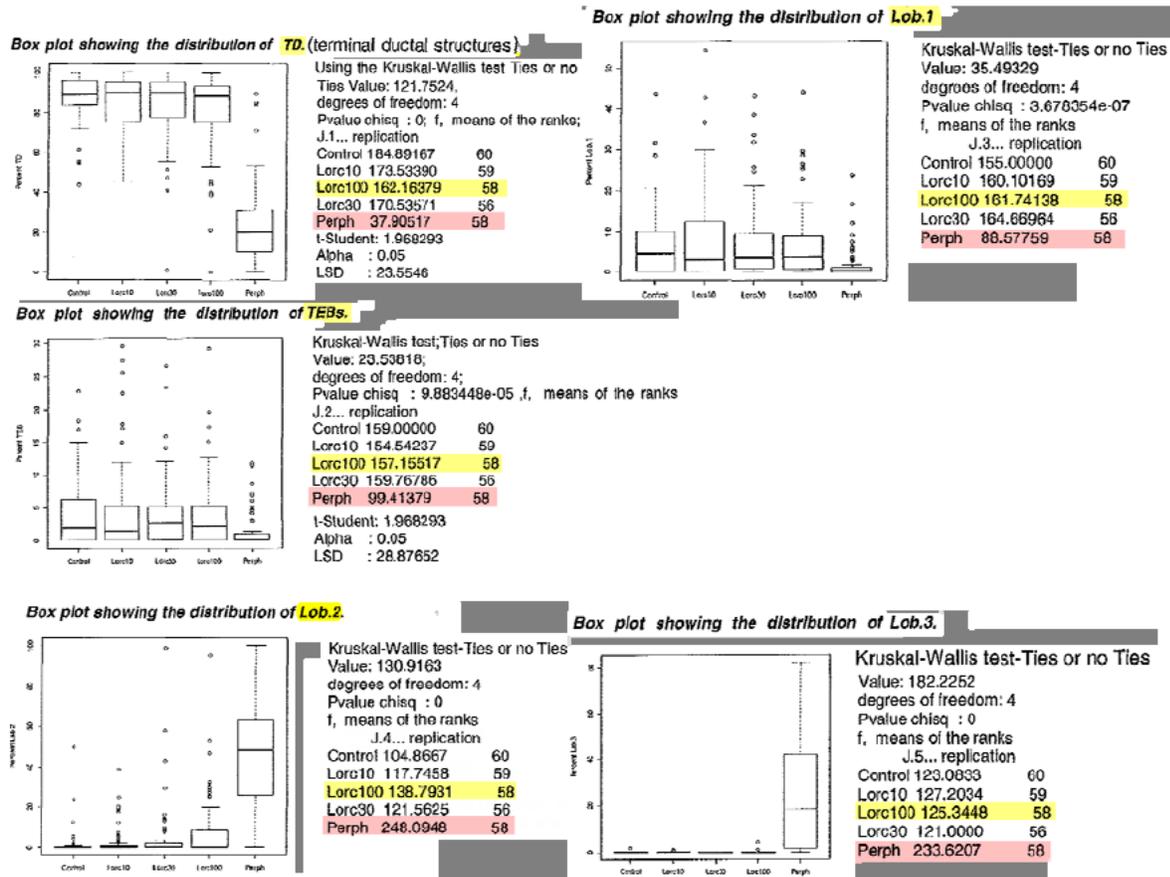
evidence that lorcaserin was producing an effect similar to drugs acting via prolactin. However, examining the animal ID, the reviewer found significant errors in the animal ID and treatment group designation. Only three rats appear to belong to the right treatment group.

Prevalence of Mammary Gland Structures after 28 Days of Treatment

Day	Structure	Treatment Group and Dose (mg/kg)	Frequency of Structure (% of total) ^a
28	Terminal ducts	Vehicle	87.4 ± 1.5
		Lorcaserin 10	84.3 ± 1.8
		Lorcaserin 30	82.4 ± 2.5
		Lorcaserin 100	80.2 ± 2.7
		Perphenazine	24.4 ± 2.7 ^c
28	Terminal end buds	Vehicle	4.3 ± 0.7
		Lorcaserin 10	4.6 ± 0.9
		Lorcaserin 30	3.9 ± 0.7
		Lorcaserin 100	4.2 ± 0.8
		Perphenazine	1.4 ± 0.4 ^c
28	Lobule type 1	Vehicle	6.6 ± 1.1
		Lorcaserin 10	8.2 ± 1.5
		Lorcaserin 30	7.8 ± 1.4
		Lorcaserin 100	7.2 ± 1.2
		Perphenazine	1.8 ± 0.6 ^c
28	Lobule type 2	Vehicle	1.8 ± 0.9
		Lorcaserin 10	2.8 ± 0.9
		Lorcaserin 30	5.9 ± 2.2
		Lorcaserin 100	8.4 ± 2.2 ^c
		Perphenazine	46.8 ± 3.3 ^c
28	Lobule type 3	Vehicle	0.0 ± 0.0
		Lorcaserin 10	0.0 ± 0.0
		Lorcaserin 30	0.0 ± 0.0
		Lorcaserin 100	0.1 ± 0.1
		Perphenazine	25.6 ± 3.2 ^c
28	Total lobular structures	Vehicle	8.4 ± 1.5
		Lorcaserin 10	11.1 ± 1.8
		Lorcaserin 30	13.7 ± 2.6
		Lorcaserin 100	15.7 ± 2.9
		Perphenazine	74.2 ± 2.9 ^c

Box plot of mammary histopath data collected from female rats treated with 10, 30, 100 mg/kg lorcaserin and 5 mg/kg perphenazine for 28 days are shown in figures below. Kruskal-Wallis one way ANOVA was used to compare structure percentages

among five groups. Post hoc analysis adjusting for multiple comparisons was performed between pairs of groups (i.e. treatment vs. control).



In addition to mammary lobular differentiation, Dr. Russo also examined mammary tissues samples that he considered 'suspicious'. The findings at these suspicious areas on the mammary tissue were used as weight of evidence in favor of a hormonal effect (prolactin) for lorcaserin on increased lobular differentiation. However, close examination of the plasma prolactin and animal treatment identification found errors in properly identifying the treatment these animals had received.

Histological diagnosis of the suspicious areas detected in the whole mount preparations.

ANIMAL NUMBER	TREATMENT	LESION
26	LORCASERIN 10	Periductal and interlobular fibrosis
120	LORCASERIN 10	Periductal and interlobular fibrosis
38	LORCASERIN 30	Periductal and interlobular fibrosis
49	LORCASERIN 100	Periductal and interlobular fibrosis
155	LORCASERIN 100	Periductal and interlobular fibrosis
2	PERPHENAZINE	Lobules 3 with Cystic dilatation
24	PERPHENAZINE	Lobules 3 with Cystic dilatation
57	PERPHENAZINE	Ductal hyperplasia
130	PERPHENAZINE	Lobules 3 with Cystic dilatation
244	PERPHENAZINE	Lobules 3 with Cystic dilatation
276	PERPHENAZINE	Lobules 3 with Cystic dilatation
288	PERPHENAZINE	Lobules 3 with Cystic dilatation and interlobular fibrosis

Matching the animal ID to the treatment in the raw data set provided by the sponsor found only three rats in the correct treatment groups (rats **38, 49 and 130**). Examining the plasma prolactin levels and animals with suspicious mammary lesions found no consistent relationship between prolactin and mammary lesions in lorcaserin treated rats.

Prolactin levels at 1 hr and 20 hr post dose on Day 28 in animals with lesions in suspicious areas.

Animal ID	Treatment, mg/kg	Cohort	Plasma prolactin levels, ng/ml	
			1 hr	20 hr
26	Vehicle	Day 28	14	13
120	Perphenazine, 5	Day 28	275	93
38	Lorcaserin 30	Day 28	14	12
49	Lorcaserin 100	Day 28	14	13
155	Perphenazine 5	Day 28	727	40
2	Lorcaserin, 10	Day 28	14	13
24	Lorcaserin, 100	Day 28	15	13
57	Lorcaserin, 10	Day 28	24	12
130	Perphenazine, 5	Day 28	117	159
244	Lorcaserin, 100	Day 28	14	13
276	Vehicle	Day 28	24	22
288	Lorcaserin, 30	Day 28	61	134

Dr. Russo's Interpretation of Day 28 Data:

Based on increased lob type 2 phenotype at 100 mg/kg, Dr. Russo concluded that lorcaserin was initiating a hormonal response, probably a prolactin response. Since analysis of selected suspicious tissues found some periductal and interlobular fibrosis in couple of rats at 10, 30 and 100 mg/kg of lorcaserin, it was interpreted as indicative of early evidence of fibroadenoma formation. However, since the animal numbers mismatched the treatment groups, any conclusion based on the suspicious areas is unfounded.

Lorcaserin clearly produced changes in the mammary tissue but since there was no reduction in TD or TED in lorcaserin treated rats at Day 28, lorcaserin was not producing a differentiation effect known to result in mammary tumors in rat. Indeed, only a slight increase in the number of lobule 2 was observed, which could have been coincidental. Perphenazine clearly initiated mammary differentiation (reduction in TD and TEB) by Day 28.

Dr. Russo's conclusion that the mammary changes in the lorcaserin-treated groups were consistent with prolactin changes at Day 38 is unfounded, because: a) there was no consistent relationship between plasma prolactin and mammary findings in individual rats treated with lorcaserin (unlike perphenazine) b) total lobular structures were not increased (unlike perphenazine), c) the suspicious areas used to support the prolactin effect were inaccurate and unreliable (see statement below), and finally, d) there was no clear relationship between plasma prolactin and lobular 2

incidence in lorcaserin-treated rats. The duration of the study may not have been long enough to show meaningful lorcaserin related changes in the mammary tissue.

Direct Statement from the sponsor's report (Dr. Russo).

Lorcaserine produces periductal and interlobular fibrosis, although in few animals (Two per each group), could be interpreted as an early evidence of fibro adenoma formation. If this is the case this type of lesions will increase with longer treatment.

The 28 days treatment shows morphological response that needs to be put in perspective with other data generated such as; cell proliferation, apoptosis, and prolactin level.

The evaluation of cell proliferation, apoptosis or other applied morphological criteria needs to be examined at the light of the different histological structures present such as TD, TEB, LOB 1, 2 and 3, because it could be misleading if an average of these parameters (cell proliferation and apoptosis for example) are given without considering the particular structures.

Blinded mammary tissue samples from Day 90 (60 rats /group) were sent by the sponsor to Dr. Russo's lab for whole mount histomorphological analysis similar to day 28 discussed earlier. The report was completed and signed by Dr. Russo on Nov 11, 2011. Again, the objective was to determine the percentage of undifferentiated terminal duct (TD) and terminal end buds (TEB) in the whole mount preparations as sign of prolactin related preneoplastic changes in the mammary tissue samples.

Whole Mount Mammary Findings on Day 90:

- **Lorcaserin doses of 10, 30 and 100 mg/kg slightly decreased the number of the terminal ductal structures (TD) relative to control. There was no dose-response relationship.**

The effect of Lorcaserine at 10, 30 and 100mg/kg bw decreases the number of the terminal ductal structures (TD and TEB) when compared to control. The most significant effect is observed with 100mg/kg bw. (Tables 1 and 2).

- Perphenazine strongly reduced terminal duct structures (TD) in female rats, consistent with robust increases in prolactin and hormonal differentiation of mammary structures.
- Lorcaserin at 10 and 100 mg/kg significantly increased lobular type 1. Perphenazine induced a significant increase in lobular type 1, 2 and 3.

The main finding of this study is that Lorcaserine at 10, 30 and 100 mg/kg of body weight induces changes in the number of lobules type 1 and 2 over the control indicating that this compound at that dose exerts a stimulatory hormonal effect in lobular development (Figure 1).

- The total lobular structures were increased slightly at 10 and 100 mg/kg lorcaserin and perphenazine.
- Histological examination of main tissues found no evidence of intraductal carcinoma, adenocarcinoma or fibroadenoma. However, when selected suspicious areas were examined, according to the sponsor couple of animals (643 and 640) treated with 10 and 100 mg/kg had periductal and interlobular fibrosis, respectively. The findings were used to further argue that lorcaserin's effect on prolactin was producing mammary changes. However, this interpretation is wrong, because these two animals were in fact treated with 30 mg/kg lorcaserin and 5 mg/kg perphenazine, respectively.
- The 3-month study appeared to have been too short to show significant and consistent dose-dependent changes that are supportive of prolactin hypothesis. However, the study did show some lorcaserin related changes in the mammary gland, thus the role of prolactin in lorcaserin-induced increase in mammary tumors is plausible.

Whole mount mammary tissue evaluation of female SD rats on Day 91. The positive control rats received 5 mg/kg perphenazine.

Prevalence of Mammary Gland Structures after 91 Days of Treatment

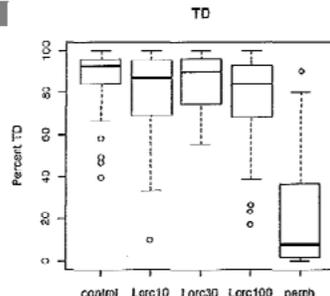
Day	Structure	Treatment Group and Dose (mg/kg)	Frequency of Structure (% of total) ^a
91	Terminal ducts	Vehicle	87.9 ± 1.7
		Lorcaserin 10	79.6 ± 2.6 ^b
		Lorcaserin 30	85.1 ± 1.7
		Lorcaserin 100	78.2 ± 2.7 ^c
		Perphenazine	20.6 ± 3.1 ^c
91	Terminal end buds	Vehicle	1.8 ± 0.4
		Lorcaserin 10	1.8 ± 0.5
		Lorcaserin 30	1.3 ± 0.3
		Lorcaserin 100	0.9 ± 0.2
		Perphenazine	0.8 ± 0.2
91	Lobule type 1	Vehicle	9.6 ± 1.5
		Lorcaserin 10	16.1 ± 2.0 ^b
		Lorcaserin 30	12.5 ± 1.5
		Lorcaserin 100	19.8 ± 2.6 ^c
		Perphenazine	26.0 ± 2.4 ^b
91	Lobule type 2	Vehicle	0.7 ± 0.4
		Lorcaserin 10	2.3 ± 1.1
		Lorcaserin 30	1.0 ± 0.3
		Lorcaserin 100	1.0 ± 0.4
		Perphenazine	38.0 ± 0.4 ^b
91	Lobule type 3	Vehicle	0.1 ± 0.1
		Lorcaserin 10	0.2 ± 0.1
		Lorcaserin 30	0.0 ± 0.0
		Lorcaserin 100	0.1 ± 0.0
		Perphenazine	14.6 ± 2.5 ^b
91	Total lobular structures	Vehicle	10.4 ± 1.7
		Lorcaserin 10	18.6 ± 2.7 ^b
		Lorcaserin 30	13.6 ± 1.7
		Lorcaserin 100	20.9 ± 2.7 ^b
		Perphenazine	78.6 ± 3.2 ^b

^a Mean ± standard deviation ^b P < 0.05 versus vehicle control ^c P < 0.01 versus vehicle control

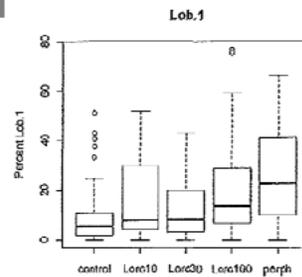
Box plot of mammary histopath data collected from female rats treated with 10, 30, 100 mg/kg lorcaserin and 5 mg/kg perphenazine for 90 days are shown in figures below.

Kruskal-Wallis one way ANOVA was used to compare structure percentages among five groups. Post hoc analysis adjusting for multiple comparisons was performed between pairs of groups (i.e. treatment vs. control).

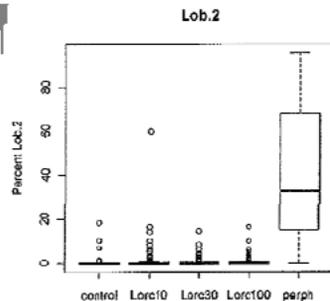
TD-Kruskal-Wallis test's
Ties or no Ties
Value: 130.6984
degrees of freedom: 4
Pvalue chisq : 0
f, means of the ranks
J.1... replication
control 197.06557 61
Lorc10 168.57500 60
Lorc100 158.59821 56
Lorc30 181.88333 60
perph 38.71867 60



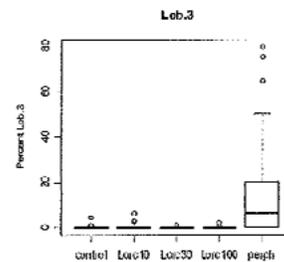
Lob1-Kruskal-Wallis test's
Ties or no Ties
Value: 33.71858
degrees of freedom: 4
Pvalue chisq : 8.510733e-07
f, means of the ranks
J.3... replication
control 109.8197 61
Lorc10 146.2417 60
Lorc100 162.8571 56
Lorc30 132.5750 60
perph 195.0833 60
t-Student: 1.968121



Lob2-Kruskal-Wallis test's
Ties or no Ties
Value: 161.6987
degrees of freedom: 4
Pvalue chisq : 0
f, means of the ranks
J.4... replication
control 111.3770 61
Lorc10 127.9333 60
Lorc100 125.5446 56
Lorc30 122.6000 60
perph 256.6083 60



Lob3-Kruskal-Wallis test's
Ties or no Ties
Value: 153.7561
degrees of freedom: 4
Pvalue chisq : 0
f, means of the ranks
J.5... replication
control 128.7787 61
Lorc10 131.2583 60
Lorc100 129.0357 56
Lorc30 126.6083 60
perph 228.3250 60



Several female rats with suspicious areas were also identified on Day 90. Based on the protocol, animals from Cohort Day 1 were not sent for evaluation. The treatment group listed by the sponsor did not match the animal ID identified in Dr. Russo's data set. In the table below, only rat #467 was correctly identified. Using the suspicious areas with mammary findings, the sponsor claimed as more evidence that prolactin was the intermediary hormone in lorcaserin-induced increase in mammary tumors in female rats.

Histological diagnosis of the suspicious areas detected in the whole mount preparations.

ANIMAL NUMBER	TREATMENT	LESION
650	CONTROL	Ductal structures (TD)
377	CONTROL	Ductal structures (TD)
263	LORC 10	Ductal structures (TD)
380	LORC 10	Lob1 and Lob2
634	LORC 10	Lob 2
467	LORC 10	Lob 2 and Lob3
643	LORC 10	Local inter ductal fibrosis
640	LORC 30	Local inter ductal fibrosis and Lob 2
635	LORC 100	Lob 1

Since the treatments were assigned in error, any conclusion based on the suspicious areas is unjustified. The correct treatment assignments to the animals with suspicious areas is provided in the following table:

Prolactin levels at 1 hr and 20 hr post dose on Day 1, 28 and 90 in animals with lesions in suspicious areas.

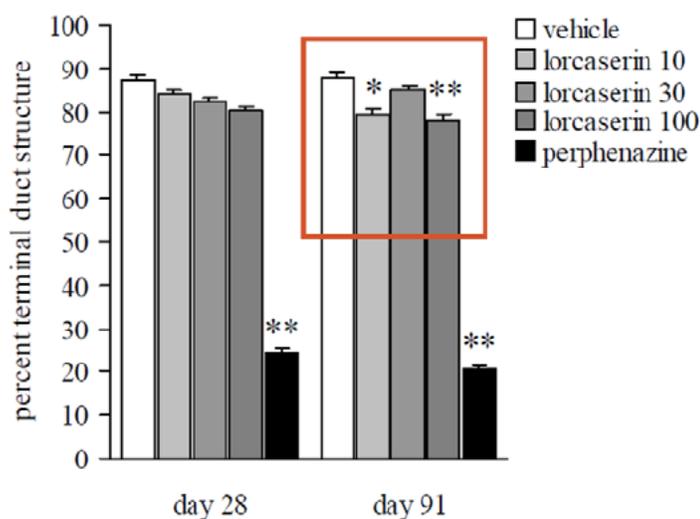
Animal ID	Treatment, mg/kg	Cohort	Plasma prolactin, ng/ml	
			1 hr	20 hr
650	Perphenazine, 5	Day 1	1653	27
377	Lorcaserin, 10	Day 90		19
263	Lorcaserin, 30	Day 28	18	12
380	Perphenazine, 5	Day 90		238
634	Lorcaserin, 100	Day 1	32	124
467	Lorcaserin, 10	Day 90		21
643	Lorcaserin, 30	Day 1	20	28.5
640	Perphenazine, 5	Day 1	475	118
635	Perphenazine, 5	Day 1	786	136

Whole mount Day 90 interpretation:

Dr. Russo stated that all doses of lorcaserin produced a significant increase in lobules type 1 relative to control, an indication of “hormone response”. Since the increase was very slight relative to perphenazine, lorcaserin had a very weak prolactin-related effect but perhaps enough to consider prolactin as a possible mechanism. In contrast, perphenazine substantially lowered the number of TD structures and increased lobular type 1, 2 and 3, consistent with significant elevation in prolactin. Given the minimal effect of lorcaserin which occurred without a clear dose-dependency, it is just as likely that the signal generated with lorcaserin was due to normal variation rather than being related to drug.

Prevalence of Terminal Ducts in the Mammary Gland as a Percentage of Total Structures after 28 and 91 Days of Dosing

(* P<0.05, ** P<0.01 vs. vehicle)



Direct Statement from the sponsor's report (Dr. Russo).

The 90 days treatment of rats with lorcaserin shows clearly that all the doses increase significantly the lobules type 1 over the control level that is an indication of hormone response. The effect observed with perphenazine is significantly higher not only at the Lob1 formation but also at the percentage of Lob 2 and 3.

Of relevance is that the increase in lobular differentiation induced by Lorcaserine has been more significant at 90 days than at 28 days treatment. This is shown by a steady increase in lob 1 over the control and that at all the doses LOB 2 were also increased although not statically significant. This is a demonstration that this compound has a hormonal influence in the mammary tissue. The fact that the TD and TEB are reduced supports this conclusion. It is possible to speculate that based on these data a continuous level of Lorcaserine beyond 90 days period will continue exerting effect on lobular development that may result in the formation of lobular hyperplasia, adenomas and probably conversion to adenocarcinomas as has been observed in Sprague Dawley rats treated with chemical carcinogens (1, 2).

The 90 days treatment shows morphological response that needs to be put in perspective with other data generated such as; cell proliferation, apoptosis, and prolactin level.

The evaluation of cell proliferation, apoptosis or other applied morphological criteria needs to be examined at the light of the different histological structures present such as TD, TEB, LOB 1, 2 and 3, because it could be misleading if an average of these parameters (cell proliferation and apoptosis for example) are given without considering the particular structures.

(b) (4)
Standard Mammary Tissue Morphology on Day 28 and Day 91 in female rats

(b) (4) had also evaluated both mammary tissue morphology by H& E and PCNA methods in blinded manner. The number of animals processed at (b) (4) per group is shown below:

Dose Material	Group *	Dose Level (mg/kg/day)	Day 28	Day 90
			Number of females to block (number of females processed to slide)	Number of females to block (number of females processed to slide)
Vehicle – Water	1	0	60 (60)	60 (60)
Positive Control - Perphenazine	2	5	59 (30)	60 (30)
Test Substance - Lorcaserin	3	10	60 (0)	60 (60)
Test Substance - Lorcaserin	4	30	58 (58)	60 (60)
Test Substance - Lorcaserin	5	100	58 (58)	56 (56)

* Group numbers were assigned by (b) (4) for tabulation purposes.

Mammary H&E Histopath Findings on Day 28 and 91

- Lorcaserin doses of 30 and 100 mg/kg did not increase the incidence of lobular hyperplasia at Day 28 or Day 91. However, few rats in the LD (382, 572 and 447) and HD (30, 324) on Day 90 appeared to have minimal to mild hyperplasia, which were usually associated with hemorrhage or atypia (558, 364). LD lorcaserin was not evaluated on Day 28. The absence of hyperplasia in this study is consistent with the 3- and 6-month rat toxicology studies where no notable changes in the mammary tissue were identified by the sponsor. The only case of an animal with mammary hyperplasia seen in the 6-month study was considered incidental by the sponsor.
- Perphenazine significantly increased lobular hyperplasia on Day 28 and Day 90 (mild to marked). Lobular hyperplasia was observed in 100% of perphenazine rats (30/30 rats) on Day 28 and Day 90. Plasma prolactin levels were consistently elevated in perphenazine treated rats.
- Perphenazine increased perialveolar hemorrhage on both Day 28 and 91. There was a nonsignificant increase in perialveolar hemorrhage on Day 91 in lorcaserin treated rats. Significance of this was not clear.
- Lorcaserin treated rats appeared to have increased secretory product on Day 28. There was no lorcaserin related increase in secretory product on Day 91. The significance of secretory product is not clear since some of the controls also had secretory product.
- There was only one incidence of **adenoma** (#421) which occurred in control rats on Day 90.

Per published articles by Dr. Russo, the reviewer picked the Day 10 plasma prolactin to correspond to time of greatest sensitivity in rats (rat age at the start of the study + 10 days in the treatment). The table also lists prolactin levels at few time points where an increase in prolactin levels were seen in lorcaserin treated rats. There was no apparent relationship between prolactin and lobular hyperplasia in lorcaserin treated rats. Since prolactin threshold is unknown, it is difficult to conclude for or against prolactin hypothesis based on data below.

Rat ID	Group	Prolactin, ng/ml (day)				Histopath
		14 (D10)	59 (D28)	82 (D84)	217 (D91)	
382	LD	14 (D10)		82 (D84)	217 (D91)	lob hyperpl/hemmr
447	LD	15 (D10)	309 (D70)	774 (D77)	258 (D91)	lob hyperpl/hemmr
572	LD	15 (D10)	110 (D63)		53 (D91)	lob hyperpl/hemmr
309	HD	18 (D10)	59 (D28)	46 (D84)		lob hyperpl/hemmr
324	HD	14 (D10)	60(D77)	617(D84)		lob hyperpl/hemmr
364	HD	21 (D10)		44 (D83)		lob hyperpl/atypia
558	MD	21 (D10)		35 (D84)		lob hyperpl/atypia
580	PZ	287(D10)	118(D70)	84(D84)	167(D91)	lob hyperpl/atypia
421	Cont	20 (D10)	77 (D70)		221 (D90)	adenoma

The data in table below shows the number of animals and severity of lobular hyperplasia in female rats treated with lorcaserin (10, 30, 100 mg/kg: LD, MD and HD) and positive control, perphenazine (5 mg/kg). There was no significant increase in lobular hyperplasia in lorcaserin treated rats. However, perphenazine significantly increased mammary lobular hyperplasia in all rats (30 in the study). Prolactin levels in nearly all perphenazine rats were significantly above the control values (Day 1 through Day 90). The increase in prolactin in the perphenazine rats corresponded to minimal to marked lobular hyperplasia.

Summary Microscopic Findings on Day 28

Group	Dose	No. animals examined	1	2	4	5
			Neg Control	Pos Control	Mid Dose	High Dose
			60	30	58	58
Mammary Gland (Total No. sections examined)			(278)	(142)	(270)	(268)
Hyperplasia, lobular (Total No. Animals)			0	30	0	3
	Minimal		0	11	0	6
	Mild		0	65	0	0
	Moderate		0	53	0	0
	Marked		0	13	0	0
Secretory Product (Total No. Animals)			5	0	10	13
	Minimal		18	0	43	57
Hemorrhage, Perialveolar (Total No. Animals)			2	18	3	3
	Minimal		2	30	3	4
	Mild		0	2	0	0

Summary Microscopic Findings on Day 90

	Group Dose	No. animals examined				
		1 Neg Control	2 Pos Control	3 Low Dose	4 Mid Dose	5 High Dose
		60	30	60	60	56
Mammary Gland (Total No. sections examined)		(281)	(147)	(279)	(274)	(254)
Hyperplasia, lobular (Total No. Animals)		1	30	3	0	2
Minimal		5	9	8	0	2
Mild		0	115	5	0	5
Moderate		0	23	0	0	0
Secretory Product (Total No. Animals)		1	0	5	2	2
Minimal		5	0	17	10	7
Hemorrhage, Perialveolar (Total No. Animals)		5	14	8	6	12
Minimal		8	25	12	8	13
Mild		0	2	0	0	0
Hyperplasia with cellular atypia, focal						
Minimal		0	1	0	1	1
Adenoma		1	0	0	0	0

(b) (4) Number 900-046
 APD356: 6-Month Oral Toxicity Study in Rats With A 4-Week Recovery Period

Individual Animal Data Record: Pathology - FEMALE
 Terminal

Group,	Animal Number	Fate	Tissue	Observations
<u>0 mg/kg/day (Vehicle)</u>				
	1135	S	Microscopic mammary gland	- galactocele, minimal
<u>50 mg/kg/day</u>				
	1198	S	Microscopic mammary gland	- hyperplasia, lobular, minimal
	1205	S	Microscopic mammary gland	- adenoma, benign, primary, <u>incidental</u> , scheduled sacrifice - galactocele, mild corresponds to macroscopic observation (mammary gland - galactocele)
S - Scheduled Necropsy				

(b) (4)
PCNA Immunohistochemistry of Mammary Gland on Day 28 and 91 in female rats

- There was no dose-dependent lorcaserin related increase in the PCNA score. The slight increase in the number of positive nuclei at 10 mg/kg lorcaserin on Day 91 and 30 mg/kg lorcaserin on Day 28 were likely coincidental since there was no change at 100 mg/kg lorcaserin.
- Perphenazine significantly increased PCNA score (a marker of early cell proliferation) on 28 and Day 91 corroborating mammary hyperplasia seen in the histological examination and epithelial prolactin score (next page).

PCNA Staining in the Mammary Gland after 28 or 91 Days of Treatment

Treatment	Day 28 ^a	Day 91 ^a
Vehicle	6.7 ± 3.0	8.01 ± 3.8
Lorcaserin 10 mg/kg	-	10.3 ± 4.7 ^c
Lorcaserin 30 mg/kg	8.3 ± 3.6 ^b	9.1 ± 3.5
Lorcaserin 100 mg/kg	5.8 ± 3.3	8.1 ± 3.0
Perphenazine 5 mg/kg	15.9 ± 6.7 ^c	16.6 ± 5.2 ^c

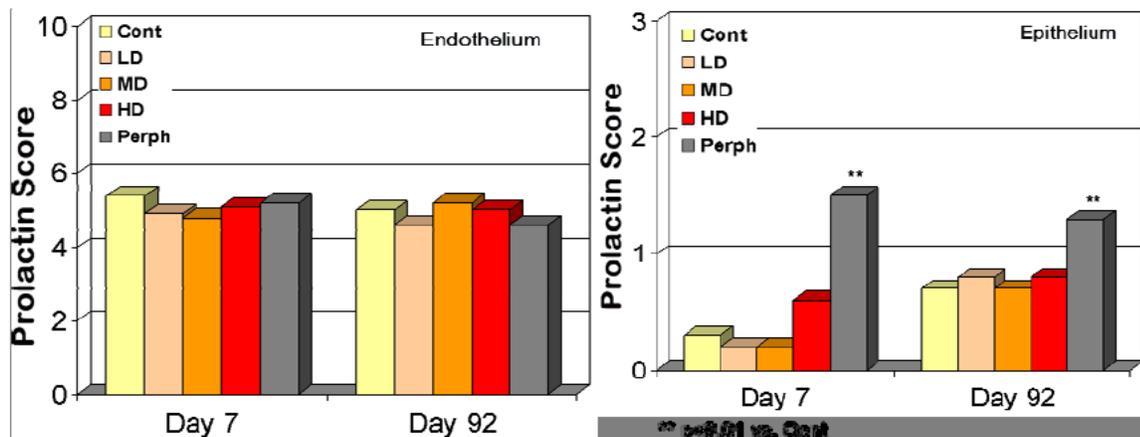
^a Mean ± standard deviation ^b P < 0.05 versus vehicle control ^c P < 0.01 versus vehicle control

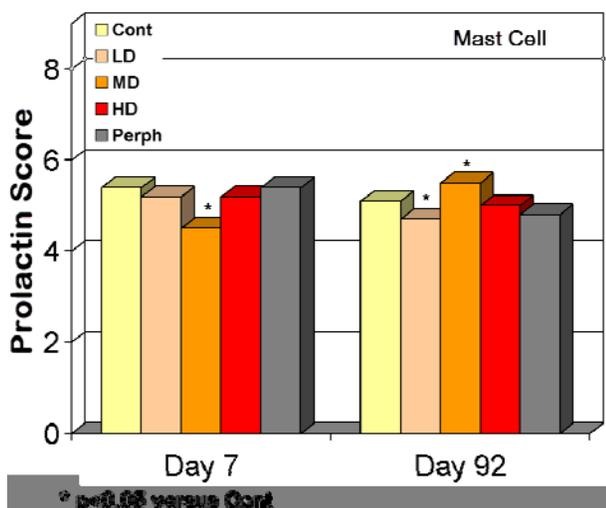
The sponsor had stated that there was no consistent effect of lorcaserin or perphenazine on PCNA when in reality perphenazine had significantly increased PCNA staining on both Day 28 and 91.

There were no consistent effects of lorcaserin or perphenazine on PCNA immunohistochemical staining of the mammary gland as a whole after 28 or 91 days of dosing, although significant increases in staining were observed after 28 days (10 mg/kg) and 91 days (30 mg/kg) of lorcaserin treatment.

Mammary Prolactin

- Lorcaserin appeared to have no effect on mammary epithelial and endothelial cell prolactin scores in female rats. Occasional non-dose dependent changes were seen in mast cell prolactin score.
- The positive control, perphenazine, significantly increased **epithelial** prolactin score.





Note. The objective of prolactin score was to determine whether there was a local prolactin release by the cells residing in mammary tissue. Although mast cells have been reported to be capable of releasing prolactin, nonspecific staining could have occurred (according to the sponsor) since staining also occurred in negative control mast cells as well.

Study Title: three-month evaluation of Lorcaserin effects on prolactin concentrations and mammary gland histology in MALE Sprague (DBR-11-004)

Study initiated July 28, 11, completed on Dec 14, Final Report Dec 22, 2011

Key Findings:

- Both lorcaserin (30 and 100 mg/kg) and perphenazine resulted in significantly lower BW than control at the end of the 3-month study in male rats. The effect of perphenazine was greater than the highest dose of lorcaserin (100 mg/kg).
- There were three deaths early in the 100 mg/kg lorcaserin group.
- Plasma prolactin measured 15-min post dose was not significantly increased in lorcaserin or perphenazine treated male rats.
- There was no clinically relevant or dose-dependent change in pituitary prolactin in lorcaserin or perphenazine treated male rats.
- There was no notable increase in mammary hyperplasia or increase in PCNA score in lorcaserin or perphenazine treated male rats.
- Absence of mammary tissue pathology is consistent with lack of rise in prolactin in lorcaserin and perphenazine treated rats.
- Analysis of plasma drug levels found some irregularities in drug exposure suggesting improper dosing of male rats, i.e. some control and perphenazine rats had lorcaserin in blood suggesting significant dosing error.
- The 3-month male rat study was considered a failed study, possibly due to a) significant dosing error, b) inadequate blood and mammary tissue sample collection method, time and analysis, c) limited sensitivity of male SD rats to lorcaserin and perphenazine type drugs, d) relatively short duration of treatment.

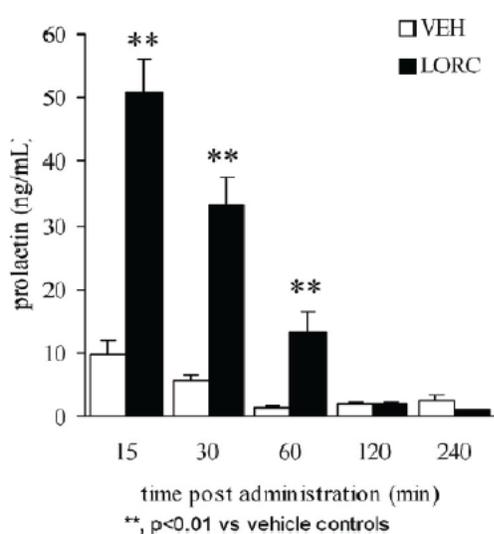
3-Month Male Rat Study Protocol

The 3-month non-GLP study in male SD rats was carried out by the sponsor to show that chronic administration of lorcaserin will result in persistent and robust increase in prolactin similar to positive control, perphenazine. The protocol was submitted to

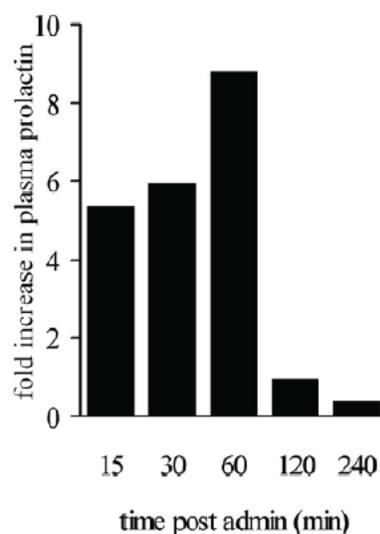
the Division for review. The sponsor had planned to collect plasma samples at 15 min post based on preliminary data showing sharpest rise in prolactin occurring at 15 min post dose in males. The Division had recommended 60 min post dose to allow lorcaserin reaching brain Tmax (45 to 60 min post dose) and to reduce any potential gavage induced stress effect on prolactin release. As noted, timing of blood sample collection appeared to be based on single dose study where maximal increase in prolactin was seen at 15 min post dose at 100 mg/kg lorcaserin. It is not clear why the maximum rise in prolactin did not follow the lorcaserin pharmacokinetic profile with Tmax of 45 to 60 min.

Time Course of Serum Prolactin Response to a Single Oral Lorcaserin Dose of 100 mg/kg in Male Sprague-Dawley Rats

A. Plasma Prolactin Concentration



B. Fold Increase in Prolactin



Hypothesis: “The sponsor had hypothesized that lorcaserin induced increase in mammary hyperplasia and tumors is mediated by increase in circulating prolactin concentrations in male rats⁸.” Prolactin is not only synthesized in pituitary gland but also in other areas in the CNS, immune cells and uterus.

Objective: The study was designed to evaluate plasma and tissue prolactin and mammary tissue morphology (H&E and PCNA stain) at different time intervals in male SD rats treated with 10, 30 and 100 mg/kg lorcaserin and 5 mg/kg perphenazine for 3 months.

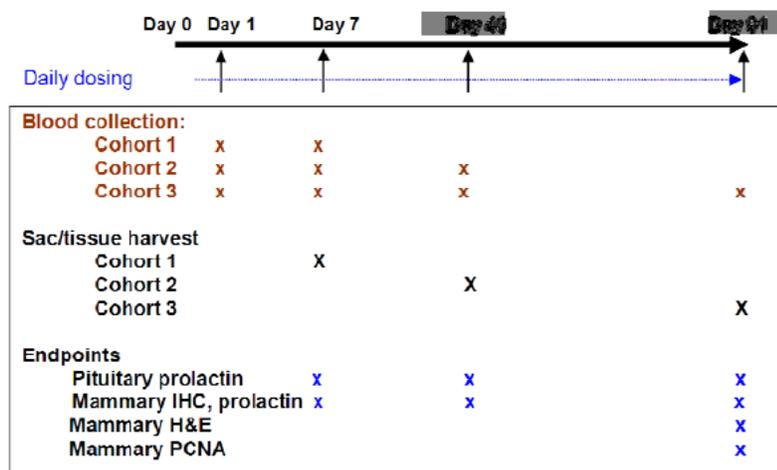
Method: In this non-GLP 3-month pivotal mechanistic study (Arena # DBR-11-004 or (b) (4)), 165 healthy 8 WK old male SD rats ((b) (4)) were individually housed (12-hr light/dark cycle, 6 AM-6PM light) with ad lib access to food and water and acclimated for a week prior to the study at (b) (4).

⁸ Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: Structure, Function, and Regulation of Secretion. *Physiol Rev* 2000 January 10;80(4):1523-631.

(b) (4) At the time of drug dosing, rats were 8-9 weeks old (190 to 250 g). Rats were treated daily with lorcaserin (0, 10, 30 and 100 mg/kg, in 1 ml/kg water) or positive control, perphenazine (5 mg/kg in acidified water) for 7 days (cohort 1), 43 days (cohort 2) or 91 days (cohort 3).

Blood collection: 15 min post dose on Day 1, 7, 43 and 91 by tail nick (100 -150 µl). On schedules sacrifice day 90, blood was collected by tail nick at 20 hr post dose. Animals were then sacrificed (within 24 hr of post dose) on schedules sacrifice days 7, 43 and 91 days. No trunk blood was collected in this study.

Mammary tissue morphology (H&E stain) and PCNA staining of tissue samples collected on Day 91 were analyzed in blinded manner at (b) (4). (The 3-month female rat evaluations were done by Dr. Russo and by (b) (4). Pituitary and plasma prolactin levels as well as plasma drug levels were determined at Arena. Mammary prolactin was not determined.



Animals were dosed between about 9:00 AM and 4:30 PM. There were total of 165 male SD rats, 50 rats per cohort (10 rats /group).

Experimental design as stated in the Study Protocol (b) (4)

Cohort #	Group	# of Animals	Treatment (QD)	Route	Dose Volume (mg/mL)	Termination Day
1	1	10	Vehicle	PO	10	Day 7
	2	10	APD356 10 mg/kg	PO	10	Day 7
	3	10	APD356 30 mg/kg	PO	10	Day 7
	4	10	APD356 100 mg/kg	PO	10	Day 7
	5	10	Perphenazine 5 mg/kg	PO	10	Day 7
2	1	10	Vehicle	PO	10	Day 45
	2	10	APD356 10 mg/kg	PO	10	Day 45
	3	10	APD356 30 mg/kg	PO	10	Day 45
	4	10	APD356 100 mg/kg	PO	10	Day 45
	5	10	Perphenazine 5 mg/kg	PO	10	Day 45
3	1	10	Vehicle	PO	10	Day 90
	2	10	APD356 10 mg/kg	PO	10	Day 90
	3	10	APD356 30 mg/kg	PO	10	Day 90
	4	10	APD356 100 mg/kg	PO	10	Day 90
	5	10	Perphenazine 5 mg/kg	PO	10	Day 90

Animal Information

Cohort #	Group	# of Animals	Treatment	Total # of Animal	DOB	Age at the First Dose
1 7-day	1	11 (10 +1 extra)	Vehicle	55	8/10/2011	60 days (8 weeks, 4 days)
	2	11 (10 +1 extra)	APD356 10 mg/kg			
	3	11 (10 +1 extra)	APD356 30 mg/kg			
	4	11 (10 +1 extra)	APD356 100 mg/kg			
	5	11 (10 +1 extra)	Perphenazine 5 mg/kg			
2 45-day	1	10	Vehicle	55	7/6/2011	58 days (8 weeks, 2 days)
	2	10	APD356 10 mg/kg			
	3	10	APD356 30 mg/kg			
	4	14 (10 +4 extra)	APD356 100 mg/kg			
	5	11 (10 +1 extra)	Perphenazine 5 mg/kg			
3 90-day	1	11 (10 +1 extra)	Vehicle	55	6/10/2011	60 days (8 weeks, 4 days)
	2	11 (10 +1 extra)	APD356 10 mg/kg			
	3	11 (10 +1 extra)	APD356 30 mg/kg			
	4	11 (10 +1 extra)	APD356 100 mg/kg			
	5	11 (10 +1 extra)	Perphenazine 5 mg/kg			

The original study was designed to terminate rats in cohort 1, 2 and 3 on Day 7, 45 and 90 but at end rats were killed on Day 7, 43 and 91.

Primary:

- Concentration of circulating prolactin at selected time points (Days 1, 7, 45 and 90) during 90 days of dosing with lorcaserin as compared to vehicle.

Secondary:

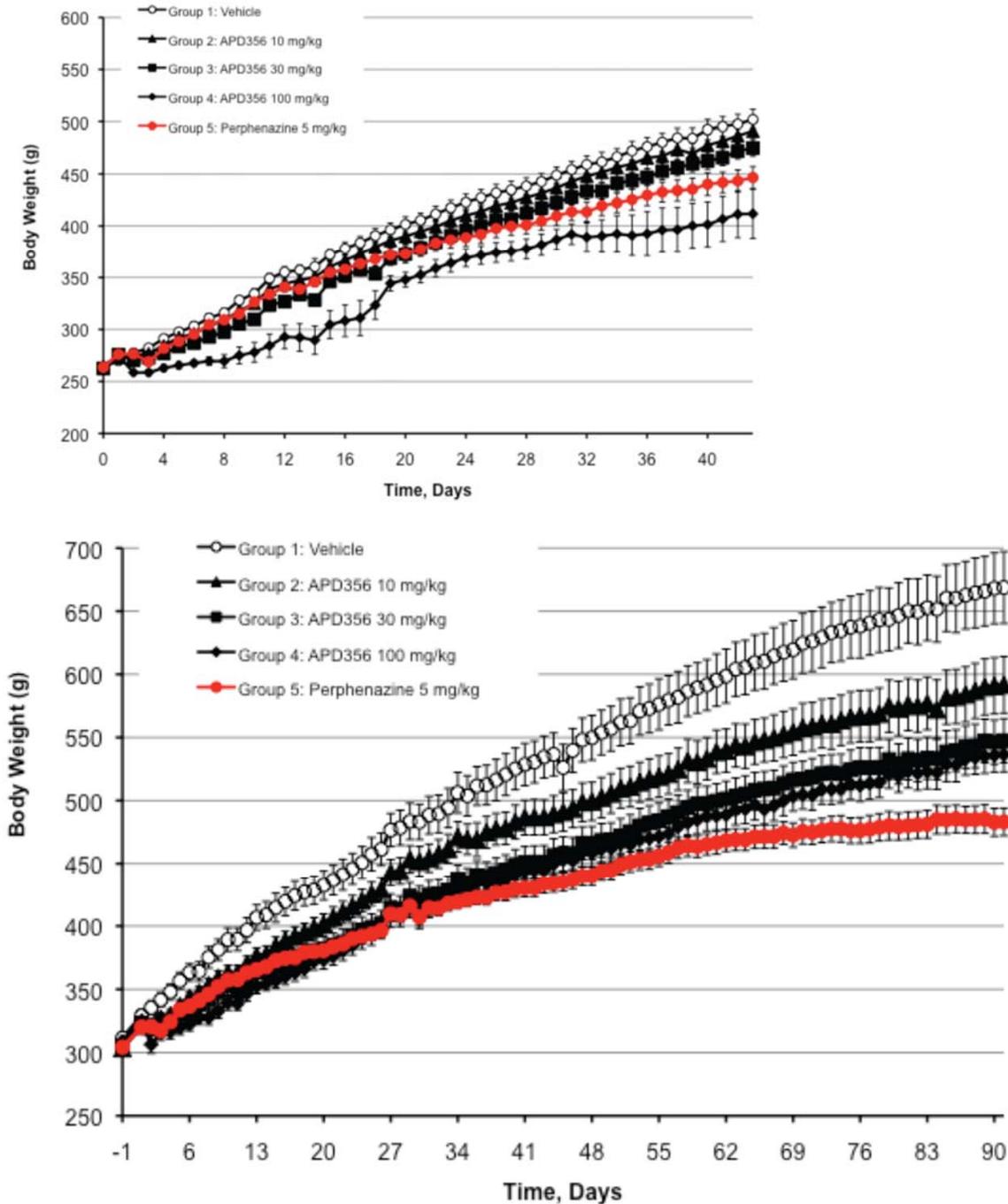
- Histopathological evaluation of hematoxylin/eosin (H&E) and proliferating cell nuclear antigen (PCNA) stained mammary tissue after 90 days of lorcaserin administration.
 - Qualitative histological changes in mammary tissue.
 - Quantitative proliferative changes in mammary tissue.
- Concentration/content of prolactin in pituitary tissue after 7, 45 and 90 days of dosing.

Study Results

Mortality and Clinical signs

- There were 3 deaths in cohort 2 at 100 mg/kg (#3911, 3930 and 3939) on Day11, 18 and 19. They were marked by dark discoloration of liver, heart and thymus and abdominal distention. **There were no deaths in cohort 1 or 3.**
- Both 30 and 100 mg/kg lorcaserin resulted in a significant decrease in BW of rats in cohort 3 at Day 91.
- Perphenazine dose of 5 mg/kg resulted in a significant decrease in BW in cohort 3 rats by Day 91. The impact of perphenazine appeared to be greater than 100 mg/kg lorcaserin (see figures below).

Body weight of SD male rats treated with lorcaserin (10, 30 and 100 mg/kg) and perphenazine (5 mg/kg) for 43 days (cohort 2) and 91 days (cohort 3)



Changes in prolactin, mammary histology and PCNA data

- There were no lorcaserin related changes in 15-min post dose plasma prolactin levels on Day 1, 7, 43 or 91 in male SD rats. It is not clear why there was no lorcaserin effect even after single dose on day 1 since the only supportive evidence for prolactin in the 2009 application was the rise in the 15-min post dose plasma prolactin levels in male SD rats after single dose of 100 mg/kg lorcaserin.

- There were no changes in 15-min post dose plasma prolactin levels in perphenazine (positive control) treated male rats. Lack of change in prolactin levels were likely due to, a) dosing error, b) stress induced increase in basal levels of prolactin level 15 min after gavage, b) inadequate time to allow maximal prolactin release. Closer examination of the data found significantly higher than normal prolactin in control males (up to 75 ng/ml) likely due to gavage or some other kind of stress, measurement, or dosing error. The normal prolactin levels should have ranged between 15 to 25 ng/ml in male and female SD rats. The sponsor was advised against collecting sample so soon after drug administration. Lower prolactin levels in cohort 2 on Day 43 and cohort 3 on Day 90 were likely due to acclimation to the procedure
- There were no mammary tissue changes in either lorcaserin or perphenazine treated rats. It is not clear why perphenazine had no effect.
- There were no PCNA changes in either lorcaserin or perphenazine treated rats
- Lorcaserin at 10 mg/kg on Day 43 and lorcaserin at 30 mg/kg on Day 91 slightly increased pituitary prolactin levels in male SD rats.
- There was a slight ($P>0.05$) increase in pituitary prolactin content in perphenazine rats on Day 90.

Changes in plasma prolactin I in male rats at 15-min post treatment.

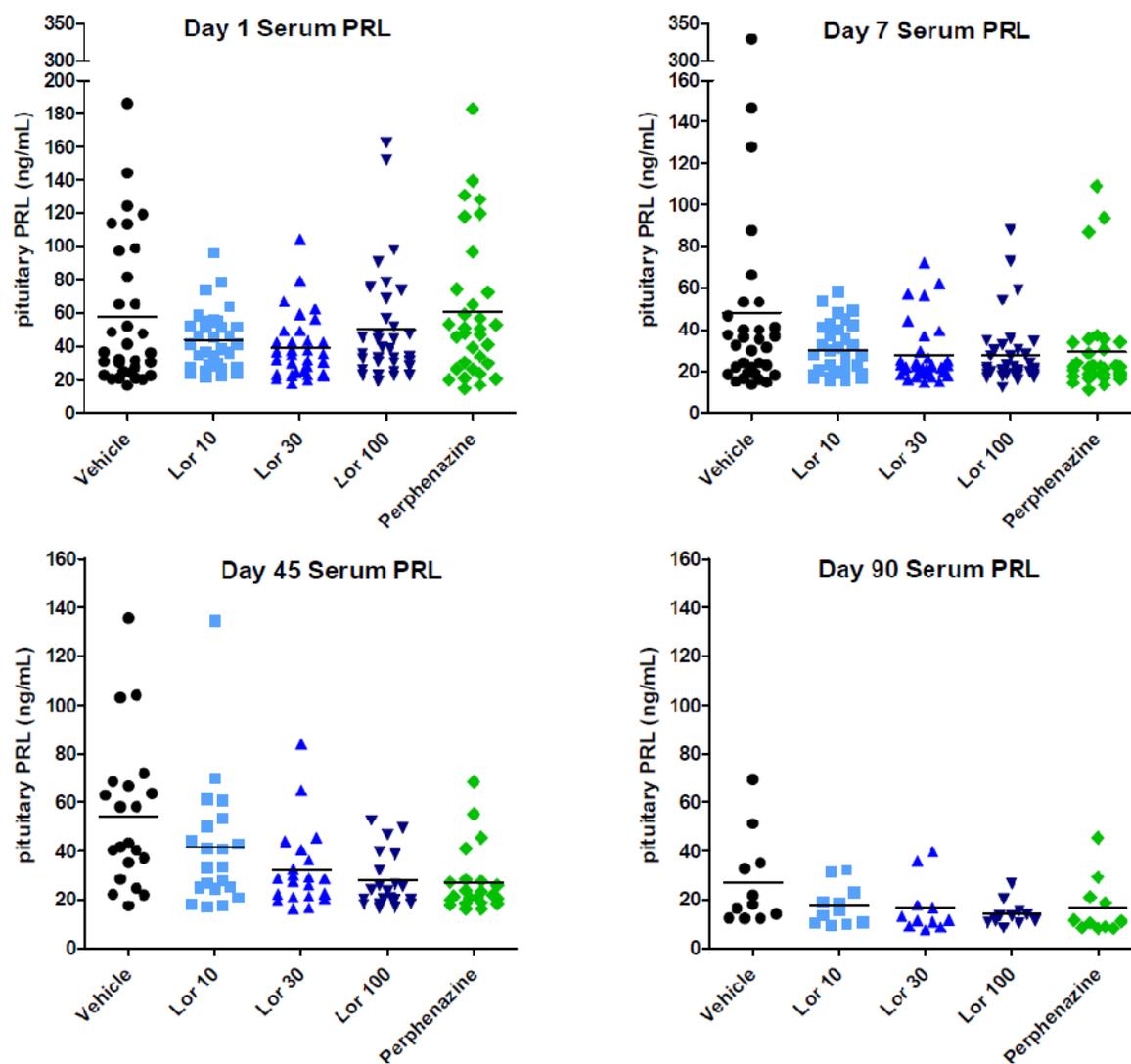
Plasma Prolactin Concentration at 15 min Post-dose

Mean (sd) ng/mL	Vehicle	Perphenazine 5 mg/kg/day	Lorcaserin Dose (mg/kg/day)		
			10	30	100
Cohort 1:					
Day 1	75.4 (43.6)	52.6 (25.0)	49.0 (17.8)	42.1 (22.7) ^a	69.9 (49.3)
Day 7	50.0 (92.8)	20.7 (1.9)	30.4 (13.8)	32.4 (18.1)	29.2 (20.1)
Cohort 2:					
Day 1	26.1 (6.8)	32.6 (20.7)	26.8 (5.0)	26.0 (5.1)	37.1 (17.8) ^a
Day 7	27.2 (10.0)	26.3 (23.6)	28.7 (10.6)	22.5 (12.3)	22.5 (6.7)
Day 45	37.1 (18.3)	31.8 (18.1)	28.7 (9.2)	23.5 (4.4)	22.0 (9.3) ^a
Cohort 3:					
Day 1	68.5 (51.1)	93.3 (50.6)	53.5 (51.3)	47.9 (18.6)	46.8 (25.6)
Day 7	64.7 (40.3)	40.4 (29.7) ^a	31.0 (13.7) ^a	28.0 (13.0) ^a	33.1 (19.8) ^a
Day 45	70.4 (31.3)	22.5 (3.3) ^a	52.7 (32.0)	40.3 (19.6) ^a	33.7 (10.4) ^a
Day 90	26.9 (18.8)	16.4 (11.8) ^a	17.5 (8.3)	16.6 (11.0) ^a	14.1 (5.2) ^a

^a $p<0.05$ from nonparametric (rank-based ANOVA) analysis vs. vehicle

N=10-12 per group

Graphical representation of plasma prolactin levels in male rats. The lines in the graphs represent the mean prolactin levels.



Pituitary tissue levels of prolactin were measured on Day 7, 43 and 91 in lorcaserin and perphenazine treated male rats. There were no clinically meaningful changes in pituitary prolactin levels in perphenazine rats or a dose-dependent change in lorcaserin treated rats in the 3-month study.

Pituitary Prolactin Content

Mean (sd) ng/mL ^a	Vehicle	Perphenazine 5 mg/kg/day	Lorcaserin Dose (mg/kg/day)		
			10	30	100
Cohort 1 (7 days)	6.1 (1.3)	7.9 (3.5)	7.1 (2.6)	6.4 (1.3)	5.8 (1.1)
Cohort 2 (45 days)	6.0 (1.1)	6.5 (1.2)	7.9 (1.4) ^b	6.6 (1.0)	6.3 (0.8)
Cohort 3 (90 days)	7.1 (2.5)	10.1 (2.0) ^b	7.3 (2.7)	16.0 (13.6) ^b	7.1 (1.5)

N=10-11 per group

^a Pituitary prolactin is expressed as ng/mL; equivalent volumes were assayed, and all homogenate samples had equal total volumes. Hence, concentrations can be compared across groups as representative of relative total prolactin content.

^b p<0.05 vs. vehicle control

Mammary tissue histopathology and PCNA in male rats

There was no significant lorcaserin or perphenazine effect on mammary hyperplasia in male rats. PCNA staining found no significant dose-dependent preneoplastic changes in lorcaserin or perphenazine treated rats.

Effects of Lorcaserin, Perphenazine and Vehicle on Mammary Gland Histopathology of Male Rats

Parameter	Vehicle	Perphenazine	Lorcaserin 10 mg/kg/day	Lorcaserin 30 mg/kg/day	Lorcaserin 100 mg/kg/day
Hyperplasia Score					
Mean (sd)	2.1 (0.5)	1.8 (0.1) ^{a,b}	2.0 (0.4)	2.2 (0.3) ^d	2.0 (0.5) ^{c,e}
n(%) of rats ≥1	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)
n(%) of rats ≥2	10 (90.9)	11 (100)	10 (90.9)	11 (100)	9 (81.8)
n(%) of rats ≥3	3 (27.3)	0	3 (27.2)	0	3 (27.2)
Fibrosis Score					
Mean (sd)	2.1 (0.4)	2.2 (0.3)	1.9 (0.3)	2.1 (0.3)	2.2 (0.3)
Neoplasia Score					
Mean (sd)	0	0	0	0.1 (0.3)	0
Inflammation score					
Mean (sd)	1.0 (0.1)	1.0 (0)	1.0 (0.1)	1.0 (0)	1.0 (0.2)
Atrophy Score					
Mean (sd)	0.1 (0.2)	0.7 (1.2)	0.2 (0.3)	0.1 (0.4)	0.2 (0.4)

^a Hyperplasia in 2 animals described as resembling female

^b Micro-cyst in 1 animal

^c Hyperplasia in 1 animal described as resembling female

^d Ductal carcinoma with squamous differentiation in 1 animal

^e Cystic structure lined with squamous epithelium, filled with eosinophilic secretion in 1 animal

Effects of Lorcaserin, Perphenazine and Vehicle on Mammary Gland PCNA Staining

Mean (sd) % of Nuclei with PCNA Stain:	Vehicle	Perphenazine	Lorcaserin 10 mg/kg/day	Lorcaserin 30 mg/kg/day	Lorcaserin 100 mg/kg/day
N	11	11	11	11	11
% PCNA Positive	5.8 (8.7)	3.8 (2.1)	5.7 (10.1)	9.9 (14.6)	7.7 (9.9)
% Strongly Stained	0.8 (2.2)	0.2 (0.2)	1.2 (3.4)	1.8 (3.6)	0.7 (1.7)
% Moderately Stained	1.6 (3.1)	0.7 (0.6)	1.7 (3.7)	3.0 (5.1)	2.0 (3.5)
% Weakly Stained	3.4 (3.4)	2.9 (1.4)	2.8 (3.1)	5.1 (6.0)	5.0 (4.8)

Plasma lorcaserin and perphenazine concentrations in 3-month male rat study

Plasma concentration of lorcaserin and perphenazine were measured at 0.25 hr post dose in male SD rats on Day 1, 7, 43 and 91. Several vehicle treated rats as well as perphenazine treated rats appear to have detectable levels of lorcaserin suggesting dosing error. Whether a dose mix-up would have resulted in poor study outcome is not certain but clearly dosing errors were relatively common.

APD356 Plasma Concentrations at 0.25 Hour on Day 1, 7, 45, and 90 after Oral Administration of APD356 at 10, 30 and 100 mg/kg/day for 90 Days to Fed Male Sprague-Dawley Rats (n = 11-36/group).

Group	Treatment	Plasma Concentration of APD356 (ng/mL)				N
		Dose (mg/kg/day)	Mean	SD	CV %	
Day 1						
2	APD356	10	65.5	66.0	101	32
3	APD356	30	149	145	97.5	31
4	APD356	100	294	214	72.8	32
Day 7						
2	APD356	10	194	115	59.3	32
3	APD356	30	550	300	54.5	31
4	APD356	100	2480	1640	66.1	36
Day 45^a						
2	APD356	10	111	59.0	53.1	21
3	APD356	30	369	176	47.8	21
4	APD356	100	1490	731	49.0	22
Day 90^b						
2	APD356	10	178	69	38.9	11
3	APD356	30	427	133	31.2	11
4	APD356	100	1920	609	31.7	11

^a Day 45 cohort - samples collected on Day 43

^b Day 90 cohort - samples collected on Day 91

Perphenazine Plasma Concentrations at 0.25 Hour on Day 1, 7, 45, and 90 after Oral Administration of Perphenazine at 5 mg/kg/day for 90 Days to Fed Male Sprague-Dawley Rats (n = 11-33/group).

Day	Treatment	Plasma Concentration of Perphenazine (ng/mL)				N
		Dose (mg/kg/day)	Mean	SD	CV %	
1 ^a	Perphenazine	5	6.33	7.53	119	32
7 ^a	Perphenazine	5	6.41	5.21	81.3	33
45 ^b	Perphenazine	5	13.1	10.4	79.3	22
90 ^c	Perphenazine	5	21.7	11.7	53.8	11

^a Although animal #3900 had high concentrations of APD356 it was included in the descriptive statistics.

^b Day 45 cohort - samples collected on Day 43

^c Day 90 cohort - samples collected on Day 91

Study Title: Effects of Prolactin Receptor Antagonism on Lorcaserin-mediated Changes in Mammary Glands of Female Sprague Dawley Rats (DBR-11-003)

Key Findings

- Oral administration of 100 mg/kg lorcaserin had no effect on plasma prolactin levels at any time point in this study.
- HD lorcaserin (100 mg/kg) had no effect on mammary hyperplasia. However, unlike the 3-month female rat study, 100 mg/kg lorcaserin (100 mkd) significantly increased mammary PCNA on Day 25 even though there was no increase in prolactin.
- Perphenazine significantly increased mammary tissue hyperplasia consistent with increase in prolactin and “positive” PCNA score consistent with increase in prolactin.
- The PCNA score may not be reliable in this study for the following reasons:
 - a) S179 did not reduce PCNA score in the negative control rats;
 - b) S179 did not block the effect of perphenazine on PCNA score or mammary hyperplasia
 - c) significantly higher PCNA score in the lorcaserin group alone should have been associated with hyperplasia, similar to perphenazine
 - e) More rats in the lorcaserin treated group (i.e, larger sample size) may have biased PCNA score outcome.
- Overall, the prolactin antagonist reduced the effect of lorcaserin on PCNA staining, but again the interpretation is not clear because lorcaserin did not increase prolactin or mammary hyperplasia, and also failed to prevent the effect of perphenazine, which clearly acts through prolactin.

Study Protocol

The objective of this study was to block the prolactin’s biological action by blocking the prolactin receptor. If prolactin is the intermediary hormone by which lorcaserin induces mammary tissues changes, then prolactin blockade should prevent mammary hyperplasia caused by lorcaserin and perphenazine.

Study start Date: Sept 20th 2011 and completion Date: Oct 22, 2011

Method:

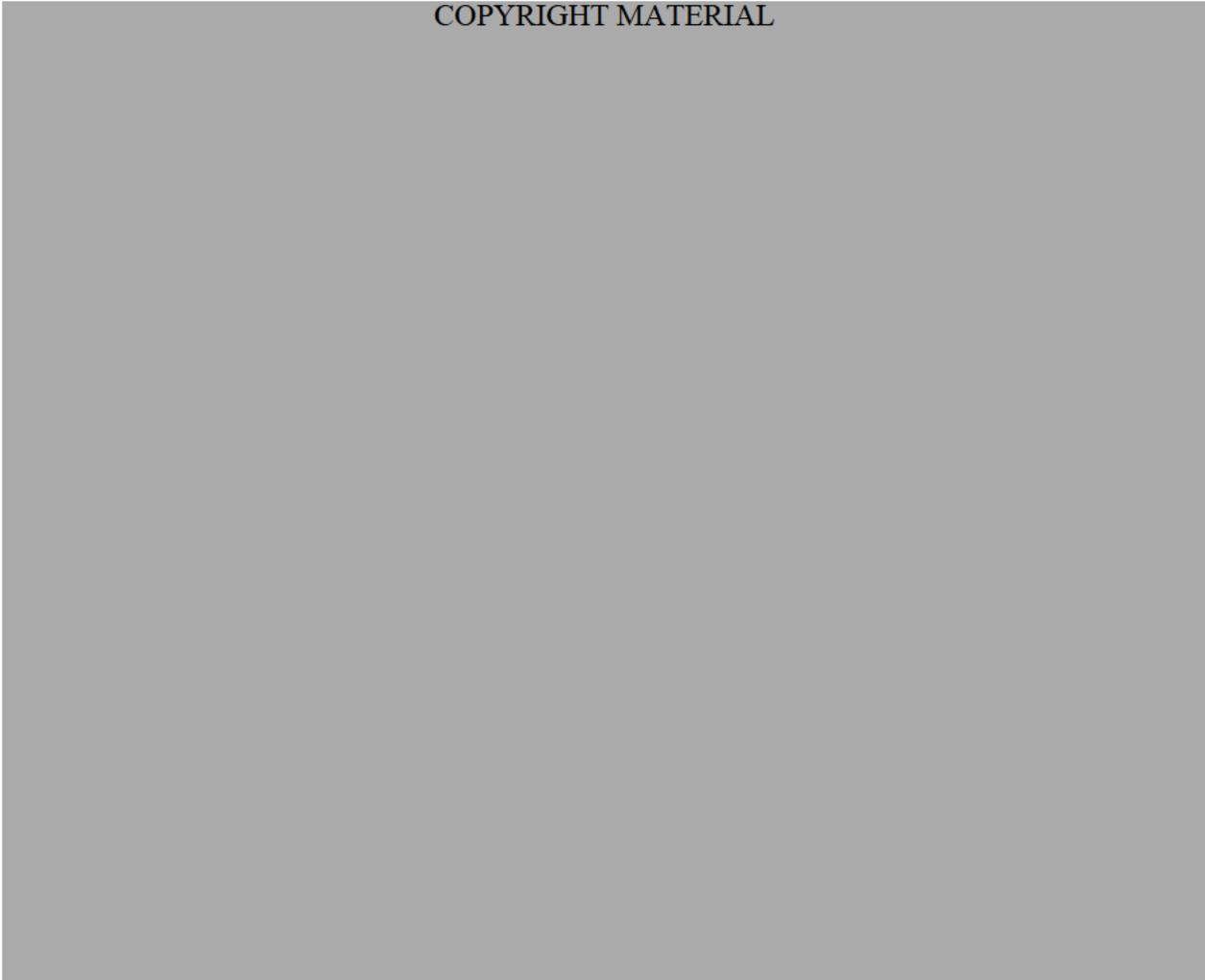
In this non-GLP study, 110 eight week old female SD rats (25/group for lorcaserin, 15/group for others, weighting 200-250 g from (b) (4) were individually housed with ad lib access to food and water. They were then gavaged with vehicle (controls), lorcaserin or perphenazine (positive control) for 25 days. Gavage doses (1 ml/kg) were delivered two hours after light cycle. The 12 hour light cycle was between 10:45 AM and 10.45 PM.

Group	Pump treatment	PO treatment
1	vehicle	vehicle
2	vehicle	lorcaserin (100 mg/kg)
3	vehicle	perphenazine (5 mg/kg)
4	S179D (6µg/day)	vehicle
5	S179D (6µg/day)	lorcaserin (100 mg/kg)
6	S179D (6µg/day)	perphenazine (5 mg/kg)

Rats were implanted with osmotic minipump 4 days before the start of the study under isoflurane anesthesia. The pumps containing vehicle or prolactin receptor antagonist, S179D were to deliver at a rate of 6 μ l/day (1 mg/ml of S179D) for 29 days total. Based on the rate and concentration, rats received 6 μ g/day of S179D (~ 25 μ g/kg). The biological activity of S179D was determined by [REDACTED] (b) (6) [REDACTED] (b) (4) [REDACTED] (b) (6) S179D is considered a partial or selective prolactin antagonist by the author [REDACTED] (b) (6)

1 2 3 4

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The figure is from Dr. Walker's publication listed on previous page

Sample Collection

Blood samples were collected at 20 hr post dose on Day 7, 10 and 24 from the tip of the tail. Earlier studies by the sponsor had found lorcaserin related increase in plasma prolactin in female SD rats at 20 hr post dose up to Day 10. Trunk blood was also collected (23 hr post final dose) after decapitation before necropsy. Plasma and mammary tissue prolactin levels were measured by ELISA kit from ALPCO (Salem, NH). Left mammary microscopic evaluation (H&E stain) and cell

⁹ Walker AM. S179D prolactin: antagonistic agony! *Mol Cell Endocrinol* 2007 September 30;276(1-2):1-9.

proliferation (proliferating cell nuclear antigen, PCNA) were carried out at (b) (4)

PCNA staining is generally used to determine the number of proliferating cells (positive staining cells) in the target tissue. The right mammary tissue samples were frozen on dry ice for storage. Prolactin data was analyzed by nonparametric methods. The data was also categorized as low (<100 ng/ml) or high (>100 ng/ml) prolactin concentrations. PCNA and prolactin immunohistochemistry data were analyzed by one-way ANOVA. Mammary tissue slides were evaluated by (b) (6). He used the following criteria in analysis of the H&E slides.

Samples: H&E sections of female rat mammary glands, five glands per animal/slide; 109 slides.

Histopathology: A subjective, semi-quantitative scoring system was utilized: 0 = no significant lesion; minimal change = 1; mild change = 2; moderate change = 3; and marked change = 4. See

Five lesions were scored: inflammation, fibrosis, hyperplasia, atrophy and neoplasia.

Inflammatory changes were generally minimal, consisting of mainly a few lymphocytes that were present in the fibrous connective tissue around ducts, within the duct epithelium and occasionally in glands. A rare PMN was also noted.

~~Fibrosis consisted of increased concentric fibrous collars around ducts and increased fibrous connective tissue adjacent to or outlining alveoli, which sometimes extended and blended into the surrounding adipose tissue. Fibrosis was generally minimal to mild, although focally the increase in fibrous connective tissue was occasionally moderate.~~

Hyperplasia was characterized by increased numbers of alveoli, which were sometimes associated with intracellular lipid droplets, as well as with either amphophilic or eosinophilic secretory material within alveolar lumens and/or within ducts. A second type of hyperplasia was characterized by increased numbers of ducts and/or duct epithelial cells, present as nests, or irregular arrays of pleomorphic cells obscuring the normal low cuboidal duct lining. Some of these pleomorphic cells could have arisen from myoepithelial cells. Hyperplasia generally varied from minimal to mild to moderate. In some instances, hyperplasia involved mainly ducts (e.g. #92) whereas in other instances, hyperplasia involved mainly alveoli (e.g. #93)

Atrophy of mammary gland structures was not identified in these sections.

Study

Results:

- 100 mg/kg lorcaserin increased mean plasma prolactin levels on Day 7 and 10 which returned to basal levels by Day 25. Several rats appeared to have the greatest contribution to the mean prolactin levels (4/25 rats in lorcaserin alone and 3/25 in lorcaserin +antagonist had high plasma prolactin levels, 157 to 521 ng/ml).
- Plasma prolactin levels in perphenazine treated rats were significantly increased (3.5 to 8.8 fold) on Day 7, 10 and 25 relative to vehicle control. A statistically significant number of rats in the perphenazine group had high prolactin levels (>100 ng/ml prolactin).
- As expected, prolactin receptor antagonist (S179D, 24 µg/kg/day) had no effect on plasma prolactin levels.

Plasma Prolactin after Daily Treatment with Lorcaserin or Perphenazine in the Presence or Absence of the Prolactin Receptor Antagonist S179D

Treatment Number	Treatment Group	Plasma Prolactin (ng/mL)	% of Animals with Prolactin >100ng/mL
7	vehicle-vehicle	18.4 ± 0.7	0.0
	vehicle-S179D	19.9 ± 1.5	0.0
	Lorcaserin-vehicle	45.2 ± 11.7	16.0
	Lorcaserin-S179D	65.9 ± 26.6	12.0
	Perphenazine-vehicle	161.8 ± 20.3 ^a	66.7 ^a
	Perphenazine-S179D	213.7 ± 39.6 ^b	80.0 ^b
10	vehicle-vehicle	19.7 ± 1.8	0.0
	vehicle-S179D	21.0 ± 2.6	0.0
	Lorcaserin-vehicle	43.9 ± 12.7	12.0
	Lorcaserin-S179D	63.8 ± 19.9	16.0
	Perphenazine-vehicle	117.0 ± 13.4 ^a	60.0 ^a
	Perphenazine-S179D	144.1 ± 23.7 ^b	73.3 ^b
25	vehicle-vehicle	20.8 ± 5.8	6.7
	vehicle-S179D	20.8 ± 3.0	0.0
	Lorcaserin-vehicle	16.2 ± 0.8	0.0
	Lorcaserin-S179D	20.6 ± 2.0	0.0
	Perphenazine-vehicle	65.7 ± 6.2 ^a	13.3
	Perphenazine-S179D	90.6 ± 14.5 ^b	40.0 ^c

^a P<0.01 versus vehicle-vehicle ^b P<0.01 versus vehicle-S179D ^c P<0.05 versus vehicle-S179D

- Mammary tissue hyperplasia (H&E stained tissues) significantly increased in both perphenazine (5 mg/kg/day) alone and perphenazine + S179D. Hyperplasia was greater on Day 28 in the 3-month study than observed here.
- Lorcaserin (100 mg/kg) alone had no effect on mammary tissue hyperplasia. Infusion of prolactin receptor antagonist had no effect on hyperplasia score in lorcaserin treated rats. The finding is similar to the 3-month study data.

Effect of the Prolactin Antagonist S179D on Lorcaserin-mediated Hyperplasia in the Rat Mammary Gland: Mean Hyperplasia Score

Treatment		Hyperplasia score (mean ± SE)
Minipump	PO treatment	
Vehicle	Vehicle	1.70 ± 0.11
Vehicle	Perphenazine (5 mg/kg)	2.59 ± 0.07 ^a
Vehicle	Lorcaserin (100 mg/kg)	1.88 ± 0.07
S179D	Vehicle	1.72 ± 0.08
S179D	Perphenazine (5 mg/kg)	2.57 ± 0.10 ^b
S179D	Lorcaserin (100 mg/kg)	1.83 ± 0.04

^a P<0.01 vs vehicle-vehicle ^b P<0.01 versus S179D-vehicle.

- PCNA staining of the mammary glands on Day 25 found a significant increase in cell proliferation in perphenazine and 100 mg/kg lorcaserin treated rats. This is opposite to the findings from the prior 3-month study, where the same dose of lorcaserin increased prolactin but did not increase PCNA staining. The PCNA score in this study appeared to be higher for lorcaserin while perphenazine matched the score in the previous study.

- Chronic infusion of S179D appeared to significantly reduce the number of positive nuclei in lorcaserin treated rats. The small decrease in positive nuclei in the perphenazine rats was not statistically significant [(it should be noted that there were more rats in lorcaserin treated groups than other groups, (n=25 vs. 15)].
- Chronic infusion of S179D had no effect on PCNA vs. vehicle control rats.
- There was no significant change in mammary prolactin levels in lorcaserin treated rats.

Analysis of Average Percent Positive Nuclei from PCNA Staining of Mammary Tissue

Descriptive Statistics						
Treatment	N	Mean	SD	Median	Minimum	Maximum
Vehicle/Vehicle	15	22.430	8.301	21.436	8.1254	36.7662
Perph/Vehicle	15	35.617	6.124	36.200	21.6199	43.9085
Lorc/Vehicle	24	32.229	13.619	35.688	5.1542	51.2252
Vehicle/Antagonist	15	23.870	10.091	20.932	5.6864	41.8230
Perph/Antagonist	15	30.745	6.262	32.044	19.7978	42.5570
Lorc/Antagonist	25	24.449	9.006	23.604	10.8274	50.4329
Analysis of Variance						
Least Square Mean						
Treatment	LS Mean			95% CI for LS Mean		
Vehicle/Vehicle	22.430			(17.467, 27.394)		
Perph/Vehicle	35.617			(30.653, 40.580)		
Lorc/Vehicle	32.229			(28.305, 36.153)		
Vehicle/Antagonist	23.870			(18.906, 28.833)		
Perph/Antagonist	30.745			(25.782, 35.709)		
Lorc/Antagonist	24.449			(20.605, 28.294)		
Between Group Comparisons	Difference in LS Means		95% CI		p-Value	
Lorc/Antagonist vs Lorc/Vehicle	-7.780		(-13.273, -2.286)		0.0060	
Lorc/Vehicle vs Vehicle/Vehicle	9.799		(3.472, 16.126)		0.0027	
Lorc/Antagonist vs Vehicle/Antagonist	0.580		(-5.699, 6.858)		0.8551	
Perph/Antagonist vs Perph/Vehicle	-4.871		(-11.891, 2.148)		0.1717	
Perph/Vehicle vs Vehicle/Vehicle	13.186		(6.167, 20.206)		0.0003	
Main Effect	p-Value					
Treatment	0.0003					
Root Mean Square Error = 9.693						
CI=Confidence Interval; LS=Least Squares; SD=Standard Deviation						

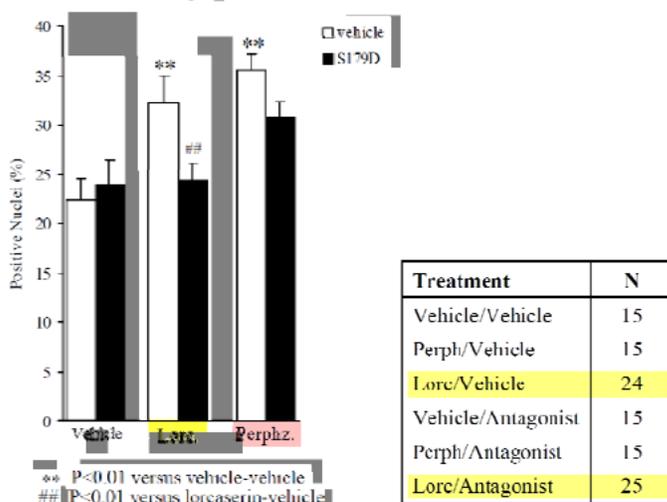
In the 3-month study (DBR-11-002, page 139) mammary tissue PCNA stained slides evaluated by (b) (4) labs found no positive signal with 100 mg/kg on Day 28

PCNA Staining in the Mammary Gland after 28 or 91 Days of Treatment

Treatment	Day 28 ^a	Day 91 ^a
Vehicle	6.7 ± 3.0	8.01 ± 3.8
Lorcaserin 10 mg/kg	-	10.3 ± 4.7 ^c
Lorcaserin 30 mg/kg	8.3 ± 3.6 ^b	9.1 ± 3.5
Lorcaserin 100 mg/kg	5.8 ± 3.3	8.1 ± 3.0
Perphenazine 5 mg/kg	15.9 ± 6.7 ^c	16.6 ± 5.2 ^c

^a Mean ± standard deviation ^b p < 0.05 versus vehicle control ^c p < 0.01 versus vehicle control

Effect of the Prolactin Antagonist S179D on Lorcaserin-mediated PCNA Staining in the Rat Mammary Gland



To get a better understanding of the relationship between experimental treatment and the endpoints of plasma prolactin, the reviewer examined mammary histopathology and PCNA scores, several female rats in each group in detail. It should be noted that these rats at the beginning of the study were 8 to 9 weeks old (56 to 63 days). Technically, the first 10 days where an increase in prolactin had been observed with 100 mg/kg lorcaserin should have led to changes at the end of the 30-day study.

Evaluation of these animals found no relationship between PCNA score and plasma prolactin levels. The incidences of mammary hyperplasia and fibrosis were seen in all sampled animals with severity of hyperplasia and fibrosis slightly higher in the perphenazine-treated rats. The prolactin antagonist did not appear to have an effect on mammary histopathology.

Animal ID	Treatment	Prolactin levels, ng/ml			Histopath	PCNA, % positive			
		Day 7	Day 10	Day 25		Mean	Strong +	Mod +	Weak+
43	Veh/Veh	19	17	100	hyper/fib	23.8	1.6	7.5	14.9
105	Veh/Veh	14	14	17	hyper/fib	21	0.5	4	16.5
91	Veh/Veh	19	17	18	hyper/fib	19.8	1.8	5.2	12.7
37	Lor/Veh	182	154	17	hyper/fib	11.7	0.23	2.3	9.2
57	Lor/Veh	170	270	15	hyper/fib	51	9	22.7	19
70	Lor/Veh	189	183	11	hyper/fib	5.1	0.1	0.8	4.3
92	Lor/Veh	17	16	17	hyper/fib	10.6	0.5	2.5	7.6
22	Lor/Veh	18	15	12	hyper/fib	44.9	6.5	16.5	21.9
30	Lor/Veh	18	21	17	hyper/fib	49	9.9	20.8	18.7
54	Lor/Ant	454	279	24	hyper/fib	10.6	0.5	2.48	7.6
5	Lor/Ant	209	174	12	hyper/fib	25.3	1.5	7.8	16
13	Lor/Ant	19.2	17.7	51	hyper/fib	21.5	0.5	5.0	16
20	Per/Antg	246	166	154	hyper/fib	32	3.9	13.2	15
6	Per/Antg	208	222	40	hyper/fib	19.8	2.1	6.03	11.7
24	Per/Veh	128	121	93	hyper/fib	42	7.2	16.8	17.8
45	Per/Veh	698	305	150	hyper/fib	41.5	10.0	14.9	16.5
100	Per/Veh	175	117	70	hyper/fib	21.6	1.6	6.9	13.1

Data tables provided for reference.

Number (%) of Rats with Mean Diagnosis Score \geq Various Cutpoint of H and E Slides: Fibrosis from All Sections Combined

Cutpoint	Treatment Group (N)	Incidence n (%)	%-Diff. Between Group (95% CI), Fisher's Test pValue
Mean Score \geq 1	Vehicle/Vehicle (N=15)	14 (93.3)	
	Perph/Vehicle (N=15)	14 (93.3)	P/A vs P/V: 6.67 (-5.96, 19.29), p>0.9999
	Lorc/Vehicle (N=24)	22 (91.7)	L/V vs V/V: -1.67 (-18.45, 15.11), p>0.9999
	Vehicle/Antagonist (N=15)	15 (100.0)	
	Perph/Antagonist (N=15)	15 (100.0)	
	Lorc/Antagonist (N=25)	25 (100.0)	L/A vs L/V: 8.33 (-2.72, 19.39), p=0.2347
Mean Score \geq 2	Vehicle/Vehicle (N=15)	3 (20.0)	
	Perph/Vehicle (N=15)	5 (33.3)	P/A vs P/V: 20.00 (-14.73, 54.73), p=0.4621
	Lorc/Vehicle (N=24)	7 (29.2)	L/V vs V/V: 9.17 (-18.04, 36.38), p=0.7110
	Vehicle/Antagonist (N=15)	5 (33.3)	L/A vs V/A: -13.33 (-41.88, 15.21), p=0.4568
	Perph/Antagonist (N=15)	8 (53.3)	
	Lorc/Antagonist (N=25)	5 (20.0)	L/A vs L/V: -9.17 (-33.18, 14.84), p=0.5202

Number (%) of Rats with Mean Diagnosis Score \geq Various Cutpoint of H and E Slides: Hyperplasia from All Sections Combined

Cutpoint	Treatment Group (N)	Incidence n (%)	%-Diff. Between Group (95% CI), Fisher's Test pValue
Mean Score \geq 1	Vehicle/Vehicle (N=15)	14 (93.3)	
	Perph/Vehicle (N=15)	15 (100.0)	
	Lorc/Vehicle (N=24)	23 (95.8)	L/V vs V/V: 2.50 (-12.44, 17.44), p>0.9999
	Vehicle/Antagonist (N=15)	15 (100.0)	
	Perph/Antagonist (N=15)	15 (100.0)	
	Lorc/Antagonist (N=25)	25 (100.0)	L/A vs L/V: 4.17 (-3.83, 12.16), p=0.4898
Mean Score \geq 2	Vehicle/Vehicle (N=15)	1 (6.7)	
	Perph/Vehicle (N=15)	15 (100.0)	
	Lorc/Vehicle (N=24)	11 (45.8)	L/V vs V/V: 39.17 (15.57, 62.76), p=0.0131
	Vehicle/Antagonist (N=15)	3 (20.0)	L/A vs V/A: 16.00 (-11.64, 43.64), p=0.4774
	Perph/Antagonist (N=15)	15 (100.0)	
	Lorc/Antagonist (N=25)	9 (36.0)	L/A vs L/V: -9.83 (-37.25, 17.58), p=0.5672
Mean Score \geq 3	Vehicle/Vehicle (N=15)	0	
	Perph/Vehicle (N=15)	2 (13.3)	P/A vs P/V: 0.00 (-24.33, 24.33), p>0.9999
	Lorc/Vehicle (N=24)	0	
	Vehicle/Antagonist (N=15)	0	
	Perph/Antagonist (N=15)	2 (13.3)	

The combined weak, moderate, and strongly positive stained nuclei in the lorcaserin + S179D rats were significantly lower than S179D alone. Although perphenazine had significantly increased positive nuclei, in the presence of S179D, there was no significant reduction in the number of positive nuclei.

Analysis of Average Percent Strongly Positive Nuclei from PCNA Staining of Mammary Tissue

Descriptive Statistics						
Treatment	N	Mean	SD	Median	Minimum	Maximum
Vehicle/Vehicle	15	1.644	1.457	1.513	0.0828	6.1174
Perph/Vehicle	15	6.052	2.787	6.409	1.6358	11.3728
Lorc/Vehicle	24	3.788	2.973	3.295	0.0861	9.8990
Vehicle/Antagonist	15	1.932	1.908	1.009	0.2363	5.9330
Perph/Antagonist	15	4.566	2.029	4.404	1.2104	9.3264
Lorc/Antagonist	25	2.142	1.904	1.475	0.3458	7.2588
Analysis of Variance						
Least Square Mean						
Treatment	LS Mean			95% CI for LS Mean		
Vehicle/Vehicle	1.644			(0.475, 2.814)		
Perph/Vehicle	6.052			(4.882, 7.221)		
Lorc/Vehicle	3.788			(2.864, 4.713)		
Vehicle/Antagonist	1.932			(0.763, 3.102)		
Perph/Antagonist	4.566			(3.396, 5.736)		
Lorc/Antagonist	2.142			(1.236, 3.048)		
Between Group Comparisons	Difference in LS Means		95% CI		p-Value	
Lorc/Antagonist vs Lorc/Vehicle	-1.646		(-2.941, -0.351)		0.0132	
Lorc/Vehicle vs Vehicle/Vehicle	2.144		(0.653, 3.635)		0.0053	
Lorc/Antagonist vs Vehicle/Antagonist	0.210		(-1.270, 1.689)		0.7791	
Perph/Antagonist vs Perph/Vehicle	-1.486		(-3.140, 0.168)		0.0778	
Perph/Vehicle vs Vehicle/Vehicle	4.407		(2.753, 6.062)		<0.0001	
Main Effect					p-Value	
Treatment					<0.0001	
Root Mean Square Error = 2.284						
CI=Confidence Interval; LS=Least Squares; SD=Standard Deviation						

Analysis of Average Percent Moderately Positive Nuclei from PCNA Staining of Mammary Tissue

Descriptive Statistics						
Treatment	N	Mean	SD	Median	Minimum	Maximum
Vehicle/Vehicle	15	6.675	3.403	7.025	1.1094	12.8950
Perph/Vehicle	15	12.935	2.867	12.240	6.9056	16.8395
Lorc/Vehicle	24	11.522	6.663	12.619	0.7871	22.7221
Vehicle/Antagonist	15	7.350	4.713	5.904	1.3442	16.9850
Perph/Antagonist	15	11.137	3.169	10.497	6.0387	18.2897
Lorc/Antagonist	25	7.818	4.471	7.273	2.1236	20.7858
Analysis of Variance						
Least Square Mean						
Treatment	LS Mean			95% CI for LS Mean		
Vehicle/Vehicle	6.675			(4.293, 9.058)		
Perph/Vehicle	12.935			(10.532, 15.317)		
Lorc/Vehicle	11.522			(9.638, 13.405)		
Vehicle/Antagonist	7.350			(4.968, 9.733)		
Perph/Antagonist	11.137			(8.755, 13.520)		
Lorc/Antagonist	7.818			(5.973, 9.664)		
Between Group Comparisons	Difference in LS Means		95% CI		p-Value	
Lorc/Antagonist vs Lorc/Vehicle	-3.703		(-6.340, -1.067)		0.0064	
Lorc/Vehicle vs Vehicle/Vehicle	4.847		(1.810, 7.884)		0.0020	
Lorc/Antagonist vs Vehicle/Antagonist	0.468		(-2.545, 3.482)		0.7586	
Perph/Antagonist vs Perph/Vehicle	-1.798		(-5.167, 1.572)		0.2925	
Perph/Vehicle vs Vehicle/Vehicle	6.239		(2.890, 9.629)		0.0004	
Main Effect					p-Value	
Treatment					0.0002	
Root Mean Square Error = 4.652						
CI=Confidence Interval; LS=Least Squares; SD=Standard Deviation						

Analysis of Average Percent Weakly Positive Nuclei from PCNA Staining of Mammary Tissue

Descriptive Statistics						
Treatment	N	Mean	SD	Median	Minimum	Maximum
Vehicle/Vehicle	15	14.111	4.065	14.788	5.9042	21.4268
Perph/Vehicle	15	16.630	1.957	16.724	13.0784	21.3519
Lorc/Vehicle	24	16.919	4.908	18.551	4.2811	23.7984
Vehicle/Antagonist	15	14.587	4.195	14.231	4.1059	20.0640
Perph/Antagonist	15	15.042	2.371	15.047	11.5470	19.7984
Lorc/Antagonist	25	14.489	3.446	14.103	8.3580	22.3883
Analysis of Variance						
Least Square Mean						
Treatment	LS Mean			95% CI for LS Mean		
Vehicle/Vehicle	14.111			(12.190, 16.032)		
Perph/Vehicle	16.630			(14.710, 18.551)		
Lorc/Vehicle	16.919			(15.401, 18.438)		
Vehicle/Antagonist	14.587			(12.667, 16.508)		
Perph/Antagonist	15.042			(13.122, 16.963)		
Lorc/Antagonist	14.489			(13.001, 15.977)		
Between Group Comparisons		Difference in LS Means		95% CI		p-Value
Lorc/Antagonist vs Lorc/Vehicle		-2.430		(-4.556, -0.305)		0.0255
Lorc/Vehicle vs Vehicle/Vehicle		2.808		(0.360, 5.257)		0.0250
Lorc/Antagonist vs Vehicle/Antagonist		-0.098		(-2.528, 2.331)		0.9361
Perph/Antagonist vs Perph/Vehicle		-1.588		(-4.304, 1.128)		0.2489
Perph/Vehicle vs Vehicle/Vehicle		2.519		(-0.197, 5.236)		0.0687
Main Effect				p-Value		
Treatment				0.0965		
Root Mean Square Error = 3.751						
CI=Confidence Interval; LS=Least Squares; SD=Standard Deviation						

Analysis of Average Positivity from Prolactin Staining of Mammary Tissue

Descriptive Statistics						
Treatment	N	Mean	SD	Median	Minimum	Maximum
Vehicle/Vehicle	15	18.912	6.138	19.289	9.1674	30.5112
Perph/Vehicle	15	21.246	6.920	21.615	9.8391	32.1783
Lorc/Vehicle	24	18.365	7.080	17.689	7.9199	33.1261
Vehicle/Antagonist	15	16.532	7.512	15.958	9.1393	37.3718
Perph/Antagonist	15	24.504	6.857	25.777	10.8290	33.1425
Lorc/Antagonist	25	16.973	7.387	16.150	7.4327	32.0637
Analysis of Variance						
Between Group Comparisons		Difference in LS Means		95% CI		p-Value
Lorc/Antagonist vs Lorc/Vehicle		-1.392		(-5.404, 2.621)		0.4931
Lorc/Vehicle vs Vehicle/Vehicle		-0.577		(-5.198, 4.044)		0.8048
Lorc/Antagonist vs Vehicle/Antagonist		0.111		(-4.111, 5.027)		0.8190
Perph/Antagonist vs Perph/Vehicle		3.258		(-1.869, 8.384)		0.2104
Perph/Vehicle vs Vehicle/Vehicle		2.304		(-2.823, 7.431)		0.3748
Main Effect				p-Value		
Treatment				0.0165		

Analysis of Prolactin Data at Day 7

Treatment	N	Mean	SD	Median	Minimum	Maximum	Mean Rank Scores
Lorcaserin 100 mg/kg / S179D (L/A)	25	65.956	133.190	19.300	14.1000	521.6000	48.24
Lorcaserin 100 mg/kg / Vehicle (L/V)	25	45.180	58.718	18.000	13.8000	188.9000	45.02
Perphenazine 5 mg/kg / S179D (P/A)	15	213.647	153.242	199.500	15.0000	559.7000	89.23
Perphenazine 5 mg/kg / Vehicle (P/V)	15	161.847	164.141	124.300	27.9000	697.7000	87.03
Vehicle / S179D (V/A)	15	19.873	5.659	18.100	15.1000	37.9000	38.43
Vehicle / Vehicle (V/V)	15	18.360	2.868	18.000	13.6000	24.2000	36.87

Analysis of Prolactin Data at Day 10

Treatment	N	Mean	SD	Median	Minimum	Maximum	Mean Rank Scores
Lorcaserin 100 mg/kg / S179D (L/A)	25	63.812	99.308	21.500	15.3000	420.2000	54.78
Lorcaserin 100 mg/kg / Vehicle (L/V)	25	43.928	63.255	19.400	14.9000	269.9000	42.40
Perphenazine 5 mg/kg / S179D (P/A)	15	144.140	91.647	122.100	18.7000	389.1000	88.70
Perphenazine 5 mg/kg / Vehicle (P/V)	15	116.980	72.324	121.300	20.0000	305.2000	84.87
Vehicle / S179D (V/A)	15	21.000	9.943	18.200	15.4000	56.3000	40.40
Vehicle / Vehicle (V/V)	15	19.740	7.026	16.600	14.4000	35.9000	31.07

Analysis of Prolactin Data at Day 25

Treatment	N	Mean	SD	Median	Minimum	Maximum	Mean Rank Scores
Lorcaserin 100 mg/kg / S179D (L/A)	25	20.628	10.054	18.400	11.9000	51.0000	49.56
Lorcaserin 100 mg/kg / Vehicle (L/V)	24	16.213	3.902	17.050	11.3000	27.1000	37.15
Perphenazine 5 mg/kg / S179D (P/A)	15	90.567	56.191	89.400	13.1000	183.8000	90.63
Perphenazine 5 mg/kg / Vehicle (P/V)	15	65.660	37.515	62.300	17.4000	149.5000	87.33
Vehicle / S179D (V/A)	15	20.793	11.436	17.700	11.1000	54.8000	46.17
Vehicle / Vehicle (V/V)	15	20.833	22.585	13.200	11.2000	100.4000	33.50

Study Summary:

Data for perphenazine is consistent with earlier observations of mammary hyperplasia, hyperprolactinemia and PCNA score. Prolactin receptor antagonist was unable to block the effect of perphenazine on mammary tissue changes, rendering this study inconclusive and non-interpretable. The data for lorcaserin was inconsistent with earlier observations (hyperplasia, plasma prolactin and PCNA data). In the absence of elevated plasma prolactin and hyperplasia, the mild but significant increase in PCNA score suggests that prolactin might be minimally involved in lorcaserin induced mammary tumors, but since lorcaserin failed to increase PCNA score in the 3-month study, the PCNA score in this study is not convincing.

Study # DBR-11-026: Effect of Bromocriptine on Lorcaserin-induced Increases in Plasma Prolactin

Study start date: Jan 6, 11, completion date: Jan 19, 11, Report Date Dec 20, 2011

Key Findings:

- Plasma prolactin levels measured 15-min post dose, increased significantly after single dose of 100 mg/kg lorcaserin in female rats. Multiple doses of lorcaserin were not tested. The rise in prolactin at 15 min post dose has been inconsistent in the earlier studies in female rats.
- Subcutaneous infusion of bromocriptine, a dopamine agonist, blocked the single dose effect of lorcaserin on plasma prolactin.
- This study demonstrated that the acute increase in plasma prolactin in response to a single dose of lorcaserin derives from the pituitary gland.

Study Protocol:

In this non GLP study, 9 WK old female SD rats from (b) (4) individually housed with 6 AM-6PM light cycle were implanted with a pellet to deliver continuous infusion of bromocriptine (25 and 50 mg/pellet) for 11 or 12 days. At the end of the study on Day 11 or 12, a single dose of 100 mg/kg lorcaserin was delivered by gavage (1 ml/kg). In earlier pilot studies, bromocriptine treated rats did not tolerate multiple doses of lorcaserin so multiple dosing was not carried out.

Group	Subcutaneous pellet	PO treatment
1	Placebo	vehicle
2	Placebo	lorcaserin (100 mg/kg)
3	Bromocriptine 25mg	vehicle
4	Bromocriptine 25mg	lorcaserin (100 mg/kg)
5	Bromocriptine 50mg	vehicle
6	Bromocriptine 50mg	lorcaserin (100 mg/kg)

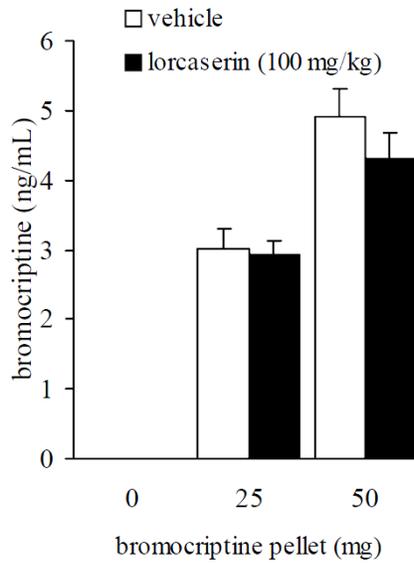
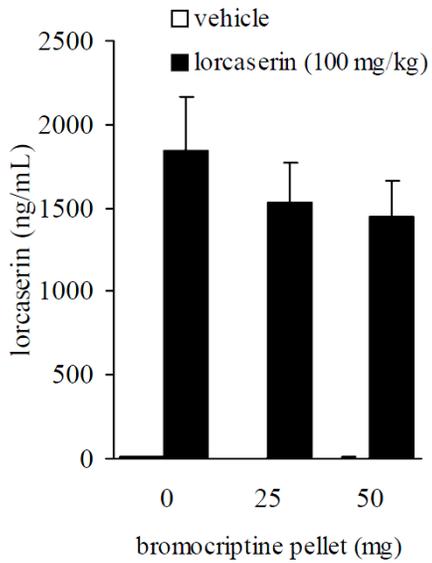
Bromocriptine (Cycloset ®) is a potent dopamine D2 receptor agonist known to inhibit prolactin release from the pituitary gland. Two dose levels of bromocriptine 25 and 50 mg pellets were used to deliver 1.2 to 2.4 µg.h/ml/day. In a pilot study, 10 mg bromocriptine pellet implant (behind the neck) was only capable of partial blockade of lorcaserin-induced increase in plasma prolactin (data not provided by sponsor).

Plasma concentrations of prolactin (ELISA, ALPCO, Salem, NH), lorcaserin and bromocriptine were measured at the end of the study by decapitation **15 min post dose**.

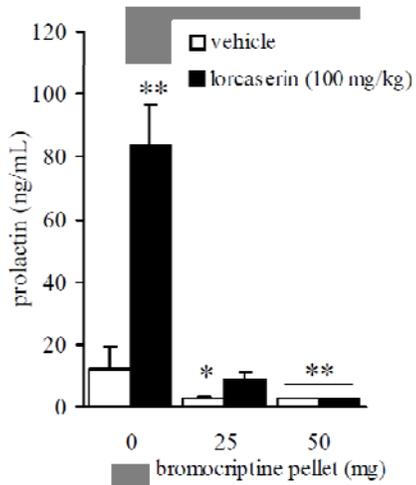
Results:

- Single dose of 100 mg/kg lorcaserin significantly increased 15 min post dose plasma prolactin levels (82 ng/ml vs. 12 ng/ml placebo) in female SD rats.
- Continuous SC infusion of 1.2 and 2.4 mg/day bromocriptine (25 and 50 mg pellet) for 11 days significantly reduced plasma prolactin levels independent of lorcaserin. The inhibition of lorcaserin induced increase in prolactin by bromocriptine demonstrates that plasma prolactin in lorcaserin rats comes from the anterior pituitary gland under control of dopaminergic system, overcoming perhaps the role of 5HT_{2C} in female rats.

Pharmacokinetic Interaction Between Lorcaserin and Bromocriptine



Effect of Bromocriptine on Lorcaserin-induced Increases in Plasma Prolactin



*P<0.05, **P<0.01 versus vehicle-vehicle.

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Study # DBR-11-025: Effect of Hypophysectomy on Lorcaserin-mediated Changes in Mammary Glands of Female Sprague Dawley Rats

Study start: Aug 22, 11, Completion: Sept 9, 11, Study report: Dec 22, 2011

In a non GLP study, 14-week old sham operated or hypophysectomized (b) (4) (b) (4) female SD rats (n=25/group) were acclimated for a week and treated with 30 mg/kg gavage doses of lorcaserin for 10 days (2 hrs into light cycle). Rats were housed individually (10 am to 10 pm light cycle) with ad-lib access to food and water. The surgical procedure was performed on rats when they were 12-WK old. To maintain good health in hypophysectomized rat, water was supplemented with 5% sucrose solution throughout the study.

Although the original study was designed for 28 days with 30 and 100 mg/kg lorcaserin, due to tolerability issues, dosing was limited to 30 mg/kg given for 10 days (due to weight loss > 10%). However, rats were

Group	Surgery	PO treatment
1	Sham	vehicle
2	Sham	lorcaserin (30 mg/kg)
3	Hypophysectomized	vehicle
4	Hypophysectomized	lorcaserin (30 mg/kg)

allowed to recover until sacrifice on Day 30. Pituitary gland plays a critical role in regulation of many hormones that play a pivotal role in homeostasis. The pituitary gland has three sections, anterior, intermediate (MSH) and posterior (Oxytocin and ADH). The acidophilic cells in anterior pituitary produce GH and prolactin while basophilic cells produce TSH, FSH and LH. The anterior chromophobic cells produce ACTH. Since pituitary is such a critical brain tissue, excising it was likely affecting their overall health.

Blood samples were collected from tail at 8 am on experimental Day 11 (20 hrs post dose) and 29. Based on 3-month study in female SD rats, 20 hr post dose was the ideal time for prolactin analysis. Mammary tissue samples were collected when animals were sacrificed by decapitation. As noted earlier, a higher dose of lorcaserin (100 mg/kg) was not tolerated and significant mortality occurred within 2 to 4 days in a pilot study in hypophysectomized female SD rats. Mammary tissue histological evaluations (H&E stain) and PCNA for cell proliferation were carried out at (b) (4). Mammary hyperplasia was characterized as an increase in the size and number of lobules/secretory product in the duct which were scored semi-quantitatively (0=normal, 1=minimal, 2=mild, 3=moderate, 4=marked change). In addition to hyperplasia, the pathologist considered the incidence of inflammation, fibrosis and atrophy in the mammary tissue slides. There was no positive control. Plasma prolactin was analyzed by nonparametric method.

Study Results:

- Lorcaserin did not increase plasma prolactin in sham or hypophysectomized rats.
- Lorcaserin increased the 'mean hyperplasia score' in sham but not in hypophysectomized rats.
- Lorcaserin did not increase PCNA staining in sham or hypophysectomized rats, which is inconsistent with the histology data showing increased hyperplasia in sham animals.
- Hypophysectomy alone substantially reduced PCNA staining and the hyperplasia score.

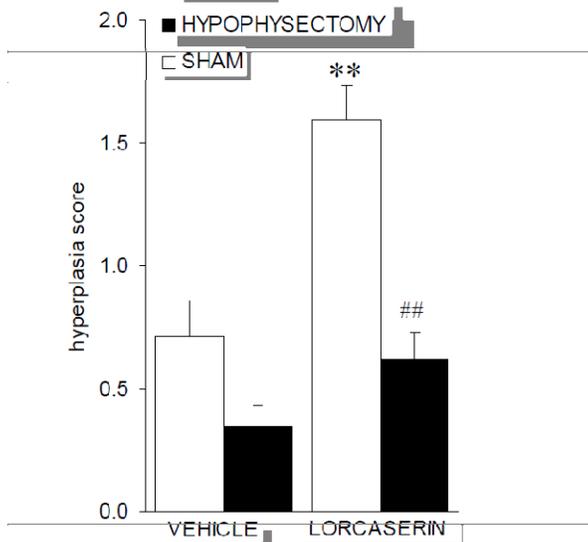
Effect of Hypophysectomy on Lorcaserin-mediated PCNA Staining in the Rat Mammary Gland

Treatment		Average PCNA positive nuclei (%)
Surgery	PO treatment	
Sham	Vehicle	15.0 ± 1.7
Sham	Lorcaserin (30 mg/kg)	11.7 ± 1.2 ^a
Hypophysectomy	Vehicle	3.6 ± 0.8
Hypophysectomy	Lorcaserin (30 mg/kg)	3.0 ± 0.5 ^b

^a P<0.05 versus sham-vehicle

^b P<0.01 versus sham-lorcaserin.

Effect of Hypophysectomy on Lorcaserin-mediated Hyperplasia in the Rat Mammary Gland: Mean Hyperplasia Score



**P<0.01 vs SHAM-VEH

##P<0.01 vs SHAM-LORC

The tables below show the histological findings reported by the pathologist. In all tissues examined, the severity of inflammation was minimal while fibrosis and hyperplasia ranged between minimal to mild.

Number (%) of Rats with Mean Diagnosis Score \geq Various Cutpoint of H and E Slides: Inflammation from All Sections Combined

Cutpoint	Treatment Group (N)	Incidence n (%)	%-Diff. Between Group (95% CI), Fisher's Test pValue
Mean Score \geq 1	Sham + Vehicle (N=25)	21 (84.0)	Vs. Sham+Lorc: 4.00 (-17.27, 25.27), p>0.9999
	Sham + Lorcasecin 30mpk (N=25)	20 (80.0)	Vs. Hypox+Lorc: 34.55 (8.49, 60.60), p=0.0179
	Hypox + Vehicle (N=25)	11 (44.0)	Vs. Hypox+Lorc: -1.45 (-29.94, 27.03), p>0.9999
	Hypox + Lorcasecin 30mpk (N=22)	10 (45.5)	

Number (%) of Rats with Mean Diagnosis Score \geq Various Cutpoint of H and E Slides: Fibrosis from All Sections Combined

Cutpoint	Treatment Group (N)	Incidence n (%)	%-Diff. Between Group (95% CI), Fisher's Test pValue
Mean Score \geq 1	Sham + Vehicle (N=25)	12 (48.0)	Vs. Sham+Lorc: -40.00 (-63.36, -16.64), p=0.0054
	Sham + Lorcasecin 30mpk (N=25)	22 (88.0)	Vs. Hypox+Lorc: 6.18 (-14.36, 26.72), p=0.6902
	Hypox + Vehicle (N=25)	24 (96.0)	Vs. Hypox+Lorc: 14.18 (-3.57, 32.04), p=0.1710
	Hypox + Lorcasecin 30mpk (N=22)	18 (81.8)	
Mean Score \geq 2	Sham + Vehicle (N=25)	0	Vs. Sham+Lorc: -4.00 (-11.68, 3.68), p>0.9999
	Sham + Lorcasecin 30mpk (N=25)	1 (4.0)	Vs. Hypox+Lorc: -0.55 (-12.15, 11.06), p>0.9999
	Hypox + Vehicle (N=25)	4 (16.0)	Vs. Hypox+Lorc: 11.45 (-5.35, 28.26), p=0.3525
	Hypox + Lorcasecin 30mpk (N=22)	1 (4.5)	

Number (%) of Rats with Mean Diagnosis Score \geq Various Cutpoint of H and E Slides: Hyperplasia from All Sections Combined

Cutpoint	Treatment Group (N)	Incidence n (%)	% Diff. Between Group (95% CI), Fisher's Test pValue
Mean Score \geq 1	Sham + Vehicle (N=25)	8 (32.0)	Vs. Sham+Lorc: -48.00 (-72.09, -23.91), p=0.0014
	Sham + Lorcasecin 30mpk (N=25)	20 (80.0)	Vs. Hypox+Lorc: 61.82 (39.33, 84.30), p<0.0001
	Hypox + Vehicle (N=25)	4 (16.0)	Vs. Hypox+Lorc: -2.18 (-23.78, 19.41), p>0.9999
	Hypox + Lorcasecin 30mpk (N=22)	4 (18.2)	
Mean Score \geq 2	Sham + Vehicle (N=25)	3 (12.0)	Vs. Sham+Lorc: -28.00 (-51.04, -4.96), p=0.0507
	Sham + Lorcasecin 30mpk (N=25)	10 (40.0)	Vs. Hypox+Lorc: 40.00 (20.80, 59.20), p=0.0008
	Hypox + Vehicle (N=25)	0	
	Hypox + Lorcasecin 30mpk (N=22)	0	

Number (%) of Rats with Maximal Diagnosis Score \geq Various Cutpoint of H and E Slides: Hyperplasia from All Sections Combined

Cutpoint	Treatment Group (N)	Incidence n (%)	%-Diff. Between Group (95% CI), Fisher's Test pValue
Max Score \geq 1	Sham + Vehicle (N=25)	18 (72.0) ^a	Vs. Sham+Lorc: -24.00 (-43.20, -4.80), p=0.0488
	Sham + Lorcasecin 30mpk (N=25)	24 (96.0)	Vs. Hypox+Lorc: 18.73 (-0.39, 37.85), p=0.0848
	Hypox + Vehicle (N=25)	15 (60.0)	Vs. Hypox+Lorc: -17.27 (-43.26, 8.72), p=0.2301
	Hypox + Lorcasecin 30mpk (N=22)	17 (77.3)	
Max Score \geq 2	Sham + Vehicle (N=25)	8 (32.0) ^b	Vs. Sham+Lorc: -52.00 (-75.26, -28.74), p=0.0004
	Sham + Lorcasecin 30mpk (N=25)	21 (84.0) ^c	Vs. Hypox+Lorc: 47.64 (22.93, 72.35), p=0.0011
	Hypox + Vehicle (N=25)	5 (20.0)	Vs. Hypox+Lorc: -16.36 (-41.86, 9.13), p=0.3279
	Hypox + Lorcasecin 30mpk (N=22)	8 (36.4)	
Max Score \geq 3	Sham + Vehicle (N=25)	2 (8.0)	Vs. Sham+Lorc: -20.00 (-40.56, 0.56), p=0.1383
	Sham + Lorcasecin 30mpk (N=25)	7 (28.0) ^c	Vs. Hypox+Lorc: 28.00 (10.40, 45.60), p=0.0104
	Hypox + Vehicle (N=25)	0	
	Hypox + Lorcasecin 30mpk (N=22)	0	

^a P<0.05 vs SHAM-LORC

^b P<0.01 vs SHAM-LORC

^c P<0.01 vs HYPOX-LORC

Number (%) of Rats with Mean Diagnosis Score \geq Various Cutpoint of H and E Slides: Atrophy from All Sections Combined

Cutpoint	Treatment Group (N)	Incidence n (%)	%-Diff. Between Group (95% CI), Fisher's Test pValue
Mean Score \geq 1	Sham + Vehicle (N=25)	2 (8.0)	Vs. Sham+Lorc: 8.00 (-2.63, 18.63), p=0.4898
	Sham + Lorcaserin 30mpk (N=25)	0	Vs. Hypox+Lorc: -81.82 (-97.94, -65.70), p<0.0001
	Hypox + Vehicle (N=25)	24 (96.0)	Vs. Hypox+Lorc: 14.18 (-3.67, 32.04), p=0.1710
	Hypox + Lorcaserin 30mpk (N=22)	18 (81.8)	
Mean Score \geq 2	Sham + Vehicle (N=25)	0	
	Sham + Lorcaserin 30mpk (N=25)	0	Vs. Hypox+Lorc: -77.27 (-94.78, -59.76), p<0.0001
	Hypox + Vehicle (N=25)	23 (92.0)	Vs. Hypox+Lorc: 14.73 (-5.76, 35.21), p=0.2276
	Hypox + Lorcaserin 30mpk (N=22)	17 (77.3)	
Mean Score \geq 3	Sham + Vehicle (N=25)	0	
	Sham + Lorcaserin 30mpk (N=25)	0	Vs. Hypox+Lorc: -50.00 (-70.89, -29.11), p<0.0001
	Hypox + Vehicle (N=25)	21 (84.0)	Vs. Hypox+Lorc: 34.00 (8.64, 59.36), p=0.0264
	Hypox + Lorcaserin 30mpk (N=22)	11 (50.0)	
Mean Score \geq 4	Sham + Vehicle (N=25)	0	
	Sham + Lorcaserin 30mpk (N=25)	0	
	Hypox + Vehicle (N=25)	1 (4.0)	Vs. Hypox+Lorc: 4.00 (-3.68, 11.68), p>0.9999
	Hypox + Lorcaserin 30mpk (N=22)	0	

Study Summary

Surgical removal of the pituitary should ablate lorcaserin-induced increases in prolactin and prevent any histological changes in mammary tissue. Unfortunately, the duration of dosing was limited to 10 days due to excessive body weight loss, and the dose was restricted to 30mg/kg lorcaserin because higher doses were toxic to hypophysectomized rats. Therefore, following hypophysectomy or a sham procedure, female rats were dosed daily for 10 days with 30mg/kg lorcaserin. The animals were allowed to recover, un-dosed, until day 28.

Prolactin measured at 20h post-dose on day 10 (end of dosing) and 28 (end of recovery) did not show an increase with lorcaserin in either sham or hypophysectomized animals.

The sponsor contends that 30mg/kg lorcaserin increased mammary hyperplasia in sham but not hypophysectomized rats within the 10 day dosing period, and is therefore evidence for an intermediary role of prolactin in the histological change to mammary tissue. Hyperplasia in this case was defined as the presence of secretory product as well as the number or size of lobules. The Agency does not agree with this conclusion, as lorcaserin did not increase prolactin in this study. Also, 18 of the 25 sham-operated animals dosed vehicle for 10 days presented with mammary hyperplasia, which is an excessively high background rate for control animals. The further increase in hyperplasia to 24 of 25 animals with lorcaserin is considered marginal given the inexplicably high background rate of mammary hyperplasia in sham-operated animals.

Integrated Summary and Safety Evaluation

Lorcaserin NDA Resubmission (Dec 2011):

Receptor Pharmacology

Lorcaserin is a 5HT_{2C} serotonin receptor selective agonist developed for treatment of obesity. Binding affinity of lorcaserin for 5HT_{2c} is within 7- to 10-fold the affinity for 5HT_{2A} and 5HT_{2B}. Selectivity for functional activation of 5HT_{2c} is within the range of 8x-15x for 5HT_{2A} and 45x-90x for 5HT_{2B}. The sponsor's resubmission addressed disparities in the receptor potency data from the original NDA and provided additional analyses addressing 5HT_{2C} selectivity of lorcaserin. The newly submitted studies demonstrate that lorcaserin is notably less potent at all 5HT₂ receptor subtypes than previously reported. As discussed in Dr. Bourcier's review in Appendix A, the weight of evidence based on non-clinical data indicates that therapeutic exposure of lorcaserin is within the selective range for activation of 5HT_{2C}, and that activation of 5HT_{2A} and 2B is unlikely both in the CNS and peripheral tissues.

Re-Adjudication of Mammary Tumors in Female SD Rats

Following the CR letter issued in the first review cycle, the Division requested re-adjudication of all available mammary tissue slides from female rats by a pathology working group (PWG). The sponsor convened a PWG comprised of 5 independent expert veterinary pathologists, and their findings are considered definitive. The PWG markedly lowered the incidence of adenocarcinoma in all lorcaserin treated female rats. The numbers of adenocarcinoma were reduced by 2 in control and by 13, 11 and 9 in LD, MD and HD, respectively. The numbers of fibroadenoma increased in all groups.

The incidence of adenocarcinoma at 10 and 30 mg/kg was no longer numerically different from the control. Only the 100 mg/kg lorcaserin significantly increased mammary adenocarcinoma in female rats with 24x fold safety margin to clinical dose of 10 mg BID based on AUC. The incidences of adenocarcinoma and fibroadenoma were not combined since there was a high degree of certainty in diagnosis and agreement among PWG. The 24x fold safety margin mitigated the Agency's concern for mammary adenocarcinoma. The incidence of fibroadenoma significantly increased at all doses of lorcaserin with no safety margin (<7x the clinical dose).

When the tumor onset and multiplicity for adenocarcinoma was re-analyzed, only the 100 mg/kg lorcaserin increased the tumor multiplicity and reduced the time for tumor onset in female rats. In case of mammary fibroadenoma, lorcaserin increased the tumor multiplicity and reduced the time for tumor onset at all doses relative to control female rats.

When the incidences of lung metastases originating from mammary adenocarcinoma were examined by the PWG, there were fewer lung metastases in female rats. The lung metastases as percent of adenocarcinoma at 10, 30 and 100 mg/kg lorcaserin were 5%, 21% and 10%, respectively. There was no lung metastasis in the control rats. The lung metastases at 30 mg/kg lorcaserin were

greater than the historical control (0-12.5%) in spite of fewer adenocarcinomas at this dose. Although the number of lung metastases in the HD female rats was within the historical range, it was not reassuring since the majority of rats in this group had died prematurely which may have reduced the spread of adenocarcinoma metastases to the lungs. The PWG considered lung metastases incidence at 10 mg/kg to be similar to control and equivocally increased at 30 and 100 mg/kg.

Prolactin Mode of Action Studies

SD rats spontaneously develop mammary and pituitary tumors. Prolactin is one of the primary driving hormones responsible for mammary tumors in aging SD rats as is considered the mode of action tumor induction associated with antidopaminergic antipsychotic drugs. Increased incidence of mammary tumors with these drugs has been consistently associated with a robust sustained increase in plasma prolactin and increased incidence of pituitary tumors. Prolactin was postulated by the sponsor to be responsible for lorcaserin-induced mammary tumors in rats. To provide evidence for the role of prolactin, the sponsor had submitted several mechanistic studies in the original NDA in 2009. Unfortunately, none of the original studies resulted in any significant change in prolactin in the intact female rats. Plasma prolactin was only increased after a single dose of 100 mg/kg in one study in male SD rats. Overall, the agency considered the prolactin role as inadequate to explain the lorcaserin-induced increase in mammary tumors. At the end of the NDA review meeting, the sponsor stated that new methodology and timing of plasma collection was likely to show a lorcaserin-related increase in prolactin. It should be noted that after PWG readjudication and the identification of a 24-fold safety margin for adenocarcinoma, the mode of action for adenocarcinoma is no longer critical. However, since the incidence of fibroadenoma was increased at all doses of lorcaserin, prolactin as the mode of action studies were considered relevant. To support the prolactin hypothesis, the sponsor conducted several mechanistic studies with lorcaserin in female and male SD rats up to 3-months. The plasma and pituitary prolactin and the preneoplastic changes in the mammary tissue samples were measured at specific time points in these studies. In the first 3-month study in female SD rats, oral administration of lorcaserin (10, 30 and 100 mg/kg) had no effect on 1 hr post dose plasma prolactin levels. However, the highest dose of lorcaserin significantly increased the 20 hr post dose plasma prolactin but only during the first 10 days of the 3-month lorcaserin treatment. With the appearance of more frequent spikes in plasma prolactin in the lorcaserin treated rats, the mean 90 day plasma prolactin was calculated and found to be significantly increased at 10 and 100 mg/kg lorcaserin, suggesting that collectively lorcaserin may minimally but nevertheless significantly increase the prolactin levels in female rats.

In comparison to lorcaserin, perphenazine (positive control, 5 mg/kg PO), robustly and consistently increased plasma prolactin at all time points throughout the study and depleted pituitary prolactin. Unlike lorcaserin, the maximum increase in daily prolactin levels at 1 hr post dose with the perphenazine corresponded to its T_{max}. The increase in prolactin at 20 hr post dose at the tail end of the lorcaserin exposure (trough levels) suggests perhaps lorcaserin recruits a cascade of steps in its stimulation of the pituitary to release prolactin.

Mammary tissue differentiates under hormonal influence (i.e. prolactin, estradiol) from terminal end buds (TEB) to mammary lobule types 1 to types 2 and 3. In the mammary tumor differentiation analysis by Dr. Russo, a small but significant decrease in TEB was seen in the lorcaserin treated rats, suggesting that lorcaserin promoted differentiation in a manner consistent with hormonal action. Perphenazine robustly increased prolactin and strongly differentiated mammary tissue to lobular types 1, 2, and 3. The small but significant decrease in terminal duct structures in lorcaserin-treated rats is consistent with the slight increase in prolactin. Thus, a plausible but weak argument can be made to support an intermediary role of prolactin in lorcaserin-induced mammary tumors in rats.

Not all studies have been supportive. When mammary tissue was examined by the standard H&E method at [REDACTED] (b) (4) there was no effect of lorcaserin on mammary hyperplasia or PCNA score, which is consistent with the prior findings of no effect of lorcaserin on mammary tissues in 3- and 6-month toxicology studies. Lorcaserin did increase mammary tissue secretory product, but only on Day 28 and not on Day 90. Whether this reflects a coincidental finding or a genuine drug effect is not clear. The robust and unequivocal response to perphenazine on mammary tissue and prolactin levels contrasts sharply with the minimal and inconsistent response to lorcaserin.

The three intervention studies were of limited value due to several inconsistencies and limitations except for demonstrating that lorcaserin can acutely provoke prolactin release from the pituitary. Interpretation of the prolactin antagonist intervention study is uncertain because, among several issues, the prolactin antagonist failed to block perphenazine. Results of hypophysectomy study is similarly mixed, with lorcaserin increasing mammary hyperplasia without evidence of an increase in plasma prolactin or the PCNA score. The 3-month study in male rats was a failure, as plasma prolactin did not change in response to lorcaserin or to perphenazine.

The prolactin mechanistic studies showed that lorcaserin minimally affected plasma and tissue prolactin and differentiation of mammary lobular structures in female rats and that these minimal changes are consistent with hormonal action on mammary tissue. No pattern of change was observed for estrogen, progesterone, or luteinizing hormone, and the Agency is not aware of a threshold of prolactin beyond which mammary tumors emerge. Given the high sensitivity of SD rats to prolactin and the absence of changes in other hormones, it is plausible that minimal increases in prolactin induced by lorcaserin contributed to the emergence of mammary tumors in female rats.

Safety Margin for Lorcaserin-Emergent Brain Astrocytoma

The second prominent clinically relevant lorcaserin- induced tumor was brain astrocytoma in male rats. Astrocytoma is a rare brain tumor. Lorcaserin induced a dose-dependent increase in brain astrocytoma tumors in male rats in the main study. Although there were tumors in female rats in the main study and toxicokinetic rats, they only reached statistical significance in male rats in the main carcinogenicity study. There were total of 20 in the rat carcinogenicity study of which 19 occurred in the lorcaserin treated rats and only one occurred in a control male. They occurred after as little as 52 weeks of lorcaserin treatment in rats.

Since lorcaserin preferentially partitions to the brain, an accurate assessment of a safety margin could not be established to determine the clinical risk in the original submission. The sponsor was requested to either establish a tumorigenic mode of action for lorcaserin-induced increases in astrocytoma or clarify the safety margin to the tumorigenic dose of lorcaserin.

The sponsor chose to conduct a clinical pharmacokinetic study to measure CSF and plasma AUC at steady state after oral administration of 10 mg BID in 10 obese volunteers. The relatively stable brain to CSF ratio in animal models was used to estimate the brain exposure from the concentration of lorcaserin in human CSF. The plasma and CSF exposures in humans were 1.02 and 0.00931 $\mu\text{g}\cdot\text{h}/\text{ml}$ AUC, respectively. This very low CSF:brain ratio in human subjects differs from the rodent and monkey studies where the unbound fraction of lorcaserin in plasma is within 2-fold the exposure in CSF, based on AUC. The brain lorcaserin exposure in human was estimated to be 1.73 $\text{ng}\cdot\text{h}/\text{ml}$ or approximately 1.7x the plasma in humans, significantly less than that observed in both rats and monkeys. The revised safety margin to the non-tumorigenic dose of lorcaserin is 70x the clinical dose, based on estimated brain exposure in rats vs. humans. A safety margin of 70-fold for astrocytoma in rats presents a negligible clinical risk and obviates the need for mode-of-action data.

In conclusion, the data in the resubmission identified a sufficient safety margin (24-fold) for lorcaserin-induced increases in mammary adenocarcinoma in female rats. Although lorcaserin increased mammary fibroadenoma at all doses with no safety margin to the clinical dose, the new mechanistic studies in female rats identified prolactin as a plausible though not definitive tumorigenic mode of action. Furthermore, as a benign mammary tumor, the potential clinical significance of fibroadenoma to humans is considered less concerning. With a 70-fold safety margin for brain tumors based on brain lorcaserin exposure, the clinical risk is negligible. Based on the new data the sponsor has submitted in response to the CR letter, the nonclinical safety signals have been resolved.

Safety margins for lorcaserin doses used in toxicology studies. The animal to human AUC exposure ratio is based on plasma AUC except for brain tumors ^c.

Species	Daily Dose, (mg/kg)	lorcaserin AUC ₀₋₂₄ (µg.h/ml)	NOAEL, (mg/kg) M/F	Exposure margins based on AUC (Animal/Human)	
				male	Female
13-Week mouse Study	25	M:3.4 F:1.0		3.3	1
	50	M:7.6 F: 2.3	50/50	7.4	2.2
	250	M:34.8 F:9.2		34.1	9
	350	M:25 F:27		24.5	26.4
13-Week rat study	1	M:0.143 F:0.33	5/1	<1	<1
	5	M:0.75 F:1.71		<1	2
	50	M:16.6 F:32.5		16	32
	100	M:33.6 F:55.8		33	55
6-Month rat study	1	M:0.20 F:0.31		0.2	0.3
	5	M:1.19 F:2.87	5/5	1.2	2.8
	50	M:22.0 F:34.4		22	34
12-Month cynomolgus monkey study	2	M: 1.0 F: 0.6	2 / 2	1	0.6
	10	M: 7.9 F: 4.5		7.7	4.4
	50	M:43.6 F:31.4		43	30.8
	125	M: 50.9 F: 51		50	50
104-Week Mouse Carci Study	5	M:0.55 F:0.32		0.5	0.3
	25	M:3.9 F: 1.6		3.8	1.5
	50	M:7.5 F:3.7	50/50	7.3	3.6
104-Week Rat Study ^c	10	M:4.78 F:6.7	5 / <7	4.7	6.6
	30	M:16.9 F:24.1		16.6	24
	100 ^b	M:55.9 F:83.8		55	82
Fertility and early embryonic development in rats	5	M:2.68 F: 4 ^a		2.6	4
	15	M:9.91 F:12 ^a	15/50	9.7	12
	50	M: 29.3 F:48.7 ^a		28.7	48
Oral Embryo-fetal development in rats	2	F:1.34			1.3
	10	F:7.99	10		7.8
	50	F:48.7			47.7
Oral Embryo-fetal development in rabbits	20	F: 0.155			0.15
	60	F: 0.443	60		0.43
	200	F:19.3			18.9
Pre- and postnatal development in rats	5	F:4 ^a	<5		4
	15	F:12 ^a			12
	50	F:48.7 ^a			48
Clinical Dose: lorcaserin, 10 mg BID		1.02			

^a The AUC value is derived from other existing similar studies.

^b The lorcaserin AUC in females in the 2-year rat study was about 60% higher than the AUC in female rats in the 13-week toxicology and 28-day prolactin mechanistic study (AUC 53 µg.hr/ml).

^c The safety margin in the table for rat carcinogenicity study does not apply to brain tumors. Since lorcaserin preferentially partitions to the brain, the safety margin for brain tumors was based on lorcaserin brain AUC in rats and humans (estimated from human CSF values).

Appendix A

Application: Lorcaserin hydrochloride, NDA 22-529

Drug Class: 5HT2c Receptor Agonist

Clinical Indication: Obesity

Reviewer: Todd Bourcier, Ph.D., Division of Metabolism and Endocrinology Products

Re: Receptor pharmacology studies included in Complete Response Resubmission for lorcaserin

Summary

Lorcaserin is a new molecular entity that targets activation of the serotonin 5HT2C receptor and is intended to promote weight loss in an obese population. Agonism at the intended target, 5HT2C, has been reasonably demonstrated to underlie the anorexigenic effect of lorcaserin. An important aspect of the non-clinical development program for lorcaserin was the assessment of receptor selectivity for 5HT2C relative to other serotonin receptor subtypes, particularly other members of the 5HT2 receptor family 5HT2A and 2B. Relative to drug action, the 5HT2A and 2B receptors are implicated in contributing to the hallucinogenic and addictive responses to drugs of abuse (5HT2A) and to drug-induced cardiac valvulopathy including that associated with use of dexfenfluramine in humans (5HT2B).

The selectivity of lorcaserin for 5HT2C was assessed by a series of *in vitro* and *in vivo* pharmacology studies and by toxicological assessments of neurobehavioral and cardiac/valvular histological endpoints.

Lorcaserin preferentially activates 5HT2C with 8- to 15-fold greater potency compared to 5HT2A, and 45- to 90-fold greater potency compared to 5HT2B. Depending on the studies one considered in the original NDA submission, off-target activation of 5HT2A and 2B appeared unlikely (2002/04 data) or possible (2009 data) when compared to clinically relevant plasma levels of lorcaserin due to differing *in vitro* estimates of receptor potency.

In their resubmission, Arena presents additional studies to clarify discrepancies in the receptor potency data reported in the original NDA. The new studies were designed to address potential receptor reserve effects in the *in vitro* assay systems that may have overestimated receptor potency of lorcaserin in the prior studies.

The new studies (referred to as 2011 data) reduced receptor density to levels more consistent with expression levels reported in the literature for 5HT2 receptors in human neurological and cardiovascular tissues. The 2011 data show that lorcaserin is at least 3- to 5-fold less potent than originally reported at all three 5HT2 receptor subtypes. Based on the new estimates of receptor potency, maximal concentrations of lorcaserin (free fraction) observed in human plasma and anticipated in human brain tissue is notably lower than the EC50 for activation of 5HT2A and 2B, while remaining above the EC50 for activation of 5HT2C *in vitro*. Plasma concentrations of lorcaserin at the therapeutic dose are thus expected to remain within the selective range for activation of 5HT2C.

Arena additionally demonstrated that based on functional activity across four *in vitro* assay platforms, lorcaserin grouped with low-potency 5HT2B agonists that are not known to be associated with clinical valvulopathy. By comparison, compounds known to cause clinical valvulopathy such as nordexfenfluramine and pergolide showed substantially higher 5HT2B receptor potency in these assays.

The 2011 receptor potency data provides supportive evidence that off-target activation of the 5HT2A or 2B receptors is unlikely at the proposed clinical dose of lorcaserin (10mg bid). This is consistent with neurological and cardiac assessments in animals which did not identify major toxicities that would be anticipated if 5HT2A and 2B were activated by lorcaserin. However, limitations in neurological assessments and the lack of validated models for drug-induced valvulopathy in animals preclude a definitive prediction that lorcaserin will be devoid of such toxicities should it be approved for marketing.

Serotonin receptor selectivity profile of Lorcaserin

Background

The original NDA submission included two sets of studies which addressed the binding affinity and receptor activation kinetics for lorcaserin against the human 5HT2A, 2B, and 2C receptors. The first set of studies was conducted in 2002/04 in support of early clinical trials, and the second was conducted in 2009 in the course of characterizing metabolites of lorcaserin. The 2009 data resulted in ~10-fold greater potency at all three receptor subtypes compared to the 2002/04 data. When compared to clinically relevant plasma drug levels, ‘off-target’ activation of 5HT2A and 2B appeared either plausible or unlikely, depending on which dataset one considered. The sponsor stated that the discrepant potency data was likely due to higher expression of the 5HT2 receptors by the transfected HEK293 cells used in the 2009 study which left-shifted the dose response and overestimated lorcaserin’s potency. It is a known phenomenon that higher receptor density in transient expression systems may result in greater ligand potency without a substantial change in binding affinity¹⁰, but the studies conducted by the sponsor did not control for potential effects of receptor reserve. Although this issue was not a Complete Response item, the Sponsor after consultation with the Division undertook additional studies to further characterize the functional potency of lorcaserin for human 5HT2 receptors under assay conditions that controlled for receptor reserve.

Human 5HT2 Receptor Binding Affinity

Receptor binding affinity of lorcaserin to the 5HT2A, 2B, and 2C receptors was reported in the original NDA and was not re-examined in the new pharmacology studies submitted in the Complete Response. Receptor binding affinity was similar in the 2002/04 and 2009 studies despite ~10-fold differences in receptor activation (i.e., potency) between the assays, and would not be expected to differ substantially in the 2011 studies that controlled for receptor reserve. Binding affinities for lorcaserin combined from the 2002/04 and 2009 studies and expressed as Ki values were 92, 147, and 13nM for 5HT2A, 2B, and 2C, respectively (Table 1). Lorcaserin’s affinity for 5HT2C was within 7- to 10-fold the affinity for 5HT2A and 2B.

Table 1: Lorcaserin binding affinity (Ki) for human serotonin receptors 5HT2A, 2B, and 2C <i>in vitro</i>.			
	5HT2A	5HT2B	5HT2C
Binding Affinity^{1,2} (Ki, nM)	92	147	13

¹Competitive binding with ¹²⁵I-DOI (Ki for DOI: 0.57, 5, 0.87nM for human 5HT2A, B, C).

²Ki values reflect average from studies conducted in 2002 and 2009

¹⁰ Jerman JC et al (2001) Eur J Pharmacol 414:23

Human 5HT₂ Receptor Activation Studies

Potency of lorcaserin at the human 5HT_{2A}, 2B, and 2C receptors was assessed by measuring downstream events in the phospholipase C pathway, specifically the accumulation of ³H-inositol phosphate and release of calcium in HEK293 cells expressing the recombinant human 5HT₂ receptors. Potency was determined at various levels of receptor density in an effort to eliminate the potential effects of receptor reserve. This was accomplished by assays that used the alkylating agent phenoxybenzamine to reduce 5HT₂ receptor density. Additionally, some assays titrated the amount of transfected cDNA to yield low levels of receptor expression as an alternate means to eliminate receptor reserve. The maximal response of lorcaserin relative to serotonin was also assessed to distinguish partial from full agonist activities.

Table 2a lists the potency of lorcaserin in the inositol phosphate assays from the prior studies submitted in the original NDA and the 2011 study submitted in the Complete Response. Eliminating receptor reserve in the 2011 assays shows that lorcaserin is 3- to 5-fold *less* potent than reported in the 2002/04 study and ~30-fold less potent than reported in the 2009 study at all three 5HT₂ receptor subtypes. Despite the shifts in potency across the studies, the relative selectivity of lorcaserin for 5HT_{2C} remains within the range of 8x-15x for 2A, and 45x-90x for 2B (**Table 2b**).

Table 2a: Potency of lorcaserin in inositol phosphate assays (Data from 2002/04, 2009, 2011 studies)			
	Lorcaserin, EC₅₀, nM		
Study date	5HT_{2A}	5HT_{2B}	5HT_{2C}
2002/04	133	811	9
2009	14	82	1.8
2011	553	2380	39

Table 2b: Fold Selectivity of Lorcaserin for 5HT_{2C} receptor activation¹		
Study data	vs. 5HT_{2A}	vs. 5HT_{2B}
2002/04	15x	90x
2009	8x	45x
2011	14x	61x

¹Fold selectivity determined by dividing the PI hydrolysis EC₅₀ value for 5HT_{2C} by the EC₅₀ value for 5HT_{2A} or 2B from the 2002/04, 2009, and 2011 studies.

Table 3 lists the potency of lorcaserin in the calcium release assays conducted in 2002/04 and again in 2011 under conditions that eliminated receptor reserve. Calcium release was not assessed in the 2009 studies. Lorcaserin was ~20-fold less potent at the 5HT2A and 2C receptors and ~3-fold less potent at the 5HT2B receptor compared to the potencies reported in 2002/04.

Table 3: Potency of lorcaserin in calcium release assays (Data from 2002/04 and 2011 studies)			
	Lorcaserin, EC50, nM		
Study date	5HT2A	5HT2B	5HT2C
2002/04	52	350	6
2011	948	1040	146

The maximal response of lorcaserin relative to serotonin was also assessed to distinguish partial from full agonist activities. Based on the 2011 assays that eliminated receptor reserve, lorcaserin displayed partial agonist activity at 5HT2A and partial to full agonist activity at 5HT2A and 2B (**Table 4**).

Table 4: Efficacy data for lorcaserin in 5HT2 receptor activation assays¹			
	5HT2A	5HT2B	5HT2C
Percent activity vs. serotonin	25%	67- 151%	81 – 86%

¹Efficacy data from inositol phosphate and calcium release assays conducted in 2011

Results from the 2011 studies are consistent with our prior observation that the selectivity of lorcaserin for 5HT2C versus 2A and 2B is driven by the functional receptor activation assays rather than the binding assays.

5HT2 receptor expression levels in potency assays compared to human tissues

Because potency of lorcaserin increases as 5HT2 receptor expression increases, it can be of interest to compare levels of receptor expression in the potency assays to potential target tissues of interest *in vivo*, particularly the heart and central nervous system tissues. **Table 5** lists the expression levels of 5HT2 receptors as measured by radioligand binding studies in the potency assays from 2011 and 2009, and from selected human tissues as reported in the literature. The range listed for the 2011 studies indicates the range of receptor expression where potency of lorcaserin was observed to be stable. By design, receptor expression in the 2011 assays was lower than in the 2009 assays, correlating with lower potency in cells expressing fewer 5HT2 receptors. Receptor expression in human tissues more closely aligns with expression levels achieved in 2011, suggesting that the potency data from the 2011 study is the more appropriate dataset to consider in extrapolating to the potential potency of lorcaserin *in vivo*.

Table 5: Expression level of 5HT2 receptors in potency assays and in human tissues (Bmax, fmol/mg protein)			
	5-HT2A¹¹	5-HT2B¹²	5-HT2C^b
2009 assays	7400	1100	750
2011 assays	220 – 1200	10 – 300	14 – 750
Human tissues	<u>Cortex</u> Frontal : 93 – 258 PreFrontal : 70 – 137 Temp/Parietal : 45 - 232	Left ventricle : 25 LV with CHF : 120	Hypothalamus : 13 Substantia Nigra : 35 Choroid Plexus : 625

Radioligands included ¹²⁵I-DOI for 2009/2011 assays, ³H-ketanserin or ¹²⁵I-LSD for 5HT2A, ³H-LY266097 for 5HT2B, and ³H-mesulergine for 5HT2C in human tissues

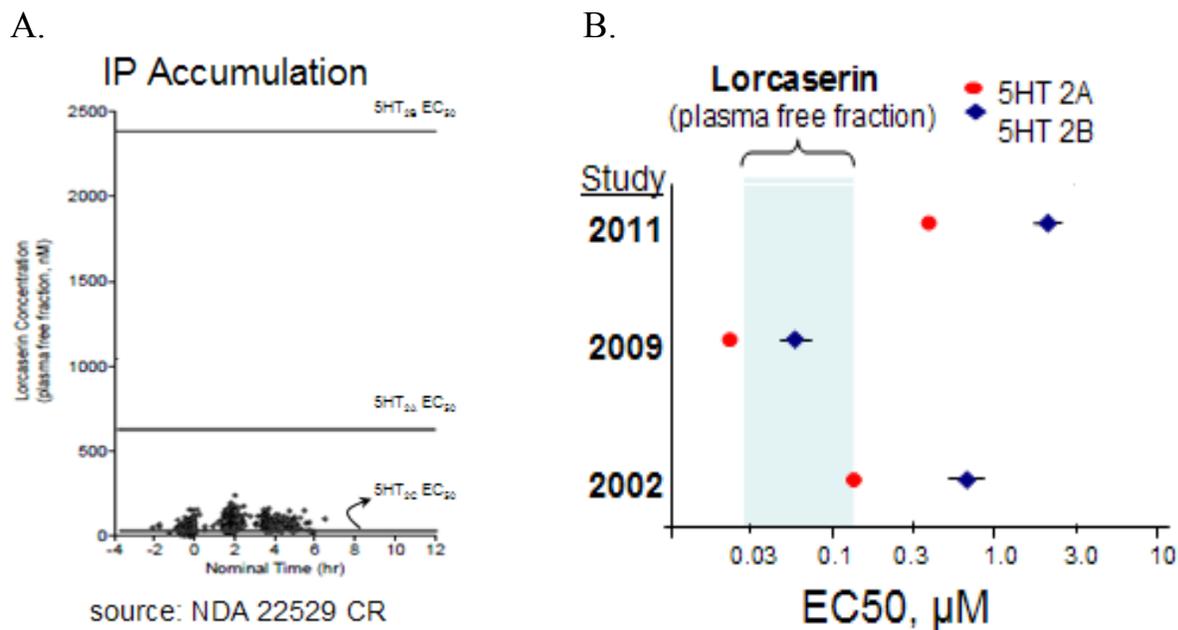
5HT Receptor Selectivity compared to clinical exposure to lorcaserin

Selectivity of lorcaserin for 5HT2C is advantageous provided that plasma drug levels fall within a selective concentration range, which can be first estimated by *in vitro* EC50 values for receptor activation. Functional selectivity would be lost, for example, if the free drug concentration *in vivo* exceeds the EC50 for all three 5HT2 receptor subtypes, which could reasonably result in partial or full receptor activation. **Figure 1** compares the observed plasma lorcaserin concentration in obese/overweight subjects to *in vitro* 5HT2 receptor activation data. **Figure 1A** (on left) shows that the plasma levels of lorcaserin at the clinical dose of 10mg bid is substantially below the EC50 for activation of 5HT2A and 2B based on estimates of potency from the 2011 study. **Figure 1B** (on right) charts the change in reported potency for lorcaserin from the three studies and their relationship to the range of lorcaserin plasma concentration at the clinical dose. Whereas the reported EC50 values from 2002/04 and 2009 suggested that activation of the 2A and 2B receptors was plausible, the revised EC50 values from 2011 indicate that off-target activation of these receptors is unlikely at therapeutic exposure.

¹¹ Marazziti D et al (1999) Eur Neuropsychopharm 10:21-26; Marazziti D et al (2003) Neurochem Int 42 :511-516; Huot P et al (2010) Movement Disor 25(10):1399-1408;

¹² Jaffe F et al (2008) Circ Res 104:113-123.

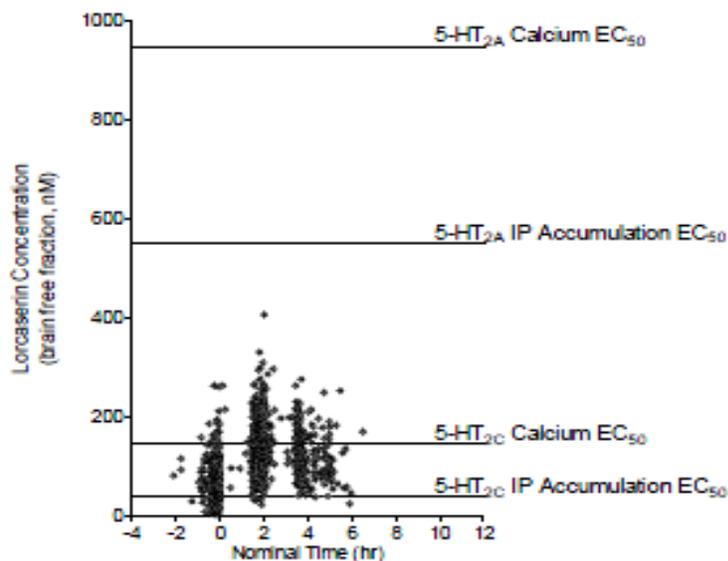
Figure 1: Lorcaserin concentration in human plasma compared to *in vitro* 5HT2 receptor potency data: **(a)** EC₅₀ (nM) for inositol phosphate accumulation (2011 study) superimposed on scatter plot of lorcaserin plasma concentration from clinical study APD356-011. **(b)** Change in potency data (pEC₅₀, nM) for calcium release and IP accumulation studies from 2002/04, 2009, and 2011 relative to lorcaserin plasma concentration from clinical study APD356-011. Blue column represents range of therapeutic plasma concentrations (free fraction) of lorcaserin at 10mg bid.



Lorcaserin’s intended pharmacological target, 5HT_{2C}, is expressed by hypothalamic nuclei within the CNS. In addition to their expression by peripheral tissues, 5HT_{2A} and 2B are also expressed in the CNS where they have a role in regulating aspects of behavior, including responses to hallucinogenic agents¹³. Under the Complete Response, Arena conducted studies to clarify the degree to which lorcaserin partitions to the CNS in human subjects. This new clinical data indicates that levels of lorcaserin are approximately 1.7-fold higher in the CNS compared to systemic blood levels. By comparison, lorcaserin was present in brain tissue an average of 25-fold and 10-fold higher than systemic levels in rodents and monkeys, respectively. **Figure 2** demonstrates that predicted brain levels of lorcaserin in human subjects aligns well with the EC₅₀ for activation of 5HT_{2C}, but falls substantially below the EC₅₀ for activation of 5HT_{2A} at the proposed clinical dose of 10mg bid.

¹³ Filip M & Bader M (2009) Pharm Reports 61:761-777; Giorgetti M & Tecott LH (2004) Eur J Pharmacol, 488: 1-9.

Figure 2: Lorcaserin free fraction predicted in human brain compared to functional potency at the human 5-HT_{2A} and 5-HT_{2C} receptors. (Figure adapted from Arena's Complete Response submission)



Neurological effects in Animals

The neurobehavioral studies conducted with lorcaserin in rats and monkeys did not identify any major adverse neurological effect considered clinically prohibitive. The most likely adverse neurological effect predicted from the rat and monkey studies would be somnolence or lethargy, particularly early after initiation of dosing. Lorcaserin did not clearly elicit 5HT_{2A}-related behavior in rats but did elicit 5HT_{2C}-related behaviors in a dedicated neurological studies submitted in the original NDA and in the Complete Response submission. These data suggest that neurological adverse events observed in clinical studies with lorcaserin at therapeutic exposure are likely initiated by activation of central 5HT_{2C} receptors.

Assessment of Valvulopathy in Animals

Several lines of evidence persuasively argue that among the 5HT₂ receptors, activation of 5HT_{2B} is the culprit mechanism underlying drug-induced valvular heart disease (VHD), such as that associated with dexfenfluramine¹⁴: 1) Cardiac valves express 5HT_{2A} & B but very little or no 5HT_{2C}, 2) Drugs associated with clinical VHD activate 5HT_{2B} with high potency (e.g., methysergide, methylergonovine, ergotamine, MDMA); 3) Parkinsonian drugs pergolide and cabergoline associated with clinical VHD also activate 5HT_{2B}, whereas structurally similar drugs (e.g., lisuride) void of 5HT_{2B} activity are not associated with VHD; 4) Fenfluramines and serotonin are mitogenic for human cardiac valve tissue *in vitro*, an effect inhibited by a 5HT_{2A/B} antagonist.

¹⁴ Hutcheson JD et al (2011) Pharmacology & Therapeutics. 132:146-157.

Huang et al¹⁵ reported that functional profiling of pharmaceuticals for 5HT2B activity using a multi-readout platform identified known valvulopathogens as high-potency 5HT2B agonists. The authors state that such functional profiling might be useful in identifying compounds ‘likely to induce valvular heart disease’. Arena adapted Huang’s approach and compared the 5HT2B receptor potency of lorcaserin to two sets of reference compounds: Compound set 1 is associated with clinical VHD and are known to activate 5HT2B with high potency, and Compound set 2 are not associated with clinical valvulopathy but activate 5HT2B with low potency. Potency of all compounds was assessed in four *in vitro* assays that measure separate signaling events downstream of 5HT2B activation.

Table 6 shows that lorcaserin grouped with Compound set 2 which are not known to be associated with clinical valvulopathy and have comparatively low potency for activating 5HT2B. By comparison, Compound set 1, which are known to cause valvulopathy, showed substantially higher potency for 5HT2B in these assays. The functional profile of lorcaserin suggests a low risk of this compound acting as a valvulopathogen like dexfenfluramine, though it must be noted that no single functional profile can conclusively predict whether a 5HT2B agonist will act as a valvulopathogen *in vivo*. Also, among the ‘low potency’ drugs in compound set 2, only guanfacine and ropinirole are relevant because both are indicated for long-term indications similar to lorcaserin.

Table 6: Potency data for lorcaserin and selected reference compounds across *in vitro* assay platforms (Table source: NDA 22529 CR submission)

	Calcium EC ₅₀	IP Accumulation EC ₅₀	β-Arrestin EC ₅₀	pERK EC ₅₀
Compounds Associated with Valvulopathy				
Serotonin	1.8 nM	9.3 nM	263 pM	2.7 nM
Pergolide	63 nM ^a	6.3 nM	912 pM	63 nM ^b
Cabergoline	209 nM ^a	759 pM	3.0 nM	110 nM ^b
Nordexfenfluramine	18 nM	26 nM	1.9 nM	158 nM
Methylergonovine	NR	63 pM	871 pM	79 nM ^b
Drugs with Lower 5-HT_{2B} Agonist Activity Identified in the Roth Study and Not Associated with Valvulopathy				
Fenoldopam	331 nM	204 nM	129 nM	331 nM
Guanfacine	776 nM	631 nM	759 nM	1.07 μM
Oxymetazoline	331 nM	676 nM	141 nM	933 nM
Quinidine	794 nM	1.20 μM	166 nM	1.55 μM
Ropinirole	NR	14.4 μM	2.82 μM	14.1 μM
Xylometazoline	1.32 μM	4.07 μM	427 nM	3.5 μM
Lorcaserin	1.04 μM	2.38 μM	119 nM	1.82 μM

NR = no response

^a Potencies likely to be significantly underestimated in the calcium assay.

^b Potencies potentially underestimated to varying degrees in the pERK assay.

Nonclinical assessment of valvular heart disease is limited in that a reproducible and robust animal model to screen for drug-induced VHD is lacking. However, there are reports in the literature that cardiac alterations suggestive of VHD were produced in rats administered serotonin¹⁶, pergolide¹⁷, and the experimental 5HT2C agonist RO3013¹⁸. Results with

¹⁵ Huang X et al (2009) *Molec Pharm* 76(4):71-722

¹⁶ Gustafsson BI et al. (2005) *Circulation*. 111: 1517-1522.

serotonin in rats have been criticized as being consistent with spontaneous age-related cardiac disease¹⁹, and have not been uniformly reproduced in the literature²⁰. Also, the FDA is unaware of any prospective toxicology study that persuasively demonstrates cardiac findings consistent with VHD in adult animals administered dexfenfluramine.

Extensive echocardiographic monitoring was conducted in the course of clinical studies with lorcaserin. For the nonclinical assessment, a comprehensive histological evaluation of cardiac tissue from preclinical species was submitted. The histological assessment included evaluation of chordae tendineae, cardiac and valve tissue, with reporting of the incidence and severity of any changes in the histopathology of these tissues.

Lorcaserin binds to human, rat, and monkey 5HT2B with similar affinity, and activates human and monkey 5HT2B with reasonably similar potency. Lorcaserin was tested over a concentration range that substantially exceeded the *in vitro* activation potency for 5HT2B in rats and monkeys, so there was a reasonable expectation that cardiac lesions might be observed at the highest doses. Histological evaluations were conducted after dosing rats for 1, 3, 6, and 24 months and in monkeys after dosing for 1, 3, and 12 months with lorcaserin.

The histological appearance of the heart, endocardium, cardiac valves, and the chordae tendineae were described by the examining veterinary pathologists as within normal limits for the species examined and at all doses of lorcaserin evaluated. This result appears reassuring, but it is noteworthy that cardiac lesions were not observed at the highest concentrations of lorcaserin, which substantially exceeded the *in vitro* potency data for activation of 5HT2B. The sponsor suggests that still higher drug levels would be required to elicit activation of 5HT2B because the potency of lorcaserin *in vivo* may still be less than that predicted by the *in vitro* activation studies. However, other limitations may be more significant. For example, the ability to detect drug-induced VHD in any one of these experiments was not demonstrated by use of a positive control such as serotonin or pergolide. Thus, inherent insensitivity of the animal model is a more likely explanation that cannot be excluded. Additionally, published studies that detected drug-induced VHD in animals included evaluation of proliferative markers and echocardiography in addition to standard histology, whereas the studies done with lorcaserin were limited to evaluation of standard histology. Thus, insufficiently sensitive detection methods also cannot be excluded.

Given the experimental limitations with the toxicological data, the more appropriate data in considering the VHD risk of lorcaserin is the receptor pharmacology data described above and the echocardiography data collected in the clinical trials with lorcaserin.

¹⁷ Droogmans S et al. (2007) Eur Heart J. 28:2156-2162.

¹⁸ Fielden MR et al (2010) Exp Toxicol Path 62:607-613

¹⁹ Donnelly KB (2008) Toxicol Path 36: 204-217

²⁰ Hauso O et al (2007) Reg Peptides 143: 39-46

Table 7: Lorcaserin activation of human, rat, and monkey 5HT2B receptors (EC50, nM)				
	Binding, Ki (nM)	EC50 (nM)		Plasma lorcaserin concentration achieved in toxicology studies
		Ca	IP	
Human	147	1040	2380	na
Rat	114	1170	195	150 to 20,000 nM
Monkey	127	2360	725	400 to 20,000 nM
Receptor binding data from 2002/04 study Potency data (EC50 for Calcium and IP accumulation) from 2011study.				

Appendix B

Comprehensive lorcaserin binding profile- (b) (4) Assay

Receptor Profile of Lorcaserin, Lorcaserin Enantiomer and Metabolites for a Collection of Human GPCRs, Ion Channels and Neurotransmitter Transporters

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
Adenosine A ₁	-11	13	-6	6	-5
Adenosine A _{2A}	7	1	-11	21	14
Adenosine A ₃	-1	1	4	13	14
α ₁ Adrenergic (nonselective) (rat)	18	16	27	22	9
α ₂ Adrenergic (nonselective) (rat)	30	41	13	65	9
β ₁ Adrenergic	21	52	-8	56	7
β ₂ Adrenergic	19	22	-3	47	10
Angiotensin AT ₁	-12	3	11	-18	-13
Angiotensin AT ₂	1	13	1	1	3
Benzodiazepine (central) (rat)	0	6	-2	6	11
Benzodiazepine (peripheral) (rat)	-3	-1	6	-1	1
Bombesin (nonselective) (rat)	-16	2	-10	6	1
Bradykinin B ₂	-3	-15	11	1	-4
CGRP	-10	-8	-9	-4	-2
Cannabinoid CB ₁	3	8	-9	5	-2
Cannabinoid CB ₂	-8	ND	-3	-6	-8
Cholecystokinin CCK _A	-2	-9	-11	29	18
Cholecystokinin CCK _B	3	-1	6	-2	3

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
Dopamine D ₁	-2	14	-1	-3	-7
Dopamine D _{2S}	-1	0	9	2	1
Dopamine D ₃	7	23	2	21	-2
Dopamine D _{4,4}	-1	-4	-5	4	4
Dopamine D ₅	-3	5	-3	8	-1
Endothelin ET _A	-8	3	-23	-2	2
Endothelin ET _B	-14	-8	-7	1	9
GABA (nonselective) (rat)	9	-3	-15	9	2
Galanin GAL ₁	5	-6	4	-1	4
Galanin GAL ₂	-18	-10	3	-10	-2
PDGF (mouse)	-9	ND	-13	1	-7
CXCR2	8	-14	12	-12	-2
TNF-α	ND	4	6	-21	-1
CCR1	-3	-2	0	-4	-3
Histamine H ₁	-4	10	8	9	8
Histamine H ₂	6	16	-12	-3	-1
Melanocortin MC ₄	-7	1	1	2	4

Receptor Profile of Lorcaserin, Lorcaserin Enantiomer and Metabolites for a Collection of Human GPCRs, Ion Channels and Neurotransmitter Transporters

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
Melatonin MT ₁	-5	5	1	6	9
Muscarinic M ₁	12	1	-27	9	6
Muscarinic M ₂	3	9	-1	-4	-7
Muscarinic M ₃	3	11	-29	10	5
Muscarinic M ₄	4	18	-2	7	2
Muscarinic M ₅	0	14	-4	4	-3
Neuronal nACh (rat)	30	ND	4	20	13
NMDA (rat)	-4	ND	17	5	1
Neurokinin NK ₁	10	-4	-4	4	2
Neurokinin NK ₂	-5	10	4	1	-3
Neurokinin NK ₃	7	-3	-6	13	11
Neuropeptide Y ₁	-9	-4	21	-6	-10
Neuropeptide Y ₂	-1	-18	-15	-3	-2
Neurotensin NT ₁	-2	-1	4	2	0
δ ₂ Opioid	4	5	10	10	0
μ Opioid	26	5	0	28	7
κ Opioid (rat)	6	24	0	18	19
Nociceptin ORL1	1	9	-2	17	10

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
PPARγ	-9	8	ND	ND	ND
PAC ₁ (PACAP)	-15	-3	-12	-18	6
PCP (rat)	8	3	-22	-14	-6
Prostanoid EP ₂	ND	7	ND	ND	ND
Prostanoid EP ₄	-3	ND	ND	-3	0
Prostanoid TP	0	11	-2	30	32
Prostanoid IP	-5	0	ND	-2	-3
P2X (rat)	9	-2	-5	-3	0
P2Y (rat)	1	-5	-4	5	11
Serotonin 5-HT _{1A}	85	92	22	72	22
Serotonin 5-HT _{1B} (rat)	69	92	13	49	4
Serotonin 5-HT _{2A}	29	50	-3	22	-6
Serotonin 5-HT _{2B}	78	78	ND	51	9
Serotonin 5-HT _{2C}	67	62	-7	32	15
Serotonin 5-HT ₃	7	14	-3	10	8
Serotonin 5-HT _{5A}	7	10	-4	17	24
Serotonin 5-HT ₆	15	47	1	17	1
Serotonin 5-HT ₇	61	82	7	40	1

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

/s/

FRED K ALAVI
05/30/2012
Approval recommended.

TODD M BOURCIER
05/30/2012
Review recommends AP

Pharm/tox review of proposed studies to assess lorcaserin effect on prolactin production in SD rats, IND 69,888 (NDA 22-529)

IND: 69,888 (NDA 22-529)

Drug: lorcaserin

Indication: Treatment of obesity

Sponsor: Arena Pharmaceuticals Inc.

DARRTS SDN # 222 eCTD Serial # 0194 Letter Date: 7/13/2011

Reference is made to the sponsor's latest amendments to the 3-month lorcaserin prolactin (DBR-11-002) protocol received by the Division on July 14, 2011. The submission has two amendments. Since amendment 2 contained all the amendment 1 and plus new additions, only the protocol amendment 2 was formally reviewed.

DBR-11-002: Three Month Evaluation of Lorcaserin Effects on Prolactin Concentrations and Mammary Gland Histology in Female Sprague Dawley Rats (amendment 2, July 8, 2011)

The Division has been reviewing and commenting on the revisions of 3-month lorcaserin prolactin protocol (DBR-11-02) ever since the sponsor submitted a preliminary draft protocol in Feb 2011. Overall, the Division has agreed that a 3-month study might be an adequate effort to show lorcaserin can robustly and persistently increase plasma prolactin levels in a dose-dependent manner in female SD rats. As highlighted in previous communications with the sponsor whether the 3-month duration will be long enough to observe a lorcaserin related change in mammary tissue pathology at the end of the 3-month study remains to be seen since there were no notable mammary findings at the end of the 3- and 6-month rat toxicology studies.

The recently amended DBR-11-02 protocol is structurally the same as the earlier drafts but with a few new small modifications (see page 14 for details). The protocol has been reviewed before thus only the notable modifications will be discussed. It should be noted that at the time of this submission, rats in the DBR-11-02 study had received at least 4 weeks of lorcaserin treatment. The most notable changes in the protocol amendments are listed below.

- The day 30 mammary tissue sample collection is changed to Day 28. The remaining time points are the same as before.
- There will be 60 rats per group for Day 28 and Day 90 as described before but 12 rats per group instead of 10 rats for Day 1, 7 and 60 day arm in earlier drafts.
- Tissue samples from the low dose as well as high dose will be evaluated.
- Mammary tissue histological, histochemical and immunochemical analyses will be carried out at (b) (4) instead of Dr. Russo's lab (Jenkintown, PA). Dr. Russo had been consulting the sponsor. According to the sponsor, some of the modifications to the mammary tissue pathology timing and details were recommended by (b) (4).
- Detailed description of study hypothesis and endpoint analysis.
- Timing of blood sampling was still unknown in the latest submission.

Discussion

The revised draft prolactin protocol (DBR-11-002) is basically similar to earlier drafts thus no specific actions needed to be taken with ongoing live phase of the study. The changes the sponsor had included in the study will have no impact on the ongoing study itself but may affect how the study results are reviewed.

The amendments include several changes. The sponsor has decided to use (b) (4) as the source for mammary tissue pathology evaluations rather than the Dr. Russo's lab originally described in the protocol. They have increased the number of animals from 10 to 12 for evaluations on Day 1, 7 and 60. The number of rats in the main arm of the study analysis on Day 28 and Day 90 has remained the same (60 rats/group). Although the time of blood collection is missing, plasma prolactin levels will be measured either by ELISA to determine immunoreactive prolactin (ALPCO Diagnostics, Inc) or by commonly used Nb2 rat lymphoma cell bioassay to determine biological active prolactin. Pituitary prolactin will be measure by the method described by Haggi et al¹.

The revised protocol also describes prolactin as the primary analysis and mammary tissue as secondary analysis. In the prolactin analysis, the sponsor will be examining the mean significant change in prolactin levels at various timepoints throughout the 90-day dosing period in lorcaserin treated rats relative to control female rats. In addition to mean changes in prolactin, they will be evaluating the number of rats with circulating prolactin levels greater than the upper limit of normal in control at each time point (page 5). In all communications with the sponsor, the agency has noted that the changes in prolactin levels need to be robust, persistent and dose-dependent. A statistically significant increase in prolactin along with greater number of rats with elevated prolactin above control range is reasonable but with out significant change in prolactin, a meager increase in individual rat prolactin levels greater than upper bound in the control rats may not be sufficient evidence to support the hypothesis that lorcaserin induced increase in mammary tumors were due to prolactin. This is supported by the fact that a significant but small increase in prolactin with dexfenfluramine was insufficient to cause mammary tumors in rats.

Recommendations:

The new amendments include additional details on mammary histopathology and prolactin analysis. According to the sponsor, changes in the mammary pathology evaluations were at the request of (b) (4) the sponsor has selected to replace Dr. Russo's labs. Although the study has already been initiated (4 weeks in to the treatment) the changes introduced in the amendments are acceptable. However, the sponsor should be advised that the increase in the prolactin in the lorcaserin treated rats should be statistically significantly greater than control in addition to the number of rats with elevated prolactin greater than control range. Without achieving a significant treatment effect on prolactin, small increases in prolactin levels in some lorcaserin treated rats above control range may not be robust enough to support the sponsor's hypothesis, especially in the absence of any mammary tissue pathology.

¹ Haggi, E. and Aoki A. Prolactin content in rat pituitary gland. RIA of prolactin after different extraction procedures. *Acta Endocrinol.*, 97(3):338-42 (1981)

External recommendations

The Division has reviewed your amendments to the prolactin protocol [DBR-11-002](#). The revised protocol appears reasonable, but we have the following comment. You list two primary analyses that presumably will serve as the basis to meet the primary end point. Your primary endpoint is stated as “Concentration of circulating prolactin throughout 90 days of dosing with lorcaserin as compared to vehicle”. We note that this definition does not specifically state “mean concentration” of circulating prolactin. We consider the first primary analysis (difference in mean concentration of circulating prolactin) as most critical to meeting the stated primary endpoint. We view the second primary analysis (number of rats with circulating prolactin above ULN) as supportive of the first analysis. Failing the first but meeting the second primary analysis will likely be viewed as failing to meet the primary endpoint of demonstrating a statistically significant difference in mean concentration of circulating prolactin.

Appendix A

1 PURPOSE

This study is designed to evaluate plasma and tissue prolactin levels and mammary tissue histology after lorcaserin treatment in the female rat.

2 HYPOTHESES

Primary: Lorcaserin increases circulating prolactin concentrations in female rats through a central mechanism.

Secondary: Lorcaserin causes histological changes in mammary tissue that are secondary to elevation of circulating prolactin.

3 ENDPOINTS

Primary:

- Concentration of circulating prolactin throughout 90 days of dosing with lorcaserin as compared to vehicle.

Secondary:

- Histological evaluation of mammary tissue after 28 and 90 days of lorcaserin repeat dose administration using a whole mount technique to include assessment of:
 - a. Lobular development, including the percentage of lobules typed 1, 2, 3 or 4.¹⁻³
 - b. Ductal branching pattern.
 - c. Ductal hyperplasia and/or lobular hyperplasia.⁴⁻⁶
 - d. Detection of ductal carcinoma in situ or invasive tumors.⁴⁻⁶
 - e. Detection of tumor masses like fibroadenomas.⁴⁻⁸
- Histopathological evaluation of hematoxylin/eosin (H&E), proliferating cell nuclear antigen (PCNA) and prolactin stained mammary tissue after 28 and 90 days of lorcaserin administration in a subset of animals.
 - Qualitative histological changes in mammary tissue.
 - Quantitative proliferative changes with PCNA immunostaining in mammary tissue.
 - Qualitative prolactin changes with immunohistochemistry.
- Concentration of prolactin in mammary tissue after 7, 28, 60, and 90 days of dosing.
- Concentration of prolactin in pituitary tissue after 7, 28, 60, and 90 days of dosing.
- Concentration of circulating hormone levels (to include progesterone, estradiol and luteinizing hormone) after 7, 28, 60, and 90 days of dosing.

4 ANALYSES

Primary:

- Difference in mean concentration of circulating prolactin at various timepoints throughout a 90-day dosing period, to include days 1, 7, 28, 60 and 90, in rats treated with lorcaserin 10, 30 or 100 mg/kg/day as compared to rats treated with vehicle (negative control), or perphenazine 5 mg/kg/day (positive control).
- Number of rats with circulating prolactin concentration greater than the upper limit of normal in lorcaserin group compared to vehicle group at each study collection day.

Secondary:

- Mammary histology scores (to include whole mount analysis, H&E analysis, PCNA analysis, prolactin immunohistochemical analysis) after 30 and 90 days of dosing in rats treated with lorcaserin 10, 30 or 100 mg/kg/day as compared to rats treated with vehicle (negative control), or perphenazine 5 mg/kg/day (positive control). H&E, PCNA and prolactin staining analyses will be performed in a subset of animals.
- The following comparisons will be made between each dose of lorcaserin and vehicle after 7, 28, 60, and 90 days of treatment:
 - Mammary tissue prolactin content
 - Pituitary tissue prolactin content
 - Levels of circulating hormones (to include estradiol, progesterone, and LH)

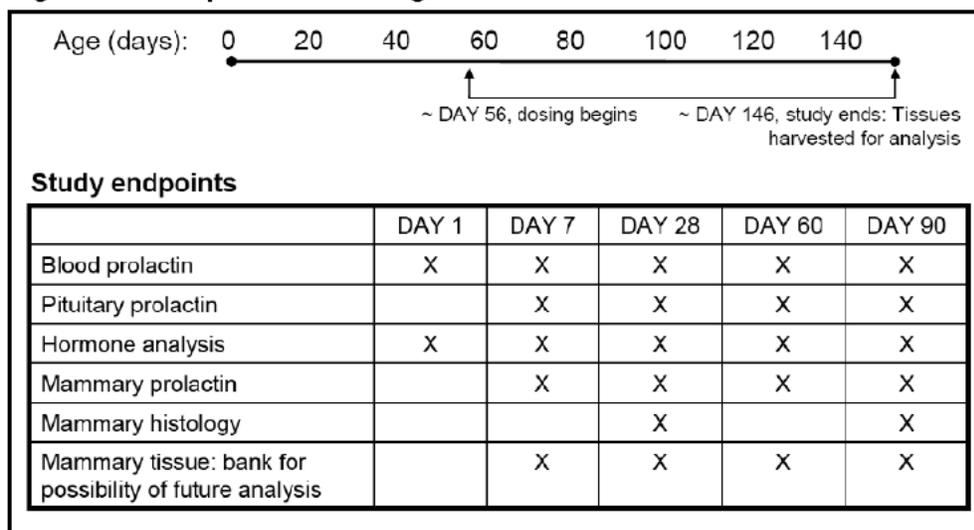
5 MATERIALS AND METHODS

5.1 Study Design

Vehicle-controlled, parallel arm study with positive control (perphenazine) and exposure durations up to 90 days as outlined in [Figure 1](#).

Four satellite treatment arms will be run in parallel to the 90 day treatment arm. Each arm will include five treatment groups dosed with vehicle, lorcaserin (3 doses) or perphenazine. The 7, 28 and 60 day treatment arms will evaluate mammary and pituitary prolactin content. Further, histopathological examination of mammary tissue will also be performed in the 28 day treatment arm similar to that described for the main treatment arm at 90 days. All animals will be used for mammary whole mount examinations; a randomly selected subset will be used for H&E, PCNA and prolactin staining endpoints, with the option of analyzing all tissues in the future. Terminal blood collections will also be performed in all arms (i.e. following 1, 7, 28, 60, and 90 days of treatment).

Figure 1. Experimental Design



5.2 Animals

Eight-week old female Sprague-Dawley rats ((b) (4)) will be used at the start of dosing. Rats will be single housed in plastic cages containing rodent bedding in a holding room controlled for temperature and light (12h:12h light-dark cycle), and will receive food and water *ad-libitum*. Rats will be housed in the facility for at least one week, and habituated to gavage and handling procedures for at least two days prior to study onset.

5.3 Experimental Procedures

5.3.1 Daily dosing and treatment groups

After habituation to administration and handling procedures, oral gavage dosing of test substance will commence. Rats will be dosed with either H₂O (vehicle), lorcaserin (10 mg/kg), lorcaserin (30 mg/kg), lorcaserin (100 mg/kg), or perphenazine (5 mg/kg). Experimental groups for the 28 day and 90 day arms will be composed of 60 rats per treatment group. Experimental groups for the 1, 7, and 60 day arms will be composed of 12 rats per treatment group. Dosing will occur daily at approximately three hours after lights on.

5.3.2 Sample collections

5.3.2.1 Plasma:

Interim bleeds: Blood will be collected by tail vein nick.

5.3.2.2 Mammary tissue:

Right abdominal mammary glands will be harvested on Day 28 and Day 90 to prepare whole mounts to assess mammary gland morphology (see below). Left mammary glands will be collected on Day 7 and Day 60 and stored for later measurement of prolactin content, and Day 28 and Day 90 for histopathology assessment. Left mammary tissue, including skin and nipple, extending from the thoracic region to the inguinal region will be excised from 60 surviving animals per treatment group at the Day 28 and day 90 endpoints. The piece of tissue will be placed (fur side up) on a piece of card or paper with the cranial aspect noted in permanent ink and fixed in 10% neutral buffered formalin. Tissue will remain in buffered formalin for 24-48 hrs then placed into 70% ethanol. Trimmed tissues will remain in 70% ethanol until they are processed and residual will be retained in 70% ethanol.

5.3.2.3 Pituitary:

Whole pituitary glands will be harvested, frozen, and stored at -80°C until analyzed for prolactin content.

5.4 Mammary tissue analysis

Mammary tissue will undergo histological evaluation by two independent laboratories. Dr. Jose Russo will conduct an analysis specific to mammary tumorigenesis. ^{(b) (4)} will conduct a traditional histopathological examination of H&E stained sections. ^{(b) (4)} will also carry out PCNA and prolactin immunohistochemistry on mammary gland sections. All assessments of mammary tissue histopathology and immunohistochemistry will be blinded. All 60 animals per group will be used for mammary whole mount examinations; a randomly selected subset will be used for H&E, PCNA and prolactin staining endpoints, with the option of analyzing all tissues in the future.

5.4.1 Whole mount analysis

Mammary tissue will be processed for whole mount and evaluated at the ^{(b) (4)} ^{(b) (4)} at the Fox Chase Cancer Center directed by Dr. J. Russo.

The fixed mammary fat pads will be dissected and separated from subcutaneous tissue before processing, staining and microscopic evaluation. Where necessary, specific areas of the whole mounted mammary gland will be excised from the whole mounts and embedded in

paraffin, stained with H&E, and studied further under the microscope for diagnostic evaluation. Microscopic evaluation will allow identification of the following end points¹⁻⁶.

- Lobular development: Percentage of structures with lobules of types 1, 2, 3 and 4 and/or degree of differentiation.
- Alteration in the ductal pattern of branching.
- Presence of ductal hyperplasia and/or lobular hyperplasia.
- Detection of ductal carcinoma *in situ* or invasive tumors.
- Detection of tumor masses such as fibroadenomas.

All histopathology evaluations will be reported and tabulated, and accompanied by an explanation and interpretation of the data. Representative photographs will also be included in the report.

5.4.2 H&E histology evaluation and PCNA and Prolactin immunohistochemistry

- Mammary gland tissue on the left side from 60 animals in all groups from the 28 day and 90 day time points will be excised to include nipple and overlying skin as detailed in [Section 5.3.2.2](#). Tissue will be fixed in 10% neutral buffered formalin for 24-48 hours. They will then be transferred to a 70% ethanol solution and shipped to (b) (4) at the address below for further processing and evaluation:

Ship tissues to:



- The tissue analyses (H&E, PCNA, prolactin immunohistochemistry) will be conducted in a stepped fashion:
 - Mammary gland tissue from all surviving animals at the 28 day and 90 day time points (up to 60 animals/group/timepoint) will be processed to paraffin blocks. A cross section of skin to include mammary gland and nipple (up to 5) will be trimmed and embedded in paraffin and saved for possible future analysis.
 - At the 28 day sacrifice, the entire lorcaserin 100 mg/kg/day group (nominally 60 rats) and a subset of the vehicle (30 rats) and positive control (30 rats) groups will be examined; pathologists will be blinded to treatment assignment. If no changes are detected in the lorcaserin 100 mg/kg/day group, the lower dose groups will not be examined unless agreed to by (b) (4) and Arena. If changes are observed, the lower dose tissues may be examined at a later date. An unblinded designee at (b) (4) who is not directly involved in study conduct will randomly

identify the 30 vehicle and 30 perphenazine treated animal samples for this analysis.

- At the Day 90 sacrifice, samples from all animals in the lorcaserin 10 mg/kg/day group and the lorcaserin 100 mg/kg/day groups will be analyzed; pathologists will be blinded to treatment assignment. Samples from 30 vehicle controls and 30 perphenazine controls will be randomly selected by (b) (4) for analysis. Samples from the remaining animals will be reserved for possible analysis at a future date.
- A cross section of skin to include mammary gland and nipple (up to 5) will be trimmed, embedded in paraffin. Tissue sections will be cut and mounted on glass slides then stained with H&E. Mammary gland sections will be referred to as: cranial thoracic, caudal thoracic, abdominal, cranial inguinal and caudal inguinal. If any of the glands are not present at trimming a note will be made. Additionally, an appropriate number of unstained slides will be prepared from each of the mammary gland paraffin blocks from all animals for PCNA and prolactin immunohistochemistry staining. After analysis of the initial samples (i.e., vehicle, lorcaserin high dose and positive control at 28 days and vehicle, lorcaserin low and high dose, and positive control at 90 days), the principal investigator(s) and the Study Director may determine that evaluation of additional samples is required. If this occurs, tissues from the 60 animals in the low and mid-dose groups at 28 days and tissues from the 60 animals in the mid-dose group (30 mg/kg) at the 90 day time point, will be processed as described above. In addition, if deemed necessary after tissue analysis and consultation with the Study Director, additional H&E sections will be taken from the high-dose group for evaluation.
- PCNA and prolactin immunohistochemistry: (b) (4) will conduct a preliminary (methods development) immunohistochemistry (IHC) staining run to optimize and/or verify staining procedures and conditions for PCNA and prolactin IHC staining on formalin fixed paraffin embedded rat tissues using a limited number of slides from at least one negative and one positive control animal. These slides will be blinded with a different numbering scheme. The blind animal number on the slide will be covered and labeled with A for negative control and B for positive control. Suitable tissues from the (b) (4) paraffin tissue bank will be used as the positive and negative control(s) for each IHC assay. IHC assays will be performed in accordance with (b) (4) SOPs, including the use of additional slides (e.g., assay control slides, inclusion of positive and/or negative control tissues or tissue elements or UV-resin spot slides, negative control antibody and/or assay control-stained slides to ensure stain specificity and reproducibility).
- H&E stained slides will be blinded according to (b) (4) SOP #s 52001 and 52100. H&E slides will be evaluated microscopically and any changes such as, but not limited to, inflammation, fibrosis, hyperplasia and neoplasia will be recorded. Subjective grading of microscopic changes based upon the intensity and extent of the changes will be employed according to the following scale: 1 = minimal, 2 = mild, 3 = moderate and 4 = marked. Mammary gland sections will be referred to as: cranial thoracic, caudal thoracic, abdominal, cranial inguinal and caudal inguinal. PCNA and prolactin IHC stained slides will be blinded according to (b) (4) SOP #s 52001 and 52100 and examined by the PI for immunohistochemistry. Quantitative analysis will be performed on all PCNA immunostained slides, and qualitative analysis will be performed on all prolactin

immunostained slides. All PCNA immunohistochemical stained slides (negative and assay control slides will not be scanned) will be scanned using the Aperio ScanScope CS and/or XT system(s) to prepare digital images. The digital images will be analyzed using Aperio's Positive Pixel Count and the resulting data will be exported to an Excel spreadsheet for reporting purposes.

- Any additional stains or evaluations, if deemed necessary by the PI for histopathology or the PI for immunohistochemistry, will be added by protocol amendment following discussion with the Study Director.
- At the completion of the study, all study-specific raw data, documentation, residual wet tissue, slides, residual paraffin blocks, and the Final Report from histopathological and IHC studies will be transferred to (b) (4) long-term repository consistent with the terms of our financial agreement. The Sponsor will be responsible for archival of all records, samples, specimens, and reports generated from phases or segments conducted by the Sponsor or Sponsor-designated subcontractors.

All histopathological and immunohistochemical evaluations will be reported and tabulated, and accompanied by an explanation and the CRO pathologists' interpretation of the data. The immunohistochemistry report will be included as an appendix to the histopathology study report.

5.5 Prolactin Extraction and Measurement

5.5.1 Plasma Prolactin

Plasma prolactin concentrations will be determined using an ELISA, to quantify immunoreactive prolactin (rat prolactin, ALPCO Diagnostics, Inc.), and/or the widely used Nb2 rat lymphoma cell bioassay^{9,10} to measure biologically active prolactin.

5.5.2 Pituitary Prolactin

Pituitary prolactin will be extracted following methods developed by Haggi et al.¹¹ Frozen pituitaries will be placed in extraction buffer (50 mM Tris buffer, pH 9, with 2.5M urea) and allowed to thaw on ice. Tissue will be disrupted using a combination of sonication followed by a snap freeze-thaw at -80°C. Homogenized tissue will then be centrifuged and the supernatant collected. Prolactin content will be evaluated using a combination of ELISA and/or Nb2 cell bioassay.

5.5.3 Drug concentrations

Drug concentrations will be assessed in terminal plasma samples using an in-house, selective LC/MS/MS method to measure lorcaserin or perphenazine.

5.6 Other hormonal analyses

Once collected and frozen, plasma aliquots will be shipped to [REDACTED] ^{(b) (4)} for analyses to include progesterone, estradiol, and luteinizing hormone content.

5.7 Test substances and dose formulation analysis

Lorcaserin will be dissolved in deionized water and dosed at a volume of 1 mL/kg orally. Lorcaserin doses will be expressed as free base.

Perphenazine will be dissolved in deionized water and the pH adjusted with HCl to pH 3-4 and dosed at a volume of 1 mL/kg orally.

Vehicle will be deionized water obtained from the in-house Barnstead water purifier.

All dose formulations and vehicles will be prepared weekly. Dose formulations are solutions and will be stored in amber bottles at 2-8°C when not in use.

Dose formulation and vehicle will be collected prior to dosing and following dosing for dose formulation analysis by HPLC. Duplicate samples will be collected from the dose formulations and vehicle control from each preparation prior to dosing and following dosing. One set of the duplicate will be analyzed and the second set will be stored at -20°C as backup. They will be disposed of once the study is complete.

Modifications in Amendment 1 and 2 submitted on July 14, 2011 are listed in tables below:

SUMMARY OF CHANGES IN AMENDMENT 1

Amendment 1 incorporates additional detail about histological and histochemical analyses that were not included in the original protocol. In addition, Amendment 1 enumerates the analysis of a subset of animals for some histological endpoints. Specific changes related to these general modifications are tabulated below:

1	Title page, and header throughout document	“Amendment 1” appended to Protocol Number. Date changed from 29 April 2011 to 30 June 2011. Histopathology laboratory investigators added
2	Page 4, Section 3	Text modified to specify quantitative PCNA and qualitative prolactin immunohistochemical analysis. Text modified to state that some histochemical endpoints will be measured initially in a subset of animals.
3	Page 5, Section 4	Text modified to state that some histochemical endpoints will be measured initially in a subset of animals.
4	Page 5, Section 5.1	Text modified to state that some histochemical endpoints will be measured initially in a subset of animals.
5	Page 7, Figure 1	“X” added to Day 30 and Day 90 mammary tissue banking cells of table
6	Page 7, Section 5.3.1	Following text was deleted: “in the 30 day and 90 day arms. All other arms will be composed of 10 rats per treatment group”. (All groups have 60 animals.)
7	Page 8, Section 5.3.2.2	Specific methodology for mammary tissue preparation has been added
8	Page 8, Section 5.4	Text modified to stipulate stains to be used and to state that some histochemical endpoints will be measured initially in a subset of animals.
9	Page 9, Section 5.4.2	Method clarified by adding/modifying text: - to clarify number of animals to be analyzed - to clarify methodology for tissue fixation and shipping - to state that subset of animals will be used and will be randomly selected
10	Page 9-10, Section 5.4.2	Text modified to update methodology to be used by (b) (4) ^{(b) (4)} for histochemical analyses
11	Page 10, Section 5.6	Language modified to include possibility that additional plasma hormones may be measured.

SUMMARY OF CHANGES IN AMENDMENT 2

Amendment 2 incorporates clarification and additional detail about histological and immunohistochemical analyses. Specific changes related to these modifications are tabulated below:

1	Title page, and header throughout document	“Amendment 2” appended to Protocol Number. Date changed from 29 April 2011 to 08 July 2011.
2	Throughout document	30 day time point corrected to indicate the duration of 28 days.
3	Page 6, Section 5.3.1	Text added, “Experimental groups for the 28 day and 90 day arms will be composed of 60 rats per treatment group. Experimental groups for the 1, 7, and 60 day arms will be composed of 12 rats per treatment group.” To clarify the number of animals in experimental groups.
4	Page 8, Section 5.4.2 , 1 st bullet point, 1 st sentence	Text added, “from the 28 day and 90 day time points” for clarification.
5	Page 8, Section 5.4.2 , 2 nd bullet point	Sub-bullet point added to indicate that all tissue will be trimmed and paraffin embedded.
6	Page 9, Section 5.4.2 , 3 rd bullet point	Text added to clarify and indicate that additional tissues at the 90 day time point will be analyzed from the low dose lorcaserin groups. Originally, only tissues from the high dose group were to be analyzed.
7	Page 9, Section 5.4.2 , 4 th bullet point	Text added, “using a limited number of slides from at least one negative and one positive control animal. These slides will be blinded with a different numbering scheme. The blind animal number on the slide will be covered and labeled with A for negative control and B for positive control.” Due to tissue blinding, lorcaserin treated animals cannot be used for assay verification as originally proposed.

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/s/

TODD M BOURCIER

07/20/2011

Comment to applicant on protocol amendment



**Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products**

SUPPLEMENT REVIEW

Date:	15 March 2011
NDA #	22529, IND 69888
Sponsor:	Arena pharmaceuticals
Drug:	Lorcaserin, 5HT2c agonist
Re:	Proposed receptor activation protocol review
Reviewer:	Todd Bourcier

In the End of Review meeting minutes from December 2010, the FDA noted that given the persistent imbalance in the FDA-defined valvulopathy in the clinical trials and the disparate in vitro receptor pharmacology data, additional and more expansive studies could be conducted to more fully characterize lorcaserin's activity at 5HT2B. Arena has submitted a summary of general approaches that they believe will address FDA's comments.

Summary of Arena's proposal:

There are many apparent inconsistencies in Arena's proposal which make it difficult to understand precisely what studies will be conducted and which receptors and drugs will be evaluated in those studies. In general, Arena's proposal consists of the following:

1. Establish cells lines expressing human, monkey, and rat 5HT2a, b, c receptors. Arena later proposes (b) (4) which seems contradictory. Cellular readouts are to include ligand binding, inositol phosphate release, calcium release, arrestin recruitment, and ERK activation. Arena will attempt to (b) (4) but is unsure of success.
2. Cellular studies will be conducted under conditions that control receptor reserve, achieved by the use of irreversible antagonists EEDQ 'and/or' phenoxybenzamine. Receptor densities would be determined by a standard binding assay with 125I-DOI. Arena later proposes (b) (4) but did not specify which compounds (Roth study used 28 compounds).
3. Investigate potencies and relative efficacies of lorcaserin and '5HT2 reference agonists' in calcium assays as a function of receptor density. Arena does not list which reference

agonists would be used, and restricts this analysis to only the calcium read-out. Arena later proposes to collect data from all cellular readouts as listed above, which again seems contradictory.

Many clarifications are required before further analysis of Arena's proposal can be completed. These issues are captured in the following draft comments to Arena.

External Comments to Sponsor:

The general approach to the receptor pharmacology studies appears reasonable, but the submission contains few experimental details and many apparent inconsistencies that require clarification before the Division concurs with the experimental plan.

- You propose to [REDACTED] (b) (4)
[REDACTED] or the purposes of this study, the highest priority is to conduct studies in cell lines expressing the *human* 5HT2A, B, and C receptors.
- You propose to investigate the potencies and efficacies of lorcaserin and unspecified reference compounds in calcium assays as a function of receptor density (Section 3.1). Later, in section 3.3, studies are described that include multiple read-outs, including IP, calcium, ERK, and arrestin assays. The proposal does not clearly state which assays will be used to evaluate lorcaserin and reference compounds. The calcium and IP assays are considered critical, as these assays would be the only bridge for comparison to the studies conducted in 2002/04 and 2009. The ERK and β -arrestin assays would provide new information and would be interpreted in the context of the larger dataset available for calcium and IP accumulation.
- You propos [REDACTED] (b) (4)
[REDACTED] Table 1 in the Roth paper lists 28 compounds that were characterized in their panel of assays. For the purpose of these experiments, the Division is most interested in binding and functional data that directly compares lorcaserin to compounds that pose a documented risk of valvulopathy presumably by their interaction with 5HT2B. Direct, comparative data between lorcaserin, norfenfluramine, and pergolide or cabergoline appears to be a reasonable and appropriate approach. It is also worth consideration to include a 'negative' control, such as phentermine, lisuride, or a selective serotonin reuptake inhibitor. Activity of these compounds as a function of receptor density at the human 5HT2A, B, and C receptors with at least the calcium and IP accumulation assays would provide a dataset that addresses the Division's concern on this issue.

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/s/

TODD M BOURCIER

03/22/2011

Review, proposed receptor pharmacology protocols

Pharm/tox review of proposed studies to assess lorcaserin effect on prolactin production in SD rats for IND 68,888 (NDA 22-529)

IND: 68,888 (NDA 22-529)

Drug: lorcaserin

Indication: Treatment of obesity

Sponsor: Arena Pharmaceutical

Sponsor Submission Serial # 0185, **SDN #** 213

Received: Feb 18, 11

Background:

Reference is made to the NDA 22-529 End of Review meeting held with Arena on Dec 12, 2010. At the meeting, the need for mechanistic studies demonstrating a robust lorcaserin dependent increase in prolactin was discussed. The meeting minutes relevant to the nonclinical studies requirements are attached to appendix A (page 9). The division agreed that mechanistic studies have to clearly show a robust dose-dependent increase in plasma prolactin or tissue changes reflective of increase in prolactin production with chronic administration of lorcaserin in SD rats (≥ 3 months in duration). The Division requested all mechanistic studies protocol to be submitted to the agency for review prior to initiation. In the meeting minutes, the Division had proposed several study design recommendation which were referenced by the sponsor in the background information submitted the protocols:

- Dosing duration of no less than 3 months.
- Dose groups to include a control, a dose range of lorcaserin, and at least one relevant positive control (e.g., dexfenfluramine, mCPP, haloperidol).
- Measure serum prolactin at multiple time points (e.g., Days 1, 7, 30, 60, 90).
- Measure tissue prolactin (mammary & pituitary) at multiple time points (e.g., Day 1, 7, and 90).
- Changes in prolactin can be assessed by comparison to untreated control and to pre-study baseline prolactin in all groups.
- Include male and female rats.
- Consider monitoring other relevant endpoints (e.g., estrogens, progesterone, LH).

In this submission, the sponsor has provided protocols for three nonclinical mechanistic studies exploring the role of lorcaserin on prolactin release in female and male SD rats. In the 28-day study (DBR-11-003), the effect of lorcaserin on prolactin will be evaluated on Day 1, 7 and 28 in female SD rats. The second study (DBR-11-002) is designed to address the effect of 90 days of lorcaserin treatment on prolactin and related tissues in female rats implanted with placebo or bromocriptine (a D2 agonist) pellets. These rats will be implanted with placebo or bromocriptine pellet (50 mg) five days before initiation of lorcaserin treatment. Data collection will be made on single time point on Day 90. The final study (DBR-11-004) will address the effect of lorcaserin on blood prolactin (single data point) in male SD rats on Day 90.

All the studies will include a placebo and positive control. The sponsor will be using antipsychotic drug perphenazine as positive control to produce a robust increase in plasma prolactin in SD rats. Single and multiple doses of perphenazine have been shown to increase and sustain high plasma prolactin in rats and humans. In rats,

perphenazine increased plasma prolactin within the earliest sample collection time of 30 minutes and up to 4 hrs suggesting that the window of data harvest is up to 4 hrs at minimum. (Van Der Gugten et al 1976; Ben-David et al, 1970, Appendix B, page 20). The initial dose of perphenazine is set at 1 mg/kg but may change based on data collected from the 28-day DBR-11-003 study. The specifics of each study design and data collection methods and reviewer's recommendations for each study are described below.

Proposed Nonclinical Study Protocol # DBR-11-003:

Title: Evaluation of lorcaserin effects on plasma prolactin concentrations after single dose or repeat doses for up to 28 days in female Sprague Dawley rats.

This appears to be a pilot study designed to determine the effect of lorcaserin on plasma prolactin levels as well as the dose of perphenazine (1 mg/kg) over 28 days in female rats. Eight-week old female SD rats (b) (4) will be divided into 5 groups (n=25/group), a placebo control (saline, 1 ml/kg), lorcaserin (10, 30 and 100 mg/kg) and a positive control group (perphenazine, 1mg/kg, route not provided). Female SD rats be housed individually with ad lib access to food and water. After a one-week acclimation, rats will be gavaged daily with saline or treatments between 0900 and 1200 hr daily for 28 days. Trunk blood and the whole pituitary gland will be collected 15-min post dose on Day 1, 7 and 28 suggesting that 1/3 of the animals will be sacrificed on each day (~ 7 per time point). There will be single data point from each animal on Days 1, 7 and 28 which might be sufficient to show a trend in prolactin release over time. The location of the testing facility was not provided.

Study Design: 28-Day study in female rats

Prolactin Study DBR-11-003	Placebo Control, saline	Lorcaserin, mg/kg			Positive Control, Perphenazine 1 mg/kg
		10	30	100	
Number of Female SD rats	25	25	25	25	25

Blood and pituitary tissue collection

- Trunk blood will be collected into K2.EDTA vacutainer after decapitation. The plasma will be collected after centrifugation (4°C) and transferred into individual 96-well PCR tubes and stored at -20°C for prolactin determination at later time point.
- Pituitary gland will be collected, frozen for prolactin content analysis.
- There will be no tissue morphology determination in the 28-day study.

Protocol DBR-11-003 Deficiencies and Recommendations:

1. The location of the testing facility is unknown.
2. Please clarify the number of rats per time point per group. As proposed, there will be 5 groups with 25 rats/group that will be bled on Day 1, 7 and 28 suggesting ~7 rats/day/group. We recommend minimum of 10 rats/day/group (30rats/group)
3. We recommend blood, pituitary and mammary tissue sample collections for determination of prolactin. As subsection of the pituitary may be saved for potential microscopic evaluation.

- Blood and tissue harvesting at 15-min post dose on Day 1, 7 and 28 is likely to be impacted by the gavage induced stress. With rat brain lorcaserin Tmax of 1 hr, we recommend blood collections at ≥ 60 min post dose. If early morning prolactin samples are desired, you may chose to collect blood/tissue samples before gavage.

Proposed Nonclinical Study Protocol # DBR-11-002:

Title: Three month evaluation of lorcaserin on prolactin concentrations and mammary gland histopathology in the presence and absence of the D2 agonist bromocriptine in female Sprague Dawley rats

The objectives of the proposed study are to determine the lorcaserin-induced changes in plasma, pituitary and mammary tissue prolactin levels and mammary morphology in presence and absence of bromocriptine, a D2 agonist at the end of the 90-day study.

Eight-week old female SD rats from (b)(4) will be implanted with either bromocriptine (50 mg) or placebo pellet on Day 1 under isoflurane anesthesia. The 50 mg bromocriptine pellets are timed to last 60-day therefore; they will be replaced on Day 50 as described by the sponsor. The sponsor expects the 50 mg bromocriptine pellet to be sufficient in blocking the lorcaserin-induced increase in prolactin. Five days after the pellets are implanted, rats (n=50 female rats/group) will be treated with placebo (saline, 1 ml/kg), lorcaserin (30 and 100 mg/kg) or positive control (perphenazine, dose/route not provided). The implanted female SD rats (placebo and bromocriptine) will be gavaged daily between 0900 and 1200 hr but for 3 months. Blood and tissues will be collected 15-min post dose on Day 90 (one time). The sponsor states that they believe the terminal bleeds are the most reliable method for plasma prolactin measurement.

Part 1 Study Design: 90-Day study in female rats

Prolactin Study DBR-11-002	Placebo control,	lorcaserin, 30 mg/kg	lorcaserin, 100 mg/kg	Positive control, Perphenazine
Number of rats with placebo pellets	25	25	25	25
Number of female rats with bromocriptine pellets	25	25	25	25

Blood, mammary and pituitary tissue collection

- Trunk blood will be collected 15-min post dose by decapitation into K2.EDTA vacutainer. The plasma will be separated and transferred into individual 96-well PCR tubes for storage at -20°C.
- Right abdominal mammary gland will be collected at the time to assess the mammary gland morphology. The left abdominal mammary gland will be collected and frozen rapidly on dry ice for future analysis of prolactin content and markers of prolactin receptor activation. Mammary tissue histopathology will be evaluated at (b)(4) directed by Dr. Jose Russo. Dr. Russo will be providing histopathology consultation. The analysis will include the following:
 - Lobular development: Percentage of structures with lobules type 1,2,3 and 4 that are different, degree of differentiation.
 - Alteration in the ductal pattern of branching

- c) Presence of ductal hyperplasia and/or lobular hyperplasia
- d) Detection of ductal carcinoma *in situ* or invasive tumors that are direct evidence of a carcinogenic effect. Detection of tumor masses such as fibroadenomas.
- The whole pituitary gland will be harvested, frozen and stored at -20°C for analysis of prolactin content at a later time. It is not clear if the pituitary gland will be frozen rapidly on dry ice first before storage in the freezer.

Protocol DBR-11-002 Deficiencies and Recommendations:

1. The location of the testing facility is unknown.
2. We recommend blood and tissues harvesting to include Day 7 and 45 in addition to the proposed Day 90. The number of animals sacrificed on each Day should be no less than 10 rats/group.
3. Blood/ tissue harvested at 15-min post dose is likely to be impacted by the gavage induced stress. With rat brain lorcaserin Tmax of 1 hr, we recommend blood collections at ≥ 60 min post dose. If early morning prolactin samples are desired, you may chose to collect blood/tissue samples before gavage.
4. Per protocol, the 50 mg bromocriptine pellet implants will last at least 60 days. Please reference studies showing that a 50 mg bromocriptine pellet will provide adequate steady dose of bromocriptine sufficient and will have no PK interaction with lorcaserin.
5. Lorcaserin had no notable effect on mammary tissue morphology in the 3- and 6-month rat studies nor had any effect on plasma prolactin or brain dopamine levels (nucleus accumbens). However, this study assumes that, a) lorcaserin will result in morphological changes in the mammary and pituitary by Day 90, b) lorcaserin will increase plasma prolactin, c) lorcaserin affects brain dopamine levels thus it's effect can be prevented by bromocriptine, a D2 agonist, d) bromocriptine dose is sufficient to block lorcaserin-induced increase in prolactin. The study will consider in negative light if there are no changes in mammary tissue morphology or plasma prolactin at Day 90 in lorcaserin treated rats without bromocriptine. Therefore, it is critical for the sponsor to show that lorcaserin will independently increase plasma prolactin and change mammary morphology by the end of the study on Day 90.

Proposed Nonclinical Study Protocol # DBR-11-004:

Title: Three month evaluation of lorcaserin on plasma prolactin concentrations in male Sprague Dawley rats

In this study, 8-WK old male SD rats (n=12/group) from (b) (4) will be treated with saline (1 ml/kg), lorcaserin (10, 30 and 100 mg/kg, 1ml/kg) or positive control (perphenazine, dose, route not provided 1 mg/kg) daily for 3 months between 0900 and 1200 hr daily. The placebo vehicle rats will be gavaged with 1 ml/kg saline. On Day 90, trunk blood and pituitary gland will be harvested 15-min after dosing.

Study design: 90-Day study in male SD rats

Prolactin study (DBR-11-004)	Placebo control	Lorcaserin, mg/kg			Positive control, Perphenazine
		10	30	100	
Number of male SD rats	12	12	12	12	12

Protocol DBR-11-004 Deficiencies and Recommendations:

1. The location of the testing facility is unknown.
2. We recommend additional blood, mammary and pituitary tissue collections on Day 7 and Day 45.
3. Mammary and pituitary tissues collected on Day 90 should be evaluated morphologically.
4. Blood/ tissue harvested at 15-min post dose is likely to be impacted by gavage stress. With rat brain lorcaserin T_{max} of 1 hr, we recommend blood collections at ≥ 60 min post dose.

Discussion

The sponsor has proposed three nonclinical mechanistic studies to address the questions regarding the role of prolactin in lorcaserin induced mammary tumors in the female and male SD rat carcinogenicity study.

In the 28-day study (DBR-11-003), the sponsor will be evaluating the effect of lorcaserin (10, 30 and 100 mg/kg, PO) on plasma prolactin and pituitary gland in intact female SD rats on Days 1, 7 and 28 at 15-min post dose. The study will also include a positive control, perphenazine (1 mg/kg). Perphenazine is an antipsychotic drug known to increase plasma prolactin in humans and rats (up to 10 fold). The study seems to be reasonable except for the limited duration of the lorcaserin treatment and timing of plasma/tissue sample collection. Since there will be longer studies, the duration is acceptable but the trunk blood collected at 15-min post dose is problematic since it is likely to be impacted by gavage-induced stress leading to greater variance in plasma prolactin levels. With rat brain lorcaserin T_{max} at 1 hr, the maximal drug effect is likely to be ≥ 1 hr post dose. Therefore, the sponsor should consider samples at 60 min post dose to reduce any potential variation in plasma prolactin. Although number of rats per data point (~ 7 rats/data point) is acceptable for a pilot study, ideally the number of animals should be ≥ 10 rats/time point considering that high variance in plasma prolactin among rats in the past studies have failed to show a consistent lorcaserin effect.

The 3-month study in female rats (DBR-11-002) is considered a pivotal study. It is designed to show lorcaserin induced (30 and 100 mg/kg) increases in plasma/tissue prolactin and related mammary tissue morphology on Day 90 and that these changes can be blocked by bromocriptine. The study makes many assumptions, a) lorcaserin will increase plasma prolactin, b) lorcaserin will result in morphological changes in the mammary and pituitary by Day 90, c) bromocriptine dose is sufficient to block lorcaserin-induced increase in prolactin. Since lorcaserin had no notable effect on mammary tissue in toxicology studies up to 6 months and plasma prolactin was not changes in studies up to 4 weeks, absence or equivocal changes in plasma prolactin or mammary tissue with lorcaserin alone will be considered a negative study. Therefore, it is critical for the sponsor to show first that lorcaserin will independently increase plasma prolactin and cause mammary changes in female SD rats by Day 90. Until the effect of lorcaserin on prolactin is clearly demonstrated, any change produced by bromocriptine +lorcaserin is of little value since bromocriptine effect via dopamine modulation on prolactin on mammary tumor may override any other mechanism. Another issue that

may limit its values as proposed is the single data collection point on Day 90. The reviewer recommends data collection at ≥ 60 min post dose on Day 7, 45 and 90. Trunk blood collected at 60 min post dose on more than occasion (Day 90) will likely to improve the reliability of the study and possible trend in plasma prolactin over the span of the proposed study.

The third study (DBR-11-004) is designed to measure prolactin levels in male SD rats at the end of the 90 days lorcaserin treatment. Similar to previous studies, perphenazine will serve as positive control. Since the study will rely on single data point 15-min post dose on Day 90, the sponsor will be advised to collect samples at ≥ 60 min post dose on from 10 animals per group on Day 7, 45 in addition to day 90. To further support the hypothesis that lorcaserin induced increase in mammary tumors in male rats was prolactin dependent, morphological evaluation of mammary and pituitary gland on Day 90 is recommended.

In the background information, the sponsor states, "*Arena is continuously considering the value of experimental modifications or additional experiments to test the overarching hypothesis that lorcaserin causes mammary tumors in female rats by increasing prolactin release. We will evaluate data as they become available from preliminary experiments, from the re-adjudication of the female rat carcinogenicity study, and from the studies outlined above. We believe that if these experiments demonstrate (1) sustained increases in circulating, pituitary, or mammary gland prolactin; and/or (2) characteristic changes in the mammary gland that are inhibited by bromocriptine, then evidence sufficient to support the prolactin hypothesis will have been generated. However, should these results indicate that additional experimentation may be important to adequately characterize the role of prolactin in rat mammary tumor pathogenesis; appropriate new protocols will be submitted to the Agency for review. Such protocols could include a 12-month study in rats to determine whether short-term exposure to lorcaserin is sufficient to induce mammary neoplasia at 1 year.*"

This statement appears to suggest that the sponsor will deem prolactin as the mechanism for lorcaserin induced mammary tumors in SD rats. The reviewer believes this statement is premature since the Division will make that decision upon review of the data. The reviewer may not accept small equivocal changes in plasma prolactin with no evidence of lorcaserin related changes in mammary gland morphology.

Recommendations:

Overall, the proposed nonclinical mechanistic study protocols are acceptable pending modifications to the timing of the data collection, number of data collection points, morphological assessment of the target tissues and robustness of the data changes in plasma prolactin levels.

The 3-month study protocol in female rats with or without bromocriptine pellet assumes that lorcaserin alone will increase plasma prolactin levels and result in morphological changes that potentially would be reversed by bromocriptine. Since lorcaserin has failed to show any change in mammary tissue in 3- and 6-month rat study or increase plasma prolactin, the assumptions are optimistic and unsubstantiated. Failure to show a change in these parameter with lorcaserin alone at Day 90 in female rats is likely to

prevent any conclusion. It is the sponsors responsibility first to show that lorcaserin can indeed increase plasma prolactin and lorcaserin dependent mammary pathology. All three studies are designed to collect samples at 15-min post dose. Since the brain Tmax for lorcaserin in rats is 60 min and oral gavage induced stress may impact prolactin levels, the sponsor is advised to collected samples at ≥ 60 min post dose at more frequent time intervals (i.e. Day 7, Day 45) to ensure more reliable data to reach a clear conclusion regarding the mechanism of lorcaserin induced mammary tumors in SD rats.

External recommendations

The Division has reviewed the protocols intended to re-address the hypothesis that mammary tumors induced by lorcaserin in rats is secondary to elevations in prolactin. The proposed approach in part addresses the issues discussed in the End-of-Review meeting minutes. The Division has the following comments and recommendations:

General Comments regarding all Protocols

1. Consider harvesting blood/tissue samples at 60 minutes post-dose in all studies instead of 15-minutes post-dose as proposed. Collecting blood and tissues at 60 minutes post-dose is consistent with the T_{max} for lorcaserin in rat brain (~1 hr), and may avoid potential undue effects of gavage-related stress on prolactin levels.
2. Identify the testing facility that will conduct the proposed studies.

Protocol DBR-11-002: Three-month study in female SD rats ± bromocriptine

1. Demonstrate that a pharmacokinetic interaction does not exist between bromocriptine and lorcaserin (e.g., exposure to lorcaserin increases or decreases with bromocriptine) after repeated exposure. It is recommended that this be determined in a pilot study before initiating the 3 month study, as a substantial change in exposure to either drug would likely invalidate the results of the 3 month study.
2. Justify the proposed dose of bromocriptine. Ideally, exposure to bromocriptine would be sufficient to blunt or prevent increases in prolactin provoked by perphenazine without severely reducing baseline prolactin. Dose-ranging studies that support the proposed 50mg dose of bromocriptine would be expected. Because bromocriptine would be administered in a slow-release pellet formulation, address whether the levels of drug at later time points (e.g., day 30-50) remain sufficient to achieve the desired pharmacological activity.
3. The protocol proposes to collect prolactin content data only at day 90, citing 'in-house' data that terminal bleeds are most reliable for prolactin analysis. The basis for this statement is unclear, as collecting such data from multiple time points would be viewed as having greater utility than from a single time point. It is preferable that in addition to the 90 day time point, you include an additional 10 females/group and collect blood, pituitary, and mammary tissue after 7 and 45 days of dosing. Tissues collected at Days 7 and 45 would be used to determine prolactin content and the mammary tissue archived for histological analysis, if needed at a future date.

4. As the protocol proposes to evaluate only 2 doses of lorcaserin, it would be preferable to evaluate 10 and 100mg/kg to cover a greater range of drug exposure than 30 and 100mg/kg.

Protocol DBR-11-003: 28-day study in female SD rats

1. Consider evaluating 10 females per time point (Day 1, 7, 28, or n=30/group) instead of the ~7/time point proposed.
2. Determine prolactin content of the pituitary and mammary tissues in addition to blood for each time point examined. One of the mammary pads could be reserved for histochemical evaluation, if such data would prove valuable at a later time.

Protocol DBR-11-004: Three-month study in male SD rats

1. We recommend additional data points to be collected on Day 7 and 45. It is recommended that the number of animals be increased to 30 males per group to allow evaluation of 10 animals each at time point of 7, 45, and 90 days of dosing.
2. Collect blood, pituitary, and mammary tissue from all animals at each time point for analysis of prolactin content.
3. Include a histological evaluation of the mammary gland at the 90 day time point.

Additional comments

1. The Division remarked at the End-of-Review meeting that the toxicology reports for lorcaserin did not describe drug-related changes in the histopathology of the mammary glands of rats even after 6 months of dosing. Concern remains that changes in mammary histology may not be observed with lorcaserin in the 3 month studies proposed herein. Small changes in prolactin in the absence of a change in mammary gland histopathology, while supportive of an effect of lorcaserin on prolactin, would fail to address the Division's request to "consider experimental designs that demonstrate the necessity of prolactin in lorcaserin-induced changes to normal or neoplastic mammary tissue (in vivo, in vitro, or both)." (End-of-Review meeting minutes, 15 of Dec 2010). We again emphasize that you need demonstrated a clear robust lorcaserin dependent increase in prolactin and related mammary tissue changes. Any change in bromocriptine arm of the study without showing first an effect on prolactin by lorcaserin will be of little value.
2. An article by Wijsman¹ describes a model of prolactin-dependent outgrowth of transplantable mammary carcinoma in rats. Perphenazine promoted tumor outgrowth in a manner sensitive to inhibition by bromocriptine. One concern expressed by the Division in the Complete Response letter was that lorcaserin

appears to increase the aggressiveness of mammary tumors based in part on the clear imbalance of lung metastases at 10, 30, and 100mg/kg lorcaserin. The model described by Wijsman appears quite appropriate to address this concern while also addressing the prolactin-dependence of responses to lorcaserin, if any are observed, and request that you consider studies in this model. ¹Wijsman JH, et al. (1991) *Br J Cancer* 64; 463-468

3. The Division notes that prior studies using similar doses of lorcaserin failed to reproducibly demonstrate a persistent increase in prolactin in intact rats, and reportedly failed to alter the histopathology of rat mammary tissue in the 6 month toxicology study. The expectation that these endpoints would now be detectable in the proposed studies of ≤ 3 months duration is presumably based on your stated improvements in methodology. A detailed discussion of these changes in methodology will be needed to address the failings of the former studies.

Appendix A

NDA 022529
Meeting Minutes

MEMORANDUM OF MEETING MINUTES

Meeting Type: Type B
Meeting Category: End-of-Review
Meeting Date and Time: December 15, 2010; 9:00 AM eastern time
Meeting Location: White Oak Campus, Silver Spring MD
Application Number: NDA 022529
Product Name: Lorcress (lorcaserin HCl) Tablets, 10 mg
Indication: An adjunct to diet and exercise for the treatment of obesity
Sponsor/Applicant Name: Arena Pharmaceuticals
Meeting Chair: Eric Colman, M.D.
Meeting Recorder: Pat Madara

CDER Attendees

Office of New Drugs

David Jacobson-Kram, Ph.D. Director for Pharmacology and Toxicology
Paul Brown, Ph.D. Associate Director for Pharmacology and Toxicology

Office of Drug Evaluation II

Curtis Rosebraugh, M.D., M.P.H. Director
Lee Ripper Associate Director for Regulatory Affairs

Office of Drug Evaluation II; Division of Metabolism and Endocrinology Products

Mary H. Parks, M.D. Director
Eric Colman, M.D. Deputy Director
Amy Egan, M.D., MPH Deputy Director for Safety
Julie Golden, M.D. Medical Officer
Todd Bourcier, Ph.D. Pharmacology/Toxicology Team Leader
Fred Alavi, Ph.D. Pharmacology/Toxicology Reviewer
Patricia Madara, M.S. Regulatory Project Manager

Office of Biostatistics; Division of Biometrics II

Todd Sahlroot, Ph.D. Deputy Director
Janice Derr, Ph.D. Statistical Reviewer

CDER Office of the Center Director, Controlled Substance Staff (CSS)

Katherine Bonson, Ph.D. Pharmacology Reviewer
John Gong, M.D., Ph.D. Medical Officer Reviewer

Arena Pharmaceuticals Attendees

Jack Lief,	Chairman, President and CEO
Mark Brunswick, Ph.D.	Senior Director, Regulatory Affairs
William Shanahan, M.D.	Senior Vice President & Chief Medical Officer
Dominic Behan, Ph.D.	Senior Vice President & Chief Scientific Officer
Christen Anderson, M.D, Ph.D.	Vice President, Lorcaserin Development
Weichao Chen, Ph.D.	Senior Director, Drug Metabolism & Pharmacokinetics
Matilde Sanchez, Ph.D.	Senior Director, Biostatistics and Data Management
Michael Kim	Director, Regulatory Affairs
Hussien Al-Shamma, Ph.D.	Senior Director Pharmacology
K. A. Ajit Simh,	Vice President Quality & Regulatory Compliance

Eisai, Inc., Attendees

Mark Taisey,	President, Global Regulatory Affairs
Lynn Kramer, M.D.	President, Neuroscience Product Creation Unit
Paul Andrews, Ph.D.	Executive Director, Global Regulatory Affairs – Nonclinical

Consultants to Arena and Eisai

[Redacted] (b) (6)

Jose Russo, MD,	Professor and Senior Member, Fox Chase Cancer Center, Philadelphia, Pennsylvania
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[Redacted] (b) (6)

Background

On December 18, 2010, Arena Pharmaceuticals submitted new drug application (NDA) 022529 for Lorqess (lorcaserin hydrochloride) tablets. Lorcaserin hydrochloride is a new molecular entity that targets activation of the serotonin 5HT2C receptor and is intended to promote weight loss in an obese population.

On September 16, 2010, the application was discussed at an Advisory Committee meeting. The panel members voted 9 to 5 that the available data do not demonstrate that the potential benefits of lorcaserin hydrochloride outweigh the potential risks, when used long-term in a population of overweight and obese individuals.

On October 22, 2010, the Agency issued a complete response letter, describing our concerns and the deficiencies in the data provided with the application. It also provided, where possible, our recommendations to address the issues.

On October 28, 2010, Arena requested an End-of-Review meeting to discuss the Complete Response letter and obtain guidance from FDA. Pre-meeting draft minutes issued on December 14, 2010. Those minutes and the additional discussion from the meeting are described below.

Note: Arena's questions are in plain text. The FDA's pre-meeting responses are in **bold text**. Discussion at the meeting is in *italicized text*.

Discussion

Nonclinical

1. Diagnostic uncertainty in the classification of mammary masses in female rats

Q1a. Arena plans to convene a Pathology Working Group of 3 to 5 independent pathologists to re-adjudicate the female rat mammary and lung tumors in a blinded fashion as requested by the Agency. The slide blinding and un-blinding will be executed by (b) (4). Arena will provide a list of proposed pathologists, working group instructions, and reporting plans to the Agency for concurrence.

FDA Response:

The revised PWG protocol that incorporated the Division's recommendations is acceptable.

Arena can provide a list of all slides prepared for female rat mammary tissues presented in the updates and the final study report with tabulations of diagnoses that changed. Documentation of the discussions between the pathologists and their notes and reasons for these changes are not available, per standard pathology review practices in the industry. Although these tabulations can be provided, we believe the plans to re-adjudicate the mammary and lung tumors with an independent Pathology Working Group will more directly address the Agency's concern. Does the Agency agree that in view of our commitment to readjudicate these tumor findings this accounting is no longer necessary?

FDA Response:

We acknowledge your explanation that the reasons for the imbalanced change in diagnoses across dose groups were not documented and therefore cannot be provided. It would be acceptable to instead provide a spreadsheet documenting all changes in the diagnosis of female rat mammary tissues from the interim updates to the final study report, listed by animal number.

In addition, we request clarification regarding the pathology records for two high-dose female rats. The histopathology report for female #4202 describes mild atypic hyperplasia of the mammary tissue despite the presence of a large (2-3.9 cm) axillary mass present about 10 weeks prior to euthanasia. The report for female #4212 lists mammary tumors as the cause of death, yet no mammary tumor is described in the histology report for this animal.

Meeting Discussion:

Arena clarified that (b) (4) is unable to provide documentation of the change in diagnoses at the level of individual slides and animals; only the change in diagnoses as a group was documented. Arena explained that interim updates of carcinogenicity studies to the FDA is not standard practice, and therefore documentation of changes in diagnosis from 'preliminary' to 'final' assessments did not capture the information requested by the FDA. Arena restated that all the histology slides that contributed to the interim updates were also examined and contributed to

the final tally provided in the NDA, indicating that (b) (4) should have knowledge of the identity of slides evaluated for the interim updates. The FDA commented that regulatory decisions were being made based on the information provided by Arena in the interim updates, and that documentation that at least specified the subsequent changes to that information was of importance to FDA's review of the final data in the NDA. The FDA requested and Arena agreed to work further with (b) (4) in tracking the animal identification of the slides examined for the interim updates, noting that such information might be derived by examining the relevant histology/cutting notebooks. It was noted that the evaluation by FDA's Division of Scientific Investigations did not include examination of the histology notebooks and was unable to provide adequate clarification on this issue. The FDA remarked that if such documentation is unattainable after further consultation with (b) (4) review of Arena's NDA resubmission would necessarily move forward without the information.

Arena noted that clarification of the histopathology records for the two high-dose female rats would be addressed by the pathology working group.

Unresolved exposure-response relationship for lorcaserin-emergent mammary adenocarcinoma

Q2a. The statistical analysis of the mammary tumor findings included combination of adenocarcinomas and fibroadenomas. This practice of combining the two tumor types is based on the publication by McConnell et al. However, there is now expert opinion based on current knowledge of mechanisms underlying mammary gland carcinogenesis that these tumor types should not be combined because fibroadenomas are known not to be a precursor to adenocarcinomas. Does the Agency agree that statistical analysis of mammary gland fibroadenomas and adenocarcinomas separately but not combined is appropriate?

FDA Response:

The FDA statistical analysis evaluated these two tumor types alone and in combination, as was done by the Sponsor (b) (4) and reported in NDA 22-529. The results of the FDA statistical analysis mirror those reported in the NDA. The FDA is aware that the human risk from these two tumor types may differ, and those differences in human risk can be taken into consideration when there is clarity of diagnosis as reflected in the pathology reports for carcinogenicity studies. Diagnostic uncertainty was evident in the classification of mammary masses in rats administered lorcaserin, for reasons summarized in the Complete Response Letter. Without confidence in the diagnoses, the more conservative approach to assessing human risk is most appropriate.

Some of the Division's recommendations for the PWG are directly targeted to clarify the degree of diagnostic certainty in distinguishing fibroadenoma from adenocarcinoma in the study conducted with lorcaserin. The Division will discuss the most appropriate approach of evaluating the mammary tumors after reviewing the PWG report and their comments on this issue.

Will establishment of a safety margin for the adenocarcinoma be sufficient to address the Agency's concerns?

FDA Response:

A ‘safety margin’ implies a dose range above therapeutic exposure within which adverse effects of a drug are not observed. As summarized in the Complete Response Letter, there is evidence of decreased latency and increased aggressiveness of adenocarcinoma at all doses of lorcaserin in female rats, and therefore, the exposure-response relationship remains unresolved. In addition to numerical increases in tumor incidence, evidence of decreased latency and increased aggressiveness of drug-induced malignancies in rodents contributes to the Division’s assessment of human risk, as does consideration of the risk/benefit associated with a given drug and clinical indication.

Q2b. If Arena provides acceptable evidence that the mammary tumors observed in the 2-year rat carcinogenicity study are due to increased prolactin either in blood or mammary gland, will the Agency consider the prolactin mechanism an adequate explanation for the tumors and their aggressiveness, and will this mechanism satisfy the Agency’s criterion that the tumors are reasonably irrelevant to human risk assessment?

Q2c. Does the weight of evidence introduced in this document provide sufficient evidence of a prolactin mechanism for rat mammary tumors? If not sufficient, would a demonstration of lorcaserin-mediated increases in serum prolactin in intact female rats or increases in prolactin in rat mammary gland be sufficient evidence for a mechanism irrelevant to human risk?

FDA Response to Q2b and 2c:

Experimental evidence with lorcaserin that demonstrably links its effects on mammary tissue in rodents to a prolactin-dependent mechanism will mitigate the Division’s level of concern for clinical risk.

As discussed in the Division’s briefing package to the Advisory Committee, the prolactin data submitted in NDA 22-529 does not provide persuasive evidence implicating this hormone in mammary tumors induced by lorcaserin. The information provided in your background package does not provide nor proposes a strategy to provide the experimental evidence with lorcaserin necessary for the Division to re-assess clinical risk.

The Division recommends that you propose experimental strategies evaluating lorcaserin which could be considered in support of prolactin as the mediating mode of tumorigenic action.

Your background document cites literature linking a 5HT_{2A/2C} pathway to negative regulation of dopamine and additional direct effects on prolactin release. These studies involved *in vitro* and *in vivo* approaches using mixed serotonergic receptor agonists, dopaminergic compounds, and pharmacological 5HTR antagonists. Lorcaserin is a new molecular entity with a unique 5HTR selectivity profile and tissue distribution characteristics. We recommend that you propose additional *in vitro* and *in vivo* studies to determine the potential for lorcaserin to alter release of pituitary/tissue prolactin and potentially its regulatory intermediaries such as dopamine and vasoactive intestinal polypeptide. Demonstration of a clear, persistent (<13 wks) increase in serum/tissue prolactin at doses of lorcaserin associated with mammary tumors would mitigate the Division’s concern of clinical risk for this target tissue. If a clear, persistent increase in prolactin is not demonstrable, the Division is interested in experimental designs that

intervene in the expression or activity of prolactin as an approach to implicate prolactin in lorcaserin's tumorigenic effect on mammary tissue.

The Division is not aware of an accepted threshold of prolactin elevation necessary for drug-induced induction or promotion of rodent mammary tumors. The Division disagrees that the data with aripiprazole provides evidence that a small elevation in prolactin may be sufficient for mammary tumor induction. Contrary to the argument presented in the briefing material, Aripiprazole did not increase mammary tumors in male mice (or rats), despite the small 2-4 fold increase in serum prolactin, as shown in Figures 2 & 4 of the background document. Also contrary to the assertion in the background document, aripiprazole increased fibroadenoma but not adenocarcinoma or metastases in female Fischer rats, unlike lorcaserin that increased the incidence and aggressiveness of both mammary tumor types. The increased incidence of adenocarcinoma in female mice with aripiprazole was associated with a 200- to 500-fold increase in serum prolactin within 4 hours after a single dose, and 8 to 9-fold increases (similar to haloperidol) after 1 week of dosing. Serum prolactin and persistent diestrus were documented after 13 weeks of dosing.

Meeting Discussion:

Arena commented that upon further evaluation and consultation with subject experts, an experimental approach has been identified that allows detection of prolactin elevation in response to lorcaserin in sexually intact female rats. Arena remarked that the failure of demonstrating such prolactin elevations in the studies provided in the NDA were due to sub-optimal experimental conditions including the timing of blood collection, animal handling (stress), and the use of isoflurane anesthesia.

The FDA inquired as to why the prior mechanistic studies were capable of detecting prolactin elevations in response to dexfenfluramine and haloperidol, but not to lorcaserin, if the prior studies were confounded by sub-optimal experimental conditions. Specifically, the FDA asked if the prolactin signal with lorcaserin was undetected due to a minimal elevation compared to that induced by haloperidol and dexfenfluramine. Arena remarked that the prolactin elevation with lorcaserin in the new studies is not necessarily minimal. Arena further explained that short-term elevations in prolactin, even minor elevations, could have a significant impact on mammary tumors over a 2-year period in rodents, citing the example involving bromocriptine. The FDA commented that the 6-month toxicology study in Sprague-Dawley rats did not result in histopathological changes in mammary tissue, despite receiving lorcaserin daily for 6 months which included the 'critical window' of exposure. Arena remarked that preneoplastic changes likely occurred in that study (though undetected), with detectable changes emerging at time points beyond six months of dosing.

The FDA specifically asked [REDACTED] (b) (6) Arena's consultant on prolactin biology, if experimental approaches are available that would intervene in prolactin expression or activity as a method to more definitively implicate prolactin in the mechanism of lorcaserin's effect on mammary tissue. [REDACTED] (b) (6) remarked that the current information in the field does not provide a consensus for how such an intervention could be done experimentally.

Arena remarked that lorcaserin induced changes in the pituitary of rats which are consistent with increased prolactin activity, citing the pathology data from the interim analysis of some rats after 12 months of dosing. The FDA commented that while lorcaserin indeed increased the

incidence of pituitary hyperplasia over the 2-year dosing period, the incidence of pituitary adenoma decreased with increasing doses, and that the incidence of mammary tumors exceeded the incidence of pituitary hyperplasia, suggesting that a relationship of the changes in pituitary to mammary tumors is not clear. (b) (4) added that local tissue expression of prolactin and negative feedback of hormones on the pituitary may confound associations of mammary tumors to changes in pituitary histopathology.

The FDA commented that the relationship of elevations in prolactin to promotion of mammary tumors in rodents is not being challenged; rather, the FDA is interested in determining the mechanism whereby lorcaserin results in mammary tumors, thereby providing a basis for risk assessment. The potential of lorcaserin to increase prolactin and how such increases, if observed, compare in magnitude and duration to other pharmaceuticals known to increase prolactin and mammary tumors in rats would be pivotal to adequately address this Complete Response deficiency. Additional experimental evidence that implicates prolactin in lorcaserin-induced changes in mammary tissue using experimental approaches that intervene in prolactin expression/activity would be particularly supportive.

2. Unidentified mode of action and unclear safety margin for lorcaserin-emergent brain astrocytoma

Q3a. Does the Agency accept that toxicity in high dose male rats, as evidenced in brain by gliosis and focal mineralization and systemically by pronounced weight loss and multiple histopathologic findings, contributed to the formation of astrocytoma?

- If so, does the Agency accept this toxicity as a mechanism that could contribute to development of astrocytoma, and that the finding of astrocytomas in male rats is reasonably unlikely to represent a relevant risk to humans?

FDA Response:

No, the Division does not agree that the weight loss or non-neoplastic findings contributed to the formation of astrocytoma at the mid and high doses of lorcaserin.

Weight loss in excess of 25% has been observed for investigational obesity compounds in 2-year carcinogenicity studies without compromising survival or inducing tumors. The FDA position is misstated in your background package. The weight loss in male rats is not viewed as secondary to tumor burden; rather, the weight loss is considered secondary to the intended pharmacodynamic effect of lorcaserin (evidenced by reduced food intake and weight loss in male but not female rats).

The brain gliosis and mild focal mineralization in high-dose males was not a pathological feature of mid-dose males that had astrocytoma, nor was it present in all high-dose males that had astrocytoma. Therefore, we do not agree that these histological findings are causally related to astrocytoma induced by lorcaserin.

The other non-neoplastic pathological findings in high-dose males listed in Table 4 do not constitute evidence that the maximum tolerated dose was exceeded, nor do they suggest a probable mechanism of tumorigenic action for the astrocytoma.

Q3b. We have been able to estimate lorcaserin brain concentrations in both monkey and human with calculations based on the minimum effective dose in the species and 5-HT_{2c} functional activity as determined by receptor EC₅₀.

- Will the Agency accept estimates of human brain exposure based on such a calculation to demonstrate exposure margin in the CNS of humans?
- Does the Agency agree that the calculated brain safety margin of approximately 30 provides an acceptable safety margin?

FDA Response:

The Division requires additional information and internal discussion before providing a definitive answer regarding the proposed predictive modeling of brain levels of lorcaserin. Please provide additional information on the assumptions and calculations used in your modeling, and on other methods that support the outcome of the model described. The Division has the following preliminary comments:

Predictability of *in vitro* EC50 receptor activation data is questionable. Variable EC50 values for 5HTR activation by lorcaserin was provided in the NDA. The variability was apparently due to uncontrolled degrees of receptor expression in the *in vitro* assays. The choice of the EC50 value has a major impact on the prediction of brain drug concentrations in the proposed predictive model. To our knowledge, the monkey EC50 of 36nM comes from a study (DBR-10-007) that the Division has not reviewed. The information in the NDA cites an EC50 of 2nM for activation of monkey 5HT2c and 1.8nM for human 5HT2c (2009 studies). Given the sensitivity of the proposed model to changes in EC50, it may be appropriate to reassess EC50 for rat, monkey, and human 5HT2C activation in the same series of assays that controls receptor reserve to a physiological range of receptor density.

As stated in your background information to the Advisory Committee, “the *in vitro* EC50 at the 5HT2c receptor (192nM, rat) significantly underestimates the *in vivo* effective concentration.” Differences in receptor density were cited as one possible explanation, which was proposed to account for the lack of 5HT2A and 2B-associated effects in rodents despite high lorcaserin concentrations. Therefore, the relationship of the *in vitro* EC50 for receptor activation to effective *in vivo* drug concentrations is not clear. The Division asks that you provide further justification for the use of EC50s in the predictive model.

Pharmacological weight loss was observed in male rats but not in female rats. Please address how the proposed predictive model would work in this situation, as the minimally effective dose appears to be substantially higher in female compared to male rats. It may be instructive to compare a predicted versus an actual value of lorcaserin levels in brain tissue of female rats.

The Division has little information regarding the mechanisms by which lorcaserin partitions to the CNS. It is feasible that if mechanisms are identified, potential differences across species may inform prediction of brain partitioning in human subjects. It may also be informative to explore relationships of drug concentrations in the CSF, brain, and plasma that are potentially consistent across rats and monkeys, as a possible means to predict brain levels of lorcaserin in human subjects. The background material did not fully discuss this or other potential approaches to more directly measuring CNS levels of

lorcaserin in human subjects. Please address whether such approaches are feasible with lorcaserin.

Meeting Discussion:

Arena explained that the choice to use the EC50 values from studies conducted in 2004/5 instead of 2009 was based on the suspected overestimation of potency from the 2009 assays which reflected higher serotonin receptor density. The EC50 of 36nM for monkey 5HT2c was not submitted in the NDA, but Arena stated that these data were obtained in the 2004/5 series of assays. The FDA acknowledged that different receptor densities for this class of receptors can result in different estimates of EC50 without much change in binding affinity, as was observed with lorcaserin. Physiological receptor density should therefore be considered in a pharmacokinetic model that relies on estimates of in vitro EC50. The FDA reiterated that further internal discussion is required before accepting the proposed model, citing the issues of concern in the pre-meeting response to Arena's question. Arena remarked that they are open to further evaluating the EC50 for lorcaserin at the serotonin receptors, using a calcium response assay, over a range of receptor density expression levels, including designs that control receptor reserve by means of receptor cross-linking. The FDA agrees that further information on receptor pharmacology of lorcaserin would be helpful.

The FDA remarked that similar pharmacokinetic models are used in drug development to predict CNS penetration of drug candidates, with the predictions sometimes and sometimes not being correct from actual endpoints measured in subsequent early clinical trials of the investigational drug. The FDA inquired whether Arena has considered more direct methods of estimating brain levels of lorcaserin in human subjects, including collection of CSF samples at steady state. The FDA further noted that a consistent relationship between CSF and brain levels of lorcaserin across species (rats and monkeys), as opposed to the variable plasma:brain ratio in these species, might allow a more reliable estimation of brain levels in human subjects based on measured CSF levels of lorcaserin. Arena remarked that such an analysis would still require assumptions of a consistent relationship from rats and monkeys to human subjects, and therefore regarded this approach as providing only incremental data to the proposed pharmacokinetic modeling prediction. The FDA responded that estimating brain levels of lorcaserin based on actual (human) CSF concentrations of lorcaserin and an apparent consistent relationship of CSF:brain drug concentrations across species is preferable to relying on a variable plasma:brain relationship or a mathematical model that relies on less-than-definitive estimates of in vitro EC50s and weight loss across species.

Post-Meeting Comments

Arena inquired what the Division thought would be an appropriate study duration to demonstrate persistency of prolactin elevation in response to lorcaserin. The Division has the following recommendations for a possible study design:

- Dosing duration of no less than 3 months.
- Dose groups to include a control, a dose range of lorcaserin, and at least one relevant positive control (e.g., dexfenfluramine, mCPP, haloperidol).
- Measure serum prolactin at multiple time points (e.g., Days 1, 7, 30, 60, 90).

- Measure tissue prolactin (mammary & pituitary) at multiple time points (e.g., Day 1, 7, and 90).
- Changes in prolactin can be assessed by comparison to untreated control and to pre-study baseline prolactin in all groups.
- Include male and female rats.
- Consider monitoring other relevant endpoints (e.g., estrogens, progesterone, LH)

The Division requests that you submit protocols prior to initiating this and other mechanistic studies with lorcaserin. Comments and recommendations regarding these protocols will be provided within approximately one month of submission.

It is the Division's understanding of Arena's argument that the profile of lorcaserin-induced increases in prolactin is sufficient to activate cellular pathways in the short term that result in prolactin-dependent mammary tumors at later time points (≥ 1 year), without histological changes to mammary tissue over a 6-month dosing period. Consistent with this argument, it is expected that lorcaserin's effect on mammary tissue requires the intervening action of prolactin. In addition to providing evidence that lorcaserin increases prolactin robustly and persistently, we ask that you consider the following experimental approaches that would lend further support to this hypothesis. Please note that the Division requests explanations for why these or similar experimental strategies are either not feasible or would be unlikely to provide relevant information, as such explanations will be included in the review of the NDA resubmission. Submission of alternative mechanistic studies is encouraged.

- Consider experimental designs that demonstrate the necessity of prolactin in lorcaserin-induced changes to normal or neoplastic mammary tissue (in vivo, in vitro, or both). It is reasonable to expect that lorcaserin produces changes in mammary tissue over the short term (e.g., acute cellular signaling events, molecular changes, gene expression), before the hyperplastic/neoplastic changes are detected beyond 1 year of dosing. Identifying such lorcaserin-induced changes in mammary tissue over the short-term may allow the use of methods that intervene in prolactin expression or activity (e.g., pituitary ablation, PRLR antagonists, PRL/PRLR deficient tissues).
- Consider experimental approaches that address whether the tumorigenic effect of lorcaserin on mammary tissue resembles the tumorigenic profile proposed for prolactin, specifically that short-term exposure to elevated prolactin is sufficient to result in mammary tumors at much later time points. For example, consider a 12-month study that doses female rats for a short period (3-6 months) with lorcaserin, with one cohort remaining on drug and a second cohort withdrawn from drug for the remaining study duration, followed by histological evaluation of mammary tissue at 12 months.

The Division understands from the meeting discussion that you intend to perform further receptor transactivation studies with lorcaserin utilizing release of calcium in place of phosphoinositol as the reporter. Given the persistent imbalance in the development of FDA-defined valvulopathy in your clinical trials with patients treated with lorcaserin as compared to placebo, we strongly encourage you to consider expanding these studies to include multiple read-outs with comparison to one or more valvulopathogens with known (published) binding/activation kinetics for 5HT_{2B} (e.g., pergolide, norfenfluramine) in order to more fully

characterize lorcaserin's activity at 5HT2B. Please refer to the publication by Huang et al. (Molec Pharm. 2009; 76:710-722) which describes an approach that would provide a more robust analysis than currently available for lorcaserin.

As stated in the pre-meeting minutes, the Division will consider further the proposed pharmacokinetic model as a method to estimating brain levels of lorcaserin in human subjects. The Division remains concerned regarding the adequacy of this approach for reasons stated in the pre-meeting minutes. In addition to the comments already provided, we ask that you attempt to apply the proposed model using EC50 values for 5HT2A or B and doses that have provoked responses associated with these receptors in animals and humans (please refer to comments by CSS). We strongly encourage Arena to reconsider strategies that provide more definitive information on brain levels of lorcaserin in human subjects, such as those raised by FDA (i.e., CSF sampling, imaging with radiolabeled lorcaserin).

Clinical

1. The weight-loss efficacy of lorcaserin 10 mg twice a day relative to placebo in overweight and obese individuals without type 2 diabetes is marginal.

Q4a. Does the Agency agree that the positive efficacy results, weight loss and blood glucose control demonstrated in the BLOOM-DM trial are sufficiently robust to increase the benefit profile of lorcaserin?

FDA Response

This is a review issue.

Meeting Discussion: *No additional discussion*

Q4b. Will the Agency consider submission of the BLOOM-DM study report prior to full submission of our complete response to alleviate concerns with regards to clinical benefit-to-risk ratio?

FDA Response

The BLOOM-DM CSR can be submitted at any time; however, we intend to review it as part of the complete response to the NDA.

Meeting Discussion: *No additional discussion*

Labeling

Q5. If Arena accepts the Schedule IV recommendation of the Agency, it is our understanding of the ^{(b)(4)} that the animal studies would not have to be repeated. Is our understanding correct?

FDA Response

No, your understanding is not correct. Repeating the animal studies and submitting the data in your complete response will provide new information that will be reviewed and considered in CSS's abuse potential assessment of lorcaserin and final scheduling recommendation. CSS is available to review the protocols for the animal studies prior to their initiation.

Appendix B

Stress-induced release of prolactin: blockade by dexamethasone and naloxone may indicate beta-endorphin mediation.

J Rossier, E French, C Rivier, T Shibasaki, R Guillemin, and F E Bloom
Proc Natl Acad Sci U S A. 1980 January; 77(1): 666–669.

Basal levels of immunoreactive (ir) beta-endorphin, corticotropin (ACTH), and prolactin (PRL) in plasma of male rats decrease after dexamethasone pretreatment (400 microgram/kg at 24 hr and 200 microgram/kg at 2 hr before). Inescapable electric footshocks increase ir-beta-endorphin, ACTH, and PRL plasma levels and this effect is blocked by dexamethasone pretreatment. Morphine (20 mg/kg) also increases ir-beta-endorphin, ACTH, and PRL levels. Dexamethasone pretreatment blocks the morphine-induced release of ir-beta-endorphin but does not prevent the morphine-induced release of PRL. Naloxone, the opiate antagonist, decreases basal plasma levels of PRL and partially blocks the stress-induced increase of PRL, but it has no effect on the basal or stress-induced release of ir-beta-endorphin. These results are consistent with the proposal that beta-endorphin may interact with an opiate receptor involved in the regulation of PRL secretion.

Prolactin stimulation test with perphenazine: an evaluation of plasma prolactin levels and pituitary secretory activity in the rat.

A. A. Van Der Gugten, P. C. Sahuleka, G. H. Van Galen and H. G. Kwa
Journal of Endocrinology (1976) 68, 355-368

Many investigations of the regulation of prolactin synthesis and release are based on single plasma prolactin determinations. The purpose of the present experiment was to ascertain whether groups of rats (i.e. young or adult, male or female animals, being either intact, gonadectomized or gonadectomized and treated with oestrone), differing in age and/or endocrine status, will react to a single dose of perphenazine by an acute release of pituitary prolactin in proportion to their initial plasma prolactin levels. No consistent relation existed between the classification of the twelve groups of rats into three categories of basal plasma prolactin levels (i.e. < 20, 25–50, > 125 ng/ml) and their response to perphenazine. Even though all groups showed a highly significant increase of plasma prolactin levels the magnitude of the maximum prolactin response at 30 min varied greatly within the groups of one category and thus was not related to the initial prolactin levels.

The effect of 14 days of oestrone treatment in increasing plasma prolactin levels in gonadectomized animals was greatest in young and adult male rats, less in young females and not significant in adult females. The results obtained after perphenazine treatment in the latter group made it clear that the effect of oestrogen treatment on prolactin release can be completely blocked by increasing synthesis and/or release of the prolactin-release inhibiting factor (PIF). Since perphenazine induces decrease of pituitary prolactin and a concomitant increase of plasma prolactin levels through lowered PIF-action, the positive effect of oestrogens on prolactin release (as observed in gonadectomized male and young female rats) apparently is caused by a different

mode of action. The implications of these findings for the regulation of prolactin release, as affected by the endocrine status of the rat, is discussed.

Moreover, comparison of prolactin lost from the pituitary and gained in the circulation of the experimental animals, with amounts of prolactin that were observed to disappear from plasma during the experiment, provided suggestive evidence that the capacity to synthesize and/or eliminate prolactin, after a sudden provoked release of the hormone, differed among the groups. The rates of synthesis by the pituitary, of release from the pituitary into the circulation as well as of elimination of the hormone from the circulation (equally involved in determining actual plasma levels) are thought, therefore, to be far more important for the elucidation of prolactin regulation than single plasma prolactin determinations.

Acute Changes in Blood and Pituitary Prolactin After a Single Injection of Perphenazine

M. Ben-David, A. Danon, F.G. Sulman
Neuroendocrinology 1970;6:336-342

The acute effects of a single injection of perphenazine on pituitary and blood prolactin levels were studied in male rats and rabbits. Prolactin from both sources was determined by bioassay. A significant rise in blood prolactin became visible as early as 0.5 h after injection of perphenazine – earlier times were not studied – and remained high for at least 4 h. The prolactin peak following intravenous injection in rabbits was attained after 1 h, while in rats injected sub-cutaneously, it was reached after 2 h. Concomitantly, pituitary prolactin in rats decreased, falling to a minimum level within 2 h, and remaining low for at least 4 h. These results are interpreted as further evidence that perphenazine induces immediate release of prolactin from the pituitary into the blood. This release is probably due to suppression of the hypothalamic prolactin-inhibiting factor. The possibility that perphenazine promotes prolactin synthesis is also discussed.

Effect of raised serum prolactin on breast development.

B M Stringer, J Rowson, and E D Williams
J Anat. 1989 February; 162: 249–261.

The effect of serum prolactin elevation on the growth and development of the rat breast was investigated. Oral administration of the dopamine antagonist, perphenazine, led to a 5-10-fold elevation of serum prolactin after two days of treatment which was maintained for the 54 days of study. A significant (P less than 0.01) 3.4-fold increase in total breast volume was seen by Day 4 of serum prolactin elevation. Breast volume continued to rise up to Day 14 reaching an 8.9-fold peak (P less than 0.001) which was maintained for the duration of the experiment. Epithelial, myoepithelial, lumen and stromal volume changes in the ductular and alveolar compartments were quantified separately. Highly significant (P less than 0.01) volume increases were seen in all components within the first few days of prolactin elevation. Similar time courses of the growth response to elevated serum prolactin were seen in the ductal tissues reaching an approximate 3-fold peak by 7 days in duct epithelium, myoepithelium and duct stroma. Time coordinated growth responses were also seen in the alveolar tissues with larger (7-15-fold) increases in alveolar epithelium, alveolar myoepithelium and alveolar stroma, reaching a peak by 14 days.

Prolactin and the small intestine. Effect of hyperprolactinaemia on mucosal structure in the rat.

E Muller and R H Dowling

Gut. 1981 July; 22(7): 558–565.

To study the mechanism for the adaptive mucosal hyperplasia which occurs independent of luminal nutrition and pancreatico-biliary secretions in isolated Thiry-Vella segments of intestine from lactating rats, and to examine the effects of prolactin on small bowel mucosal structure in the rat, we used two models of experimental hyperprolactinaemia and compared quantitative histology and several markers of mucosal mass in jejunum and ileum from control rats and from test and lactating animals. Hyperprolactinaemia, induced by perphenazine injections (5 mg/kg/day for two or seven weeks) or transplantation of four pituitary glands from donor animals to beneath the renal capsule in the recipient, was confirmed by radioimmunoassay. Proof of its biological activity was obtained by weighing the mammary pads and by demonstrating true breast hyperplasia on histological section. Median serum prolactin levels increased from 50 ng/ml in the controls to 570 ng/ml in the perphenazine treated animals and to 600 ng/ml in the pituitary transplanted rats—levels comparable with those seen in lactation (870 ng/ml). In the lactating rats, there was striking mucosal hyperplasia of both jejunum and ileum but, despite the hyperprolactinaemia, there were no such changes in villus height, crypt depth, or in mucosal wet weight, protein, or DNA/unit length intestine in the perphenazine-injected or pituitary-transplanted animals. We conclude that prolactin is not atrophic to the intestine in rats and that hyperprolactinaemia cannot explain the intestinal adaptive changes of lactation.

A prolactin-dependent, metastasising rat mammary carcinoma as a model for endocrine-related tumour dormancy

JH Wijsman¹, CJ Cornelisse, R Keijzer, CJ van de Velde and JH van Dierendonck

British Journal of Cancer (1991) 64, 463–468.

In order to study the growth kinetics of breast tumours during long-term hormonal withdrawal, we developed a transplantable, invasive mammary carcinoma EMR-86 that originated in a female WAG/Olac rat bearing a subcutaneously implanted oestrogen pellet (EP). Outgrowth of transplanted tumours occurs only in the presence of an EP, and metastases are formed in lungs and regional lymph nodes. Subsequent EP removal induces rapid regression. However, tumours do not disappear completely, as small nodules persist. These dormant tumour remnants can be restimulated even after long periods. Because EP-stimulated tumours regressed after treatment with bromocriptine (5 mg/kg) and dormant tumours in non-oestrogenized rats grew out after treatment with perphenazine (5 mg/kg), prolactin is the major growth-stimulating hormone in this model. Cell kinetics in the growing, regressing and dormant phase were studied by immunocytochemical detection of DNA-incorporated bromodeoxyuridine (BrdUrd) in tissue sections. BrdUrd labelling indices decreased from 21.6 ± 3.0% to less than 1% within 7 days after EP removal. After prolonged hormonal withdrawal (up to 90 days) BrdUrd-labelled tumour cells could always be demonstrated (range 0.4–0.8%), without a concomitant increase in tumour volume. Additional treatment either with bromocriptine

or with ovariectomy could not significantly reduce this residual proliferative activity, as demonstrated by continuous BrdUrd labelling experiments. The results indicate that in vivo dormancy may represent a steady state of cell division and cell loss, rather than an accumulation of cells in a non-cycling G0 state.

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/s/

FRED K ALAVI

03/22/2011

External comments to the sponsor regarding the nonclinical prolactin mechanistic study protocols

TODD M BOURCIER

03/22/2011

Division review of prolactin protocols

MEMO TO THE FILE

Fred Alavi, Ph.D.
Oct 25, 2010

NDA: 22-529 (IND 69,888)
Drug: Lorcaserin (LORQESS®)
Class & Mechanism: serotonin receptor 2C (5HT_{2C}) agonist
Indication: Weigh loss
Sponsor: Arena Pharmaceuticals

Attached to this memo are the tabulated histopathology data for the lorcaserin mouse (Appendix A) and rat (Appendix B) carcinogenicity studies. The memo also includes the (b) (4) historical control data for SD rats between 12/26/2002 and 1/15/2007 (Appendix C). Both rat and mouse carcinogenicity studies were reviewed with the lorcaserin NDA but due to large number of pages, only summary of the histopathology findings were included in the NDA review.

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(b) (4) Historical control Neoplastic Data in Male SD Rats	page 73
(b) (4) Historical control Neoplastic Data in female SD Rats	page 88

Appendix A

Tabulated histopath findings in male mice treated with lorcaserin

Comprehensive list of tissue histopathology findings in mice died before or at the scheduled necropsy at week 105. Lorcaserin dose was reduced to 5, 25 and 50 mg/kg/d on Day 19 following significant mortality at original top dose of 100 mg/kg/d.

(b) (4) Study Number 900-062
A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
adipose tissue		(13)	(0)	(4)	(0)	(12)	(0)	(6)	(0)
atrophy		13	0	4	0	12	0	6	0
	- minimal	0	0	2	0	5	0	1	0
	- mild	13	0	2	0	7	0	5	0
adipose tissue, brown, perirenal		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
adrenal glands		(44)	(31)	(39)	(24)	(46)	(18)	(48)	(26)
adenoma, subcapsular cell, benign, primary		0	3	2	3	0	1	1	2
amyloid		11	2	11	2	14	0	10	1
	- minimal	2	2	0	2	3	0	4	1
	- mild	8	0	8	0	8	0	4	0
	- moderate	1	0	2	0	3	0	2	0
	- severe	0	0	1	0	0	0	0	0
angiectasis/cystic degeneration, focal cortical	- minimal	1	0	0	0	2	0	0	1
bacterial colonies	- minimal	2	0	0	0	0	0	0	0
fibrosarcoma, malignant, secondary		0	0	0	0	1	0	0	0
hematopoiesis, extramedullary	- minimal	0	0	0	0	1	0	0	0
hyperplasia, focal cortical		2	8	0	7	2	5	0	3
	- minimal	0	3	0	6	0	1	0	0
	- mild	1	4	0	1	1	2	0	2
	- moderate	1	1	0	0	1	1	0	1
	- severe	0	0	0	0	0	1	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
adrenal glands		(44)	(31)	(39)	(24)	(46)	(18)	(48)	(26)
hyperplasia, focal medullary		0	0	0	2	0	1	1	0
	- minimal	0	0	0	1	0	0	0	0
	- mild	0	0	0	1	0	1	1	0
hyperplasia, subcapsular cell		19	14	16	17	22	13	19	14
	- minimal	9	6	4	6	7	2	11	3
	- mild	9	3	10	8	13	7	8	6
	- moderate	1	4	1	3	2	4	0	5
	- severe	0	1	1	0	0	0	0	0
hypertrophy, diffuse		0	0	1	0	0	1	0	0
	- mild	0	0	0	0	0	1	0	0
	- moderate	0	0	1	0	0	0	0	0
hypertrophy, focal cortical		0	7	1	11	3	5	1	7
	- minimal	0	2	1	4	1	2	1	5
	- mild	0	4	0	4	1	2	0	1
	- moderate	0	1	0	3	1	1	0	1
infiltration, lymphocytic		0	2	0	1	3	0	1	1
	- minimal	0	2	0	0	3	0	1	1
	- mild	0	0	0	1	0	0	0	0
infiltration, mixed leukocyte	- minimal	0	0	0	0	0	0	1	0
inflammation, acute	- mild	0	0	0	0	0	0	1	0
inflammation, embolic	- minimal	0	0	0	0	1	0	0	0
inflammation, peritoneal	- mild	0	0	1	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
adrenal glands		(44)	(31)	(39)	(24)	(46)	(18)	(48)	(26)
lymphoma, malignant, multicentric		3	0	1	0	2	0	2	0
mineralization	- mild	0	0	0	0	1	0	0	0
pheochromocytoma, benign, primary		0	0	0	0	1	0	0	0
thrombus		0	0	0	1	0	1	0	0
	- minimal	0	0	0	1	0	0	0	0
	- mild	0	0	0	0	0	1	0	0
within normal limits		12	6	14	3	15	2	20	5
aorta		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
infiltration, lymphocytic	- minimal	0	0	0	0	1	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	1	0	2	0	1	0
mesothelioma, malignant, secondary		0	0	0	0	1	0	0	0
within normal limits		42	31	40	24	43	18	47	27
bone marrow, femur		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
atrophy	- mild	1	0	0	0	1	0	0	0
depletion, mixed	- minimal	0	0	0	0	2	0	0	0
hemangiosarcoma, malignant, multicentric		0	1	0	0	1	0	0	0
Number of Animals Examined		44	31	41	24	47	18	48	27
bone marrow, femur		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
hyperplasia, granulocytic		29	25	18	21	13	10	16	21
	- minimal	6	9	1	5	0	0	5	5
	- mild	10	9	5	13	5	6	3	14
	- moderate	9	6	8	3	5	4	5	2
	- severe	4	1	4	0	3	0	3	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	0	0
macrophages, pigmented	- minimal	2	5	0	3	4	0	3	2
within normal limits		12	6	22	3	28	8	31	6
bone marrow, sternum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
atrophy	- minimal	0	0	1	0	0	0	0	1
depletion, mixed	- minimal	0	0	0	0	3	0	0	0
hyperplasia, granulocytic		31	21	19	21	14	10	14	21
	- minimal	8	9	3	4	1	1	1	3
	- mild	6	8	3	14	4	7	4	13
	- moderate	13	3	9	3	5	2	6	5
	- severe	4	1	4	0	4	0	3	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	0	0
macrophages, pigmented	- minimal	4	7	2	6	4	2	3	6
necrosis	- mild	0	0	1	0	0	0	0	0
thrombus	- mild	0	0	0	0	0	0	1	0
bone, sternum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
within normal limits		29	15	31	15	40	14	36	17
brain		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
astrocytoma, malignant, primary		1	0	1	0	0	0	0	0
bacterial colonies		3	0	0	0	0	0	0	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	2	0	0	0	0	0	0	0
gliosis, reactive	- minimal	1	0	0	0	0	0	0	0
hemorrhage	- minimal	1	0	0	0	0	0	1	0
inflammation, embolic		1	0	2	0	1	0	1	0
	- minimal	1	0	0	0	1	0	1	0
	- mild	0	0	2	0	0	0	0	0
inflammation, subacute	- mild	1	0	0	0	0	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
leukocytosis, vascular	- minimal	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	0	0
meningioma, benign, primary		0	0	0	0	1	0	0	0
mineralization, focal	- minimal	9	9	2	0	2	1	2	1
necrosis, focal	- minimal	0	1	0	0	0	0	0	0
polyarteritis	- minimal	0	1	0	0	0	0	0	0
within normal limits		30	21	37	24	41	17	44	26

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
cavity, abdominal		(0)	(0)	(0)	(0)	(0)	(1)	(1)	(0)
hemangiosarcoma, malignant, multicentric		0	0	0	0	0	0	1	0
necrosis, fat	- severe	0	0	0	0	0	1	0	0
cavity, cranial		(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
within normal limits		0	0	0	1	0	0	0	0
cavity, thoracic		(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
mesothelioma, malignant, primary		0	0	0	0	1	0	0	0
coagulating glands		(44)	(31)	(40)	(24)	(46)	(18)	(48)	(26)
adenoma, benign, primary		0	0	0	0	0	0	0	1
depletion, secretory		1	0	0	0	0	0	1	0
	- mild	1	0	0	0	0	0	0	0
	- moderate	0	0	0	0	0	0	1	0
infiltration, lymphocytic		2	2	1	5	2	1	1	0
	- minimal	2	1	1	4	2	1	1	0
	- mild	0	1	0	1	0	0	0	0
inflammation, acute		0	0	0	0	1	0	1	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	1	0	0	0
inflammation, chronic		0	0	1	0	0	0	1	0
inflammation, chronic-active		1	0	3	0	1	0	0	0
coagulating glands		(44)	(31)	(40)	(24)	(46)	(18)	(48)	(26)
inflammation, subacute		3	0	5	0	4	0	7	1
	- minimal	1	0	1	0	2	0	5	0
	- mild	2	0	3	0	2	0	1	1
	- moderate	0	0	1	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	1	1	0	0	2	0
secretory product, increased		2	3	2	5	3	2	0	3
	- minimal	0	0	0	0	0	1	0	0
	- mild	1	2	1	4	3	0	0	1
	- moderate	1	1	1	1	0	1	0	2
within normal limits		35	27	27	16	35	15	37	21
ears		(0)	(2)	(0)	(0)	(0)	(0)	(1)	(0)
erosion/ulcer		0	2	0	0	0	0	1	0
	- mild	0	1	0	0	0	0	0	0
	- moderate	0	1	0	0	0	0	1	0
epididymides		(44)	(31)	(40)	(24)	(47)	(18)	(48)	(27)
atrophy	- minimal	1	0	0	0	0	0	0	0
granuloma, spermatic		0	3	0	0	0	0	2	0
	- minimal	0	1	0	0	0	0	0	0
	- mild	0	2	0	0	0	0	0	0
	- moderate	0	0	0	0	0	0	2	0
epididymides		(44)	(31)	(40)	(24)	(47)	(18)	(48)	(27)
infiltration, lymphocytic		6	7	5	3	2	2	4	4
	- minimal	5	6	5	3	2	2	3	3
	- mild	1	1	0	0	0	0	1	1
inflammation, chronic	- mild	0	1	0	1	0	0	0	0
inflammation, subacute	- minimal	1	0	0	0	0	0	0	0
luminal cellular debris, bilateral		11	6	11	6	15	6	7	7
	- minimal	7	5	4	0	12	6	3	4
	- mild	3	1	7	6	3	0	4	3
	- moderate	1	0	0	0	0	0	0	0
luminal cellular debris, unilateral		2	2	0	3	3	0	2	2
	- minimal	1	2	0	2	2	0	2	1
	- mild	1	0	0	1	1	0	0	1
lymphoma, malignant, multicentric		1	0	0	0	1	0	2	0
mineralization	- minimal	0	0	0	1	1	0	0	0
oligospermia/germ cell debris, bilateral		4	4	3	2	6	2	4	2
	- minimal	0	0	0	0	0	1	0	0
	- mild	2	1	0	0	0	0	0	0
	- moderate	0	2	0	1	0	0	2	0
	- severe	2	1	3	1	6	1	2	2
oligospermia/germ cell debris, unilateral		2	3	1	4	3	2	1	3
	- moderate	1	1	0	1	2	1	1	0
	- severe	1	2	1	3	1	1	0	3
sarcoma, undifferentiated, malignant, primary		0	2	0	0	0	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal		25/5		50/25		100/50	
		0		mg/kg/day		mg/kg/day		mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
epididymides		(44)	(31)	(40)	(24)	(47)	(18)	(48)	(27)
spermatocele		0	1	1	0	2	0	0	0
- minimal		0	0	1	0	0	0	0	0
- mild		0	1	0	0	2	0	0	0
vacuolation	- minimal	0	0	0	0	0	0	1	0
within normal limits		22	10	22	7	19	8	26	12
esophagus		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
degeneration, myofiber	- minimal	0	2	1	0	0	0	0	0
dilatation	- mild	0	1	0	0	0	0	0	0
hemorrhage	- moderate	0	0	1	0	0	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	1	0
within normal limits		43	28	39	24	46	18	47	27
eyes		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
cataract		10	0	8	2	11	2	11	0
- minimal		4	0	8	1	9	0	8	0
- mild		6	0	0	1	2	1	3	0
- severe		0	0	0	0	0	1	0	0
degeneration/atrophy, retina, bilateral		0	0	0	0	1	1	0	2
- minimal		0	0	0	0	0	0	0	1
- mild		0	0	0	0	1	0	0	1
- severe		0	0	0	0	0	1	0	0
eyes		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
degeneration/atrophy, retina, unilateral		2	4	0	1	0	2	1	1
- minimal		1	2	0	0	0	2	0	1
- mild		1	2	0	0	0	0	0	0
- severe		0	0	0	1	0	0	1	0
erosion/ulcer, corneal	- minimal	0	0	0	0	1	0	0	0
fold/rosette, retinal	- minimal	0	0	0	0	1	0	0	0
infiltration, neutrophil	- mild	0	0	1	0	0	0	0	0
inflammation, acute		0	1	0	0	1	0	0	1
- minimal		0	1	0	0	1	0	0	0
- mild		0	0	0	0	0	0	0	1
keratopathy		6	1	3	3	2	5	5	3
- minimal		3	1	3	3	1	2	4	3
- mild		3	0	0	0	1	2	1	0
- moderate		0	0	0	0	0	1	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
phthisis bulbi		0	0	1	0	1	0	0	0
- mild		0	0	1	0	0	0	0	0
- severe		0	0	0	0	1	0	0	0
within normal limits		27	25	29	20	30	10	35	20
eyes, optic nerves		(41)	(31)	(39)	(24)	(45)	(18)	(45)	(27)
degeneration, axonal/myelin	- mild	0	0	0	0	0	0	0	1
inflammation, subacute	- mild	0	0	1	0	0	0	0	0
gallbladder		(44)	(29)	(41)	(24)	(47)	(17)	(48)	(27)
hyperplasia, glandular cystic		0	1	0	1	0	0	1	1
- minimal		0	0	0	0	0	0	0	1
- mild		0	1	0	1	0	0	1	0
infiltration, lymphocytic		3	3	0	2	0	2	5	1
- minimal		2	2	0	2	0	2	2	1
- mild		1	1	0	0	0	0	2	0
- moderate		0	0	0	0	0	0	1	0
inflammation, subacute	- mild	0	1	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	1	0	0	0
polyarteritis		2	0	0	0	0	1	1	0
- minimal		1	0	0	0	0	0	0	0
- mild		1	0	0	0	0	0	1	0
- moderate		0	0	0	0	0	1	0	0
within normal limits		39	24	41	21	46	14	41	25
harderian glands		(44)	(31)	(40)	(24)	(47)	(18)	(48)	(27)
adenocarcinoma, malignant, primary		0	0	0	0	0	1	0	0
adenoma, benign, primary		2	1	1	2	0	1	2	2
atrophy		1	1	0	3	2	0	1	0
- minimal		1	1	0	1	1	0	1	0
- mild		0	0	0	2	1	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal		25/5		50/25		100/50	
		mg/kg/day DOS	SNC	mg/kg/day DOS	SNC	mg/kg/day DOS	SNC	mg/kg/day DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
harderian glands		(44)	(31)	(40)	(24)	(47)	(18)	(48)	(27)
hyperplasia, focal		1	8	0	4	2	3	0	3
- minimal		0	0	0	0	1	0	0	1
- mild		1	4	0	2	1	0	0	0
- moderate		0	1	0	1	0	3	0	2
- severe		0	3	0	1	0	0	0	0
infiltration, lymphocytic		7	19	5	9	6	11	16	10
- minimal		7	18	5	9	6	11	15	10
- mild		0	1	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
pigment, porphyrin		27	17	21	17	24	14	29	21
- minimal		14	10	13	13	12	9	15	14
- mild		8	5	8	3	9	5	12	6
- moderate		4	2	0	1	3	0	2	1
- severe		1	0	0	0	0	0	0	0
within normal limits		12	7	17	2	20	4	13	3
heart		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		8	0	3	0	9	0	8	0
- minimal		6	0	2	0	7	0	5	0
- mild		2	0	1	0	2	0	3	0
bacterial colonies		2	0	2	0	0	0	1	0
- mild		2	0	1	0	0	0	1	0
- moderate		0	0	1	0	0	0	0	0
heart		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
carcinoma, bronchiolar alveolar, malignant, secondary		0	0	0	0	1	0	0	0
cardiomyopathy		18	21	11	7	10	12	15	7
- minimal		17	19	9	6	8	8	12	7
- mild		1	2	2	1	2	4	3	0
endocarditis, valvular vegetative		0	0	2	0	0	0	1	0
- mild		0	0	0	0	0	0	1	0
- moderate		0	0	2	0	0	0	0	0
exudate		0	0	1	0	0	0	1	0
- minimal		0	0	1	0	0	0	1	0
fibrous histiocytoma, malignant, secondary		1	0	0	0	0	0	0	0
hemorrhage		0	0	0	0	0	0	1	0
- minimal		0	0	0	0	0	0	1	0
hypertrophy/hyperplasia, mesothelial cell		2	0	1	1	1	0	2	0
infiltration, lymphocytic		1	0	1	0	0	0	0	0
- minimal		1	0	1	0	0	0	0	0
inflammation, acute		0	0	1	0	0	0	0	0
- minimal		0	0	1	0	0	0	0	0
inflammation, chronic		1	1	0	0	0	0	0	0
- mild		1	1	0	0	0	0	0	0
inflammation, embolic		1	0	2	0	0	0	1	0
- mild		1	0	1	0	0	0	0	0
- moderate		0	0	1	0	0	0	1	0
inflammation, subacute		1	0	4	0	3	0	1	1
- minimal		1	0	1	0	2	0	1	1
- mild		0	0	3	0	0	0	0	0
- severe		0	0	0	0	1	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
heart		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
leukocytosis, vascular	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		3	0	1	0	2	0	2	0
mesothelioma, malignant, secondary		0	0	0	0	1	0	0	0
mineralization, myofiber		0	0	4	0	0	0	1	0
- minimal		0	0	3	0	0	0	0	0
- mild		0	0	1	0	0	0	1	0
mineralization, vascular	- minimal	0	0	1	0	0	0	0	0
necrosis	- mild	1	0	0	0	0	0	0	0
polyarteritis		5	1	1	3	0	2	1	2
- minimal		1	1	0	3	0	2	1	1
- mild		3	0	1	0	0	0	0	1
- moderate		1	0	0	0	0	0	0	0
thrombus		6	0	1	3	2	0	2	0
- minimal		0	0	0	1	0	0	0	0
- mild		2	0	0	0	1	0	0	0
- moderate		4	0	1	2	1	0	2	0
within normal limits		10	9	21	14	24	5	18	18
joint, tibiofemoral		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
bacterial colonies	- mild	0	0	0	0	0	0	1	0
hyperplasia, cartilage	- mild	0	0	0	0	0	0	1	0
hyperplasia/hypertrophy, synovial	- minimal	0	0	0	0	0	0	1	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
joint, tibiofemoral		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
inflammation, chronic-active		0	0	2	0	0	0	1	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	0	0	1	0	0	0	1	0
within normal limits		44	31	39	24	47	18	45	27
kidneys		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
adenoma, tubular cell, benign, primary amyloid		0	0	0	0	1	0	0	0
	- minimal	14	4	12	2	15	0	9	3
	- mild	2	2	4	0	2	0	2	0
	- moderate	8	2	7	2	5	0	5	3
	- moderate	4	0	1	0	8	0	2	0
bacterial colonies		2	0	3	0	2	0	1	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	2	0	1	0	1	0	1	0
	- moderate	0	0	1	0	1	0	0	0
cyst		13	18	7	11	7	11	6	15
	- minimal	9	11	4	4	3	5	4	8
	- mild	4	3	3	7	4	6	2	7
	- moderate	0	4	0	0	0	0	0	0
dilatation, tubular		1	0	1	0	0	0	2	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	1	0	0	0	0	0	2	0
fibrosarcoma, malignant, secondary hematopoiesis, extramedullary, increased	- mild	0	0	0	0	1	0	0	0
	- mild	0	0	0	0	0	0	1	0
kidneys		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
hydronephrosis, bilateral		4	1	4	1	2	0	2	1
	- minimal	1	1	0	0	0	0	0	1
	- mild	3	0	2	1	1	0	1	0
	- moderate	0	0	1	0	1	0	1	0
	- severe	0	0	1	0	0	0	0	0
hydronephrosis, unilateral		1	2	1	1	1	2	1	0
	- minimal	0	0	0	1	0	1	1	0
	- mild	1	1	1	0	1	1	0	0
	- moderate	0	1	0	0	0	0	0	0
infarct		0	0	0	0	0	0	0	2
	- minimal	0	0	0	0	0	0	0	1
	- mild	0	0	0	0	0	0	0	1
infiltration, lymphocytic		11	1	13	3	17	2	17	1
	- minimal	7	1	10	2	14	2	12	1
	- mild	4	0	3	1	3	0	5	0
inflammation, embolic		0	0	1	0	1	0	0	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	0	0	0	0	1	0	0	0
leukemia, malignant, multicentric lymphoma, malignant, multicentric metaplasia, cartilaginous/osseous metaplasia, osseous		1	0	0	0	0	0	0	0
	- minimal	2	0	2	1	3	1	2	0
	- minimal	0	1	0	0	0	0	0	0
	- minimal	0	0	0	0	0	0	0	1
kidneys		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
mineralization, tubular		10	23	8	17	12	11	15	19
	- minimal	8	23	8	14	9	8	14	18
	- mild	2	0	0	3	3	3	1	1
necrosis, papillary		3	0	3	0	1	0	1	0
	- minimal	2	0	0	0	0	0	0	0
	- mild	1	0	2	0	0	0	0	0
	- moderate	0	0	1	0	1	0	1	0
nephropathy, chronic progressive		33	30	23	22	21	17	26	27
	- minimal	22	15	14	11	9	11	20	22
	- mild	5	15	6	10	8	6	3	4
	- moderate	5	0	3	1	4	0	3	1
	- severe	1	0	0	0	0	0	0	0
polyarteritis		4	5	1	6	3	4	3	2
	- minimal	3	4	1	5	2	2	1	1
	- mild	1	1	0	1	1	1	2	1
	- moderate	0	0	0	0	0	1	0	0
pyelitis		0	0	0	0	2	0	1	0
	- minimal	0	0	0	0	1	0	0	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	0	0	0	0	1	0
pyelonephritis, unilateral		1	0	2	1	1	0	1	0
	- mild	1	0	0	0	1	0	0	0
	- moderate	0	0	0	0	0	0	1	0
	- severe	0	0	2	1	0	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
kidneys		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
thrombus	- mild	0	0	1	0	0	0	0	0
within normal limits		2	0	4	0	6	0	3	0
lacrimial glands, exorbital		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(26)
amyloid		9	2	7	2	8	0	11	3
- minimal		5	1	2	2	3	0	3	1
- mild		4	1	5	0	4	0	7	0
- moderate		0	0	0	0	1	0	1	2
atrophy		5	3	1	4	2	1	2	7
- minimal		3	1	0	4	0	0	1	4
- mild		1	1	1	0	1	1	0	3
- moderate		1	0	0	0	1	0	1	0
- severe		0	1	0	0	0	0	0	0
fibrous histiocytoma, malignant, secondary		0	0	0	0	0	0	1	0
hyperplasia, stromal	- mild	1	0	0	0	0	0	0	0
infiltration, lymphocytic		32	29	16	21	23	15	30	23
- minimal		23	20	10	10	15	7	19	11
- mild		9	9	6	11	8	7	10	9
- moderate		0	0	0	0	0	1	1	3
infiltration, mononuclear cell	- mild	0	0	1	0	0	0	0	0
inflammation, chronic		0	2	0	2	0	0	0	1
- minimal		0	0	0	2	0	0	0	0
- mild		0	1	0	0	0	0	0	1
- moderate		0	1	0	0	0	0	0	0
lacrimial glands, exorbital		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(26)
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	1	0
macrophages, pigmented		1	0	0	0	1	0	0	0
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	0	0	1	0	0	0
metaplasia, harderian		0	0	0	1	1	0	0	1
- mild		0	0	0	0	1	0	0	0
- moderate		0	0	0	1	0	0	0	1
mineralization	- mild	0	1	0	0	0	0	0	1
within normal limits		7	2	19	2	17	3	14	1
large intestine, cecum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
dilatation, gland/lumen	- mild	0	0	0	0	0	0	2	0
inflammation, embolic	- minimal	0	0	0	0	1	0	0	0
inflammation, peritoneal	- minimal	1	0	0	0	0	0	0	0
inflammation, subacute	- minimal	0	1	0	0	0	0	0	0
within normal limits		43	30	41	24	46	18	46	27
large intestine, colon		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
lymphoma, malignant, multicentric		0	0	1	0	1	0	0	0
polyarteritis	- minimal	0	0	0	0	1	0	0	0
within normal limits		44	31	40	24	45	18	48	27
large intestine, rectum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
lymphoma, malignant, multicentric		0	0	0	0	1	0	0	0
polyarteritis	- minimal	0	0	0	0	0	0	1	0
within normal limits		44	31	41	24	46	18	47	27
liver		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
adenoma, hepatocellular, benign, primary		3	1	2	9	1	3	1	2
adhesion/inflammation/fibrosis, capsule	- minimal	0	0	0	0	1	0	0	0
amyloid		5	1	5	1	12	0	8	0
- minimal		3	1	1	1	6	0	8	0
- mild		1	0	3	0	4	0	0	0
- moderate		1	0	1	0	2	0	0	0
carcinoma, hepatocellular, malignant, primary		0	1	1	2	3	0	2	2
cyst, biliary		0	0	0	1	0	1	0	0
- minimal		0	0	0	1	0	0	0	0
- severe		0	0	0	0	0	1	0	0
degeneration, cystic, focal	- minimal	0	0	0	0	0	0	1	0
degeneration, hepatocellular	- minimal	0	0	0	0	0	0	0	1
focus of cellular alteration, basophilic		1	1	1	3	1	0	0	1
- minimal		0	0	0	0	0	0	0	1
- mild		1	1	1	2	0	0	0	0
- moderate		0	0	0	0	1	0	0	0
- severe		0	0	0	1	0	0	0	0
focus of cellular alteration, eosinophilic	- minimal	0	0	0	0	0	0	0	1

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal									
		0		25/5		50/25		100/50			
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC		
Number of Animals Examined		44	31	41	24	47	18	48	27		
liver		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
hemangiosarcoma, malignant, multicentric		1	0	1	0	0	0	0	0		
hematopoiesis, extramedullary		4	3	3	1	7	1	3	1		
	- minimal	3	3	1	0	4	1	1	1		
	- mild	0	0	1	1	2	0	0	0		
	- moderate	1	0	1	0	1	0	1	0		
	- severe	0	0	0	0	0	0	1	0		
hyperplasia, oval cell	- mild	0	0	1	0	0	0	0	0		
hypertrophy, hepatocyte, centrilobular	- minimal	0	0	0	0	0	0	0	1		
hypertrophy, hepatocyte, panlobular	- minimal	0	1	0	0	0	0	0	0		
hypertrophy/hyperplasia, kupffer cell		3	1	6	1	5	0	6	0		
	- minimal	2	0	6	1	3	0	4	0		
	- mild	1	1	0	0	2	0	2	0		
infiltration, mixed leukocyte		0	0	0	0	1	0	2	0		
	- minimal	0	0	0	0	1	0	1	0		
	- mild	0	0	0	0	0	0	1	0		
infiltration, mononuclear cell		23	27	22	21	25	17	22	19		
	- minimal	21	22	17	15	23	11	18	14		
	- mild	2	5	5	6	2	6	4	5		
inflammation, chronic	- minimal	1	0	0	0	0	0	0	0		
inflammation, embolic	- minimal	1	0	0	0	0	0	0	0		
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0		
leukocytosis, sinusoidal	- mild	0	0	0	0	1	0	0	0		
lymphoma, malignant, multicentric		3	0	2	0	2	0	1	1		
liver		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
mitotic figures, increased		0	1	0	1	0	1	0	1		
	- minimal	0	1	0	0	0	1	0	1		
	- mild	0	0	0	1	0	0	0	0		
necrosis	- severe	1	0	0	0	0	0	1	0		
necrosis, focal		2	1	4	2	2	1	3	1		
	- minimal	2	1	2	1	2	1	3	1		
	- mild	0	0	2	0	0	0	0	0		
	- severe	0	0	0	1	0	0	0	0		
necrosis, hepatocytes, centrilobular		3	0	2	0	3	0	0	0		
	- minimal	0	0	1	0	3	0	0	0		
	- mild	3	0	1	0	0	0	0	0		
necrosis, individual hepatocyte		0	0	2	0	3	0	2	1		
	- minimal	0	0	2	0	2	0	2	1		
	- mild	0	0	0	0	1	0	0	0		
pigment, increased kupffer cell		1	1	0	0	1	0	1	0		
	- minimal	0	1	0	0	1	0	1	0		
	- mild	1	0	0	0	0	0	0	0		
polyarteritis	- minimal	0	0	0	0	0	0	1	0		
vacuolation, diffuse	- minimal	0	0	1	0	0	0	0	0		
vacuolation, hepatocellular	- minimal	0	1	0	0	0	0	0	0		
within normal limits		11	2	12	1	8	0	14	4		
lung		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
adenoma, bronchiolar alveolar, benign, primary		3	5	4	6	3	1	1	7		
bacterial colonies		0	0	2	0	1	0	0	0		
	- minimal	0	0	1	0	1	0	0	0		
	- mild	0	0	1	0	0	0	0	0		
carcinoma, bronchiolar alveolar, malignant, primary		5	3	2	2	2	0	1	0		
carcinoma, hepatocellular, malignant, secondary		0	0	1	0	0	0	0	0		
edema	- mild	1	0	0	0	0	0	0	0		
exudate		1	0	1	1	1	0	0	0		
	- mild	1	0	0	1	0	0	0	0		
	- moderate	0	0	1	0	1	0	0	0		
fibrosarcoma, malignant, secondary		0	0	0	0	1	0	0	0		
fibrosis		2	0	0	0	1	0	0	0		
	- minimal	1	0	0	0	0	0	0	0		
	- mild	1	0	0	0	1	0	0	0		
fibrous histiocytoma, malignant, secondary		1	0	0	0	0	0	1	0		
hemorrhage		7	0	5	0	0	0	4	0		
	- minimal	4	0	4	0	0	0	2	0		
	- mild	3	0	1	0	0	0	2	0		
histiocytosis, alveolar		16	13	9	12	10	8	7	9		
	- minimal	7	7	4	5	4	6	3	8		
	- mild	5	5	3	4	4	2	2	1		
	- moderate	4	1	2	3	1	0	2	0		
	- severe	0	0	0	0	1	0	0	0		

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
lung		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
hyperplasia, bronchiolar-alveolar		0	7	2	7	5	3	3	1
- minimal		0	2	0	4	1	2	2	0
- mild		0	4	1	2	4	1	0	0
- moderate		0	1	1	1	0	0	0	1
- severe		0	0	0	0	0	0	1	0
hyperplasia, lymphoid		0	0	0	0	0	1	0	0
hyperplasia, type II cell	- minimal	1	0	0	0	0	0	0	0
hypertrophy/hyperplasia, mesothelial cell	- minimal	0	0	0	0	1	0	0	0
infiltration, lymphocytic		0	1	0	0	0	0	0	2
- minimal		0	0	0	0	0	0	0	2
- mild		0	1	0	0	0	0	0	0
infiltration, mixed leukocyte		1	0	1	1	4	0	3	0
- minimal		0	0	0	0	2	0	1	0
- mild		1	0	0	1	2	0	2	0
- moderate		0	0	1	0	0	0	0	0
inflammation, chronic	- minimal	0	1	0	0	0	0	0	0
inflammation, embolic		0	0	1	0	1	0	0	0
- minimal		0	0	0	0	1	0	0	0
- moderate		0	0	1	0	0	0	0	0
inflammation, subacute	- mild	0	0	0	1	0	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lung		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
leukocytosis, vascular		1	0	1	0	3	0	0	0
- minimal		0	0	0	0	1	0	0	0
- mild		1	0	0	0	2	0	0	0
- moderate		0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		3	0	2	0	2	1	1	0
macrophages, pigmented alveolar		1	2	0	1	1	0	0	0
- minimal		1	2	0	0	1	0	0	0
- mild		0	0	0	1	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	1	0	0	0
metaplasia, osseous	- minimal	0	0	0	0	0	0	0	1
thrombus	- mild	0	0	2	0	0	0	0	0
within normal limits		20	14	23	9	24	10	30	12
lymph node, axillary		(1)	(0)	(1)	(0)	(2)	(0)	(0)	(0)
hyperplasia, lymphocyte/plasmacyte	- mild	1	0	1	0	0	0	0	0
within normal limits		0	0	0	0	2	0	0	0
lymph node, cervical		(0)	(0)	(0)	(0)	(0)	(0)	(2)	(0)
amyloid	- mild	0	0	0	0	0	0	1	0
hyperplasia, lymphocyte/plasmacyte	- moderate	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
lymph node, hepatic		(0)	(1)	(1)	(0)	(0)	(0)	(1)	(0)
within normal limits		0	1	1	0	0	0	1	0
lymph node, iliac		(1)	(0)	(1)	(0)	(2)	(0)	(1)	(0)
hyperplasia, lymphocyte/plasmacyte	- moderate	1	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	2	0	0	0
within normal limits		0	0	0	0	0	0	1	0
lymph node, inguinal		(1)	(0)	(1)	(1)	(1)	(0)	(1)	(0)
hyperplasia, generalized lymphoid	- mild	0	0	0	1	0	0	0	0
hyperplasia, lymphocyte/plasmacyte		0	0	1	0	0	0	1	0
- mild		0	0	0	0	0	0	1	0
- moderate		0	0	1	0	0	0	0	0
within normal limits		1	0	0	0	1	0	0	0
lymph node, mandibular		(43)	(31)	(40)	(24)	(45)	(18)	(46)	(27)
abscess	- moderate	1	0	0	0	0	0	0	0
amyloid	- minimal	2	1	0	0	0	0	0	0
depletion, lymphoid	- minimal	2	0	1	0	1	0	1	0
fibrosarcoma, malignant, secondary		0	0	0	0	1	0	0	0
fibrous histiocytoma, malignant, secondary		1	0	0	0	0	0	0	0
hematopoiesis, extramedullary	- mild	0	0	1	0	1	0	1	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
lymph node, mandibular		(43)	(31)	(40)	(24)	(45)	(18)	(46)	(27)
histiocytosis, sinus		1	0	1	0	0	0	0	0
- minimal		0	0	1	0	0	0	0	0
- mild		1	0	0	0	0	0	0	0
hyperplasia, lymphocyte/plasmacyte		7	4	2	1	2	1	4	2
- minimal		1	0	0	0	0	0	1	0
- mild		3	2	1	0	0	1	2	2
- moderate		2	2	1	1	2	0	1	0
- severe		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		3	0	0	1	2	0	1	0
macrophages, pigmented		8	2	1	0	3	0	4	1
- minimal		6	2	1	0	2	0	4	1
- mild		2	0	0	0	1	0	0	0
necrosis, lymphoid		0	0	0	0	1	0	0	0
within normal limits		20	24	35	22	35	17	38	24
lymph node, mediastinal		(3)	(2)	(0)	(1)	(2)	(1)	(0)	(0)
carcinoma, bronchiolar alveolar, malignant, secondary		1	0	0	0	1	0	0	0
hyperplasia, generalized lymphoid	- mild	0	0	0	1	0	0	0	0
hyperplasia, lymphocyte/plasmacyte	- mild	0	1	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	0	0
within normal limits		2	1	0	0	0	1	0	0
lymph node, mesenteric		(44)	(31)	(39)	(24)	(46)	(18)	(48)	(27)
abscess		0	0	1	1	0	0	0	0
- minimal		0	0	0	1	0	0	0	0
- mild		0	0	1	0	0	0	0	0
amyloid		2	1	2	0	3	0	1	0
- minimal		2	1	2	0	0	0	1	0
- mild		0	0	0	0	3	0	0	0
angiectasis		7	4	6	1	5	0	2	2
- minimal		6	4	4	1	4	0	2	2
- mild		0	0	2	0	1	0	0	0
- moderate		1	0	0	0	0	0	0	0
depletion, lymphoid		4	0	2	0	4	0	2	0
- minimal		1	0	2	0	3	0	1	0
- mild		3	0	0	0	1	0	0	0
- severe		0	0	0	0	0	0	1	0
erythrocytosis/erythrophagocytosis, sinus		10	16	6	9	7	7	16	17
- minimal		4	13	3	5	2	5	8	9
- mild		6	3	3	4	4	2	7	8
- moderate		0	0	0	0	1	0	1	0
hemangiosarcoma, malignant, multicentric		0	0	0	0	1	0	0	0
hematopoiesis, extramedullary		7	3	3	3	3	0	4	3
- minimal		5	0	1	3	0	0	1	1
- mild		2	2	0	0	2	0	3	2
- moderate		0	1	2	0	1	0	0	0
histiocytosis, sinus		0	0	0	0	1	0	0	0
lymph node, mesenteric		(44)	(31)	(39)	(24)	(46)	(18)	(48)	(27)
hyperplasia, follicular lymphoid	- mild	1	0	0	0	0	0	0	0
hyperplasia, generalized lymphoid		1	3	0	3	1	0	1	1
- minimal		1	1	0	0	0	0	0	1
- mild		0	2	0	3	1	0	1	0
hyperplasia, lymphocyte/plasmacyte		2	1	2	0	1	1	1	1
- minimal		1	0	1	0	1	0	1	0
- mild		1	0	1	0	0	1	0	1
- moderate		0	1	0	0	0	0	0	0
hyperplasia, megakaryocytic		0	0	0	1	0	0	0	0
infiltration, neutrophil	- mild	2	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
- mild		1	0	0	0	0	0	0	0
inflammation, acute	- minimal	1	1	0	0	0	0	0	0
inflammation, chronic-active	- mild	0	1	0	0	0	0	0	0
inflammation, embolic	- minimal	0	0	0	0	1	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
leukocytosis, vascular	- minimal	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		4	0	2	1	2	0	2	1
macrophages, pigmented		2	2	2	0	1	0	1	0
- minimal		1	2	2	0	0	0	1	0
- mild		1	0	0	0	1	0	0	0
necrosis, lymphoid	- minimal	0	0	2	0	1	0	0	0
thrombus	- mild	1	0	0	0	1	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
lymph node, mesenteric		(44)	(31)	(39)	(24)	(46)	(18)	(48)	(27)
within normal limits		19	10	22	12	25	11	24	7
lymph node, renal		(1)	(0)	(0)	(0)	(1)	(0)	(1)	(0)
hyperplasia, lymphocyte/plasmacyte		1	0	0	0	0	0	1	0
- mild		0	0	0	0	0	0	1	0
- moderate		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	0	0
multicentric neoplasm		(5)	(4)	(5)	(1)	(5)	(3)	(3)	(2)
hemangioma, benign, multicentric		0	2	1	0	0	2	0	1
hemangiosarcoma, malignant, multicentric		1	2	2	0	2	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		4	0	2	1	3	1	2	1
nerve, sciatic		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
degeneration, axonal/myelin		19	26	12	19	19	13	23	21
- minimal		18	21	11	13	14	6	20	13
- mild		1	5	1	6	5	7	3	8
leukocytosis, vascular		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	1	0
within normal limits		25	5	29	5	26	5	24	6
pancreas		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid	- minimal	0	0	0	0	1	0	0	0
pancreas		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
atrophy, acinar	- minimal	0	0	0	0	0	0	1	0
depletion, secretory		17	0	15	0	17	0	14	0
- minimal		6	0	7	0	6	0	7	0
- mild		11	0	8	0	11	0	7	0
hyperplasia, islet cell		0	1	1	0	1	0	1	1
- minimal		0	1	1	0	1	0	0	1
- mild		0	0	0	0	0	0	1	0
hypertrophy, acinar cell, focal	- minimal	1	0	0	0	0	0	0	0
infiltration, lymphocytic		3	4	1	6	3	6	5	8
- minimal		3	4	1	5	3	6	4	8
- mild		0	0	0	1	0	0	1	0
inflammation, embolic	- minimal	0	0	0	0	1	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		2	0	2	0	2	0	2	1
mineralization, vascular	- minimal	0	0	1	0	0	0	0	0
necrosis, focal	- mild	1	0	0	0	0	0	0	0
necrosis, single cell	- minimal	0	0	0	0	0	0	0	1
polyarteritis		1	2	1	1	0	1	1	0
- minimal		1	2	0	0	0	0	0	0
- mild		0	0	1	1	0	1	1	0
within normal limits		21	25	22	17	25	11	28	17
parathyroid glands		(26)	(24)	(20)	(16)	(22)	(14)	(28)	(15)
adenoma, benign, primary		0	1	0	0	0	0	0	0
amyloid		0	0	4	0	2	0	2	0
- minimal		0	0	3	0	1	0	0	0
- mild		0	0	1	0	1	0	2	0
infiltration, lymphocytic	- minimal	0	0	0	1	0	0	0	0
within normal limits		26	23	16	15	20	14	26	15
penis		(3)	(0)	(4)	(0)	(4)	(1)	(4)	(3)
abscess	- moderate	1	0	0	0	0	0	1	0
congestion		1	0	2	0	2	1	3	1
- mild		1	0	2	0	1	1	2	1
- moderate		0	0	0	0	1	0	1	0
erosion/ulcer		1	0	2	0	0	0	1	1
- mild		0	0	1	0	0	0	0	0
- moderate		1	0	1	0	0	0	1	1
necrosis	- severe	0	0	1	0	1	0	0	0
within normal limits		0	0	0	0	1	0	0	1
peyers patch		(43)	(31)	(41)	(24)	(44)	(18)	(47)	(27)
hyperplasia, lymphoid	- mild	0	0	0	1	0	0	1	0
inflammation, chronic-active	- severe	0	0	0	0	0	0	0	1
lymphoma, malignant, multicentric		0	0	0	1	0	0	1	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
peyers patch		(43)	(31)	(41)	(24)	(44)	(18)	(47)	(27)
within normal limits		43	31	41	22	44	18	45	26
pituitary gland		(44)	(30)	(38)	(24)	(46)	(18)	(46)	(27)
abscess	- severe	1	0	0	0	0	0	0	0
adenoma, pars distalis, benign, primary		0	0	1	0	0	0	0	0
cyst	- mild	0	0	0	1	0	0	0	0
hyperplasia, pars distalis		0	2	2	1	1	5	0	1
	- minimal	0	0	1	1	0	2	0	0
	- mild	0	2	1	0	0	2	0	1
	- moderate	0	0	0	0	1	1	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
thrombus	- mild	1	0	0	0	0	0	0	0
within normal limits		42	28	34	22	45	13	46	26
preputial glands		(0)	(0)	(1)	(0)	(2)	(1)	(1)	(2)
abscess		0	0	0	0	0	1	0	1
	- mild	0	0	0	0	0	0	0	1
	- moderate	0	0	0	0	0	1	0	0
cyst	- moderate	0	0	1	0	1	0	0	1
dilatation	- moderate	0	0	0	0	1	0	0	0
inflammation, acute	- mild	0	0	0	0	0	0	1	0
metaplasia, goblet cell	- severe	0	0	0	0	1	0	0	0
prostate gland		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid	- mild	0	0	1	0	0	0	0	0
atrophy		9	17	2	10	9	7	2	12
	- minimal	4	10	1	9	8	5	2	7
	- mild	5	7	1	1	1	2	0	5
hemangioma, benign, multicentric		0	0	1	0	0	0	0	0
infiltration, lymphocytic	- minimal	2	3	2	1	2	0	3	2
inflammation, acute	- mild	0	0	0	0	1	0	0	0
inflammation, chronic	- mild	0	0	1	0	0	0	1	0
inflammation, chronic-active		1	0	3	1	2	0	2	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	1	0	0	0	2	0	2	0
	- moderate	0	0	2	0	0	0	0	0
	- severe	0	0	0	1	0	0	0	0
inflammation, subacute		4	1	3	0	4	0	6	1
	- minimal	1	1	0	0	0	0	1	0
	- mild	2	0	1	0	1	0	3	0
	- moderate	1	0	2	0	3	0	2	1
lymphoma, malignant, multicentric		1	0	0	0	1	0	2	0
within normal limits		27	14	28	13	28	11	32	13
salivary gland, mandibular		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		0	0	0	0	3	0	0	0
	- minimal	0	0	0	0	1	0	0	0
	- mild	0	0	0	0	2	0	0	0
atrophy		6	1	4	0	5	0	2	0
	- minimal	5	1	3	0	5	0	2	0
	- moderate	1	0	1	0	0	0	0	0
infiltration, lymphocytic		7	11	3	3	2	4	5	8
	- minimal	7	11	3	3	2	4	5	7
	- mild	0	0	0	0	0	0	0	1
inflammation, acute	- mild	1	0	0	0	0	0	0	0
inflammation, subacute	- minimal	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	1	0
within normal limits		31	19	34	21	38	14	39	19
salivary gland, parotid		(44)	(31)	(40)	(24)	(47)	(18)	(48)	(27)
amyloid		10	1	7	2	10	0	8	1
	- minimal	3	1	2	2	7	0	3	1
	- mild	2	0	4	0	2	0	4	0
	- moderate	5	0	1	0	1	0	1	0
atrophy		32	23	21	3	33	0	34	14
	- minimal	17	21	5	3	11	0	21	10
	- mild	15	2	16	0	22	0	13	4
infiltration, lymphocytic	- minimal	2	0	0	1	0	2	1	1

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal									
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day			
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC		
Number of Animals Examined		44	31	41	24	47	18	48	27		
salivary gland, parotid		(44)	(31)	(40)	(24)	(47)	(18)	(48)	(27)		
inflammation, subacute	- mild	0	0	1	0	0	0	0	0		
lymphoma, malignant, multicentric		0	0	0	0	1	0	0	0		
within normal limits		9	8	17	20	11	16	11	13		
salivary gland, sublingual		(44)	(31)	(40)	(24)	(44)	(18)	(48)	(26)		
atrophy	- moderate	0	0	0	0	0	0	1	0		
infiltration, lymphocytic	- minimal	0	0	0	0	1	0	0	0		
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0		
polyarteritis	- mild	0	0	1	0	0	0	0	0		
within normal limits		44	31	39	24	43	18	46	26		
seminal vesicles		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(25)		
atrophy	- minimal	1	0	0	0	0	0	0	0		
depletion, secretory	- moderate	0	0	3	0	1	0	1	0		
dilatation, gland/lumen	- moderate	0	0	0	0	1	0	0	0		
hyperplasia, glandular	- minimal	0	0	0	1	0	0	0	0		
infiltration, lymphocytic		0	2	0	1	0	0	0	2		
	- minimal	0	1	0	0	0	0	0	2		
	- mild	0	1	0	1	0	0	0	0		
inflammation, chronic		2	0	0	0	0	0	1	0		
	- minimal	1	0	0	0	0	0	0	0		
	- mild	1	0	0	0	0	0	0	0		
	- moderate	0	0	0	0	0	0	1	0		
seminal vesicles		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(25)		
inflammation, chronic-active		2	0	2	0	1	0	0	0		
	- minimal	0	0	1	0	0	0	0	0		
	- mild	1	0	0	0	0	0	0	0		
	- moderate	0	0	1	0	0	0	0	0		
	- severe	1	0	0	0	1	0	0	0		
inflammation, subacute		2	4	4	1	4	0	4	1		
	- minimal	0	4	0	0	2	0	1	0		
	- mild	1	0	2	1	2	0	2	1		
	- moderate	1	0	2	0	0	0	1	0		
lymphoma, malignant, multicentric		2	0	0	0	1	0	1	0		
polyarteritis	- minimal	0	0	0	1	0	0	0	0		
secretory product, increased		10	18	9	15	15	15	11	18		
	- minimal	1	4	2	3	2	4	0	1		
	- mild	5	7	6	8	8	5	11	10		
	- moderate	4	4	1	4	5	4	0	7		
	- severe	0	3	0	0	0	2	0	0		
within normal limits		27	13	25	9	27	3	34	7		
skeletal muscle, biceps femoris		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
degeneration, myofiber		1	0	1	1	4	1	1	0		
	- minimal	0	0	1	1	3	1	1	0		
	- mild	1	0	0	0	1	0	0	0		
fibrous histiocytoma, malignant, secondary		0	0	0	0	0	0	1	0		
infiltration, lymphocytic	- minimal	0	0	0	1	0	0	0	0		
skeletal muscle, biceps femoris		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
inflammation, acute	- mild	0	0	0	0	1	0	0	0		
polyarteritis		2	0	0	0	0	0	0	0		
	- minimal	1	0	0	0	0	0	0	0		
	- mild	1	0	0	0	0	0	0	0		
within normal limits		41	31	40	22	42	17	46	27		
skin		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
abscess	- severe	1	0	1	0	0	0	0	0		
atrophy		20	0	12	1	22	0	16	1		
	- minimal	15	0	10	1	18	0	14	1		
	- mild	5	0	2	0	4	0	2	0		
bacterial colonies	- mild	1	0	0	0	0	0	0	0		
crust, serocellular		0	1	0	0	0	0	2	0		
	- mild	0	1	0	0	0	0	0	0		
	- moderate	0	0	0	0	0	0	2	0		
edema		1	0	0	0	4	0	0	0		
	- minimal	1	0	0	0	1	0	0	0		
	- mild	0	0	0	0	3	0	0	0		
erosion/ulcer		8	1	5	0	5	0	6	0		
	- mild	2	0	0	0	0	0	0	0		
	- moderate	3	1	4	0	5	0	4	0		
	- severe	3	0	1	0	0	0	2	0		

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
skin		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
hyperkeratosis		0	2	0	0	0	0	1	0
- minimal		0	0	0	0	0	0	1	0
- mild		0	1	0	0	0	0	0	0
- moderate		0	1	0	0	0	0	0	0
hyperplasia, epidermal		3	2	1	0	1	0	3	0
- minimal		1	0	1	0	0	0	2	0
- mild		2	2	0	0	1	0	1	0
inflammation, chronic		0	0	0	0	1	0	1	0
inflammation, chronic-active		1	0	0	0	0	0	0	0
inflammation, subacute		0	1	1	0	1	0	0	0
- minimal		0	1	1	0	0	0	0	0
- mild		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	1	0
within normal limits		16	28	23	23	21	18	24	26
skin, subcutis		(4)	(0)	(3)	(2)	(5)	(0)	(3)	(0)
abscess		0	0	0	1	1	0	1	0
- moderate		0	0	0	1	1	0	0	0
- severe		0	0	0	0	0	0	1	0
bacterial colonies		0	0	1	0	0	0	0	0
edema		1	0	1	0	0	0	1	0
- mild		1	0	0	0	0	0	1	0
- moderate		0	0	1	0	0	0	0	0
skin, subcutis		(4)	(0)	(3)	(2)	(5)	(0)	(3)	(0)
fibrosarcoma, malignant, primary		0	0	0	0	2	0	0	0
fibrous histiocytoma, malignant, primary		2	0	1	1	2	0	1	0
hemorrhage		1	0	1	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
necrosis		0	0	1	0	0	0	0	0
- severe		0	0	1	0	0	0	0	0
small intestine, duodenum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		4	1	1	1	7	0	4	1
- minimal		4	1	1	1	4	0	3	1
- mild		0	0	0	0	2	0	1	0
- moderate		0	0	0	0	1	0	0	0
hemorrhage		0	0	0	0	1	0	0	0
- mild		0	0	0	0	1	0	0	0
hyperplasia, mucosal		0	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
polyarteritis		0	0	0	1	0	0	0	0
- minimal		0	0	0	1	0	0	0	0
sarcoma, undifferentiated, malignant, primary		0	1	0	0	0	0	0	0
within normal limits		39	29	40	23	38	18	44	26
small intestine, ileum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		15	4	14	4	17	0	17	5
- minimal		0	0	0	0	0	0	0	1
- mild		6	1	9	2	8	0	9	1
- moderate		9	3	5	2	9	0	8	3
dilatation, gland/lumen		0	0	0	0	0	0	0	1
- moderate		0	0	0	0	0	0	0	1
small intestine, ileum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
hemorrhage		0	0	0	0	1	0	0	0
- mild		0	0	0	0	1	0	0	0
inflammation, chronic		0	1	0	0	0	0	0	0
- mild		0	1	0	0	0	0	0	0
inflammation, embolic		0	0	0	0	1	0	0	0
- minimal		0	0	0	0	1	0	0	0
inflammation, peritoneal		1	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	1	0
within normal limits		27	26	27	20	28	18	30	22
small intestine, jejunum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		9	2	4	1	8	0	9	3
- minimal		0	1	3	0	2	0	0	0
- mild		8	1	1	1	3	0	8	2
- moderate		1	0	0	0	3	0	1	1
hemorrhage		0	0	0	0	1	0	0	0
- mild		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		34	29	37	23	38	18	39	24
spinal cord, cervical		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
bacterial colonies		0	0	0	0	1	0	0	0
- minimal		0	0	0	0	1	0	0	0
hemorrhage		2	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
- moderate		1	0	0	0	0	0	0	0
infiltration, lymphocytic		0	0	0	0	0	1	0	0
- minimal		0	0	0	0	0	1	0	0
inflammation, embolic		1	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		mg/kg/day DOS	SNC	mg/kg/day DOS	SNC	mg/kg/day DOS	SNC	mg/kg/day DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
skin, subcutis		(4)	(0)	(3)	(2)	(5)	(0)	(3)	(0)
fibrosarcoma, malignant, primary		0	0	0	0	2	0	0	0
fibrous histiocytoma, malignant, primary		2	0	1	1	2	0	1	0
hemorrhage	- mild	1	0	1	0	0	0	0	0
necrosis	- severe	0	0	1	0	0	0	0	0
small intestine, duodenum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		4	1	1	1	7	0	4	1
	- minimal	4	1	1	1	4	0	3	1
	- mild	0	0	0	0	2	0	1	0
	- moderate	0	0	0	0	1	0	0	0
hemorrhage	- mild	0	0	0	0	1	0	0	0
hyperplasia, mucosal	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
polyarteritis	- minimal	0	0	0	1	0	0	0	0
sarcoma, undifferentiated, malignant, primary		0	1	0	0	0	0	0	0
within normal limits		39	29	40	23	38	18	44	26
small intestine, ileum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		15	4	14	4	17	0	17	5
	- minimal	0	0	0	0	0	0	0	1
	- mild	6	1	9	2	8	0	9	1
	- moderate	9	3	5	2	9	0	8	3
dilatation, gland/lumen	- moderate	0	0	0	0	0	0	0	1
small intestine, ileum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
hemorrhage	- mild	0	0	0	0	1	0	0	0
inflammation, chronic	- mild	0	1	0	0	0	0	0	0
inflammation, embolic	- minimal	0	0	0	0	1	0	0	0
inflammation, peritoneal	- minimal	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	1	0
within normal limits		27	26	27	20	28	18	30	22
small intestine, jejunum		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		9	2	4	1	8	0	9	3
	- minimal	0	1	3	0	2	0	0	0
	- mild	8	1	1	1	3	0	8	2
	- moderate	1	0	0	0	3	0	1	1
hemorrhage	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		34	29	37	23	38	18	39	24
spinal cord, cervical		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
bacterial colonies	- minimal	0	0	0	0	1	0	0	0
hemorrhage		2	0	0	0	0	0	0	0
	- minimal	1	0	0	0	0	0	0	0
	- moderate	1	0	0	0	0	0	0	0
infiltration, lymphocytic	- minimal	0	0	0	0	0	1	0	0
inflammation, embolic	- minimal	1	0	0	0	0	0	0	0
spinal cord, cervical		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
within normal limits		41	31	41	24	46	17	48	27
spinal cord, lumbar		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
inflammation, embolic	- minimal	0	0	1	0	0	0	0	0
within normal limits		44	31	40	24	47	18	48	27
spinal cord, thoracic		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
inflammation, embolic	- minimal	0	0	0	0	1	0	0	0
within normal limits		44	31	41	24	46	18	48	27
spleen		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid		8	0	6	0	10	0	4	0
	- minimal	4	0	1	0	3	0	1	0
	- mild	4	0	3	0	2	0	2	0
	- moderate	0	0	2	0	3	0	1	0
	- severe	0	0	0	0	2	0	0	0
angiectasis	- severe	0	0	0	0	1	0	0	0
atrophy		3	0	6	0	6	0	2	0
	- minimal	3	0	4	0	6	0	2	0
	- mild	0	0	2	0	0	0	0	0
depletion, lymphoid		18	0	13	0	14	0	14	1
	- minimal	12	0	7	0	12	0	9	1
	- mild	5	0	6	0	2	0	5	0
	- moderate	1	0	0	0	0	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
spleen		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
fibrous histiocytoma, malignant, secondary		1	0	0	0	0	0	0	0
hemangioma, benign, multicentric		0	2	0	0	0	1	0	0
hemangiosarcoma, malignant, multicentric		0	2	1	0	1	0	0	0
hematopoiesis, extramedullary, increased		23	23	19	19	18	15	29	16
- minimal		8	11	7	10	3	8	12	9
- mild		6	10	5	7	5	7	10	6
- moderate		8	2	6	2	9	0	6	1
- severe		1	0	1	0	1	0	1	0
hemorrhage	- moderate	0	0	0	0	0	0	1	0
hyperplasia, generalized lymphoid		1	1	0	0	1	0	1	0
- minimal		1	1	0	0	1	0	0	0
- mild		0	0	0	0	0	0	1	0
hyperplasia, lymphocyte/plasmacyte		7	1	1	0	0	0	1	0
- minimal		1	1	0	0	0	0	1	0
- mild		5	0	1	0	0	0	0	0
- moderate		1	0	0	0	0	0	0	0
hyperplasia, reactive red pulp/stromal		0	0	0	1	0	1	0	0
- minimal		0	0	0	1	0	0	0	0
- moderate		0	0	0	0	0	1	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		4	0	2	1	3	1	1	1
macrophages, pigmented	- minimal	2	0	3	2	2	2	1	1
necrosis, lymphoid	- minimal	0	0	1	0	1	0	3	0
spleen		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
within normal limits		7	6	9	5	9	2	10	10
stomach, glandular		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
adenoma, benign, primary		0	0	0	0	0	1	0	0
amyloid		7	2	7	2	9	0	7	4
- minimal		2	2	1	2	4	0	2	3
- mild		5	0	6	0	5	0	5	1
cyst	- mild	0	0	0	0	1	1	0	0
dilatation, gland/lumen	- minimal	2	0	0	1	2	0	0	3
erosion/ulcer		0	0	4	1	2	0	1	0
- minimal		0	0	3	0	0	0	0	0
- mild		0	0	1	1	2	0	1	0
infiltration, lymphocytic		1	0	0	1	1	0	0	0
- minimal		1	0	0	0	1	0	0	0
- mild		0	0	0	1	0	0	0	0
inflammation, peritoneal	- mild	0	0	1	0	0	0	0	0
inflammation, subacute	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		2	0	2	0	2	0	2	1
mineralization	- mild	1	0	0	0	0	0	1	0
polyarteritis	- minimal	0	0	1	0	0	0	0	0
within normal limits		32	29	27	20	30	16	37	20
stomach, nonglandular		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
amyloid	- minimal	0	0	0	0	0	0	0	1
dilatation	- mild	0	0	0	0	0	0	1	0
hyperkeratosis	- minimal	0	0	0	0	1	0	1	1
hyperplasia, epithelial, nonglandular		1	1	0	0	0	0	0	1
- minimal		0	0	0	0	0	0	0	1
- mild		1	1	0	0	0	0	0	0
inflammation, embolic	- minimal	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		43	30	41	24	45	18	45	25
tail		(0)	(0)	(0)	(1)	(0)	(2)	(0)	(1)
cyst, epidermal inclusion	- mild	0	0	0	0	0	1	0	0
hemangioma, benign, multicentric		0	0	0	0	0	1	0	1
within normal limits		0	0	0	1	0	0	0	0
testes		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
adenoma, interstitial cell, benign, primary		0	1	0	0	1	0	0	1
amyloid		0	0	2	0	6	0	2	0
- minimal		0	0	0	0	4	0	0	0
- mild		0	0	1	0	2	0	2	0
- moderate		0	0	1	0	0	0	0	0
carcinoma, interstitial cell, malignant, primary		0	0	0	0	1	0	0	0
cyst	- moderate	0	0	0	1	0	0	0	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal									
		0		25/5		50/25		100/50			
		mg/kg/day DOS	SNC	mg/kg/day DOS	SNC	mg/kg/day DOS	SNC	mg/kg/day DOS	SNC		
Number of Animals Examined		44	31	41	24	47	18	48	27		
testes		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
degeneration, spermatocyte/spermatid, bilateral		3	0	2	0	3	0	0	0		
- minimal		1	0	2	0	1	0	0	0		
- mild		1	0	0	0	2	0	0	0		
- moderate		1	0	0	0	0	0	0	0		
degeneration, spermatocyte/spermatid, unilateral	- minimal	0	0	1	0	0	0	1	0		
degeneration/atrophy, seminiferous tubules, bilateral		5	6	6	5	11	4	8	5		
- minimal		1	1	2	1	3	2	2	2		
- mild		0	4	2	4	3	2	2	1		
- moderate		3	0	2	0	2	0	1	1		
- severe		1	1	0	0	3	0	3	1		
degeneration/atrophy, seminiferous tubules, unilateral		4	5	3	6	3	2	2	5		
- minimal		0	1	1	3	3	1	2	4		
- mild		1	4	0	3	0	1	0	1		
- moderate		2	0	1	0	0	0	0	0		
- severe		1	0	1	0	0	0	0	0		
hemorrhage	- mild	1	0	0	0	1	0	0	0		
hyperplasia, interstitial cell		4	2	3	0	1	0	6	0		
- minimal		3	2	2	0	1	0	6	0		
- mild		1	0	1	0	0	0	0	0		
hyperplasia, rete testis		0	0	0	0	1	1	2	0		
- minimal		0	0	0	0	0	1	1	0		
- mild		0	0	0	0	1	0	1	0		
testes		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)		
inflammation, embolic	- mild	0	0	1	0	0	0	0	0		
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0		
mineralization, vascular	- minimal	0	0	1	0	0	0	0	0		
polyarteritis	- minimal	0	0	0	1	0	0	0	0		
spermatocele	- mild	0	0	1	0	1	0	0	0		
vacuolation, sertoli cell, bilateral	- minimal	0	0	0	0	1	0	0	0		
within normal limits		28	19	26	12	28	11	33	16		
thymus gland		(35)	(26)	(36)	(22)	(43)	(18)	(39)	(25)		
amyloid	- minimal	1	0	0	0	0	0	0	0		
angiectasis	- minimal	0	0	0	0	0	0	0	1		
carcinoma, bronchiolar alveolar, malignant, secondary		1	0	0	0	1	0	0	0		
cyst		1	4	3	4	3	3	5	2		
- minimal		0	2	1	1	1	3	3	2		
- mild		1	2	2	3	2	0	2	0		
depletion, lymphoid		29	26	31	21	39	15	37	21		
- minimal		1	6	2	3	0	3	3	4		
- mild		6	12	8	12	14	6	9	12		
- moderate		13	6	15	5	13	5	15	4		
- severe		9	2	6	1	12	1	10	1		
fibrous histiocytoma, malignant, secondary		1	0	0	0	0	0	0	0		
thymus gland		(35)	(26)	(36)	(22)	(43)	(18)	(39)	(25)		
hyperplasia, lymphoid		2	2	0	1	0	2	1	3		
- minimal		0	1	0	0	0	2	1	0		
- mild		2	1	0	1	0	0	0	2		
- moderate		0	0	0	0	0	0	0	1		
inflammation, acute		1	0	2	0	0	0	0	0		
- minimal		1	0	0	0	0	0	0	0		
- mild		0	0	2	0	0	0	0	0		
lymphoma, malignant, multicentric		4	0	2	1	2	1	1	1		
macrophages, pigmented	- mild	1	0	0	0	0	0	0	0		
mesothelioma, malignant, secondary		0	0	0	0	1	0	0	0		
necrosis, lymphoid		1	0	0	0	2	0	2	0		
- minimal		0	0	0	0	1	0	0	0		
- mild		1	0	0	0	1	0	2	0		
polyarteritis	- minimal	0	0	0	0	0	0	1	0		
within normal limits		0	0	2	0	0	1	0	0		
thyroid gland		(44)	(31)	(39)	(24)	(47)	(18)	(48)	(27)		
adenoma, follicular cell, benign, primary		0	0	0	0	0	0	1	0		
amyloid		12	2	10	1	13	0	8	2		
- minimal		4	2	3	1	5	0	4	1		
- mild		5	0	5	0	6	0	2	1		
- moderate		3	0	2	0	2	0	2	0		
carcinoma, follicular cell, malignant, primary		0	0	0	1	0	0	0	0		

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
trachea		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		43	30	40	23	47	18	47	26
ureters		(43)	(31)	(37)	(24)	(45)	(18)	(46)	(27)
dilatation		8	6	7	2	9	3	7	3
- minimal		1	0	2	2	4	1	2	0
- mild		5	5	4	0	4	2	3	2
- moderate		2	0	1	0	0	0	2	1
- severe		0	1	0	0	1	0	0	0
infiltration, lymphocytic		3	2	1	0	4	0	4	1
- minimal		2	2	1	0	4	0	4	1
- mild		1	0	0	0	0	0	0	0
inflammation, acute	- minimal	0	0	0	0	0	0	1	0
inflammation, subacute	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		3	0	1	1	1	1	2	0
within normal limits		30	23	28	21	31	14	32	23
urinary bladder		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
dilatation		21	6	7	4	12	2	35	13
- minimal		8	3	2	1	1	0	7	5
- mild		8	3	4	2	8	2	17	6
- moderate		5	0	1	1	3	0	11	2
urinary bladder		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
hemorrhage		0	0	0	0	1	0	3	0
- minimal		0	0	0	0	0	0	1	0
- mild		0	0	0	0	1	0	0	0
- moderate		0	0	0	0	0	0	1	0
- severe		0	0	0	0	0	0	1	0
hyperplasia, simple transitional cell		3	0	6	0	4	0	2	0
- minimal		1	0	2	0	3	0	2	0
- mild		2	0	4	0	1	0	0	0
infiltration, lymphocytic		7	8	5	4	3	2	5	6
- minimal		7	8	4	4	3	2	4	6
- mild		0	0	1	0	0	0	1	0
inflammation, acute		0	0	0	0	0	0	3	0
- minimal		0	0	0	0	0	0	1	0
- mild		0	0	0	0	0	0	2	0
inflammation, chronic	- minimal	1	0	0	0	0	0	0	0
inflammation, chronic-active	- moderate	1	0	0	0	0	0	0	0
inflammation, embolic	- minimal	0	0	1	0	0	0	0	0
inflammation, subacute		2	0	5	0	3	0	7	0
- minimal		0	0	1	0	0	0	2	0
- mild		2	0	3	0	1	0	4	0
- moderate		0	0	1	0	2	0	1	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	2	0
mesenchymal tumor, benign, primary		0	1	0	1	0	0	0	0
urinary bladder		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
within normal limits		15	18	24	16	30	14	10	10

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

Tabulated histopath findings in female mice treated with lorcaserin

(b) (4) Study Number 900-062

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
adipose tissue		(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
depletion	- severe	0	0	1	0	0	0	0	0
adrenal glands		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenocarcinoma, malignant, secondary		1	0	1	0	0	0	0	0
adenoma, subcapsular cell, benign, primary		0	1	0	1	0	1	0	1
amyloid		5	1	6	4	2	0	4	2
	- minimal	0	1	2	1	0	0	1	1
	- mild	3	0	4	3	1	0	3	0
	- moderate	2	0	0	0	1	0	0	1
bacterial colonies	- minimal	0	0	0	0	0	0	1	0
carcinoma, subcapsular cell, malignant, primary		0	0	0	0	0	1	0	0
ceroid, increased		26	16	25	13	27	18	30	22
	- minimal	19	11	16	8	21	14	24	18
	- mild	7	5	9	5	5	4	6	4
	- moderate	0	0	0	0	1	0	0	0
fatty change, diffuse cortical	- mild	0	0	1	0	0	0	0	0
hematopoiesis, extramedullary		0	0	0	0	1	0	1	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	1	0	0	0
hyperplasia, focal medullary		1	1	1	1	1	1	0	1
	- minimal	0	1	1	1	0	1	0	1
	- mild	0	0	0	0	1	0	0	0
	- moderate	1	0	0	0	0	0	0	0
bone marrow, femur		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	1	1	0	0	0	1
necrosis, focal	- minimal	1	0	1	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		36	22	33	18	34	24	36	20
bone marrow, sternum		(49)	(26)	(43)	(21)	(40)	(24)	(50)	(25)
angiectasis	- mild	0	0	0	0	1	0	0	0
hyperplasia, focal lymphoid	- minimal	0	0	0	0	0	1	0	0
hyperplasia, granulocytic		11	4	8	2	5	1	13	4
	- mild	2	1	2	1	1	1	1	2
	- moderate	6	3	4	1	2	0	9	2
	- severe	3	0	2	0	2	0	3	0
hyperplasia, mixed	- mild	0	0	1	0	0	0	1	0
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	1	1	2	0	0	1
necrosis		0	0	1	0	0	0	1	0
	- minimal	0	0	0	0	0	0	1	0
	- moderate	0	0	1	0	0	0	0	0
within normal limits		38	22	32	18	31	22	35	20
bone, femur		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
fibrous osteodystrophy		4	0	6	0	1	0	5	0
	- minimal	2	0	5	0	1	0	3	0
	- mild	2	0	1	0	0	0	2	0
osteoma, benign, primary		0	0	0	0	0	0	1	0
proliferation, fibro-osseous		5	6	4	2	7	4	4	3
	- minimal	5	3	2	2	6	4	3	3
	- mild	0	3	2	0	1	0	1	0
within normal limits		40	20	33	19	32	21	40	22
bone, sternum		(49)	(26)	(43)	(21)	(40)	(24)	(50)	(25)
fibrous osteodystrophy		1	0	4	0	0	0	5	0
	- minimal	0	0	3	0	0	0	4	0
	- mild	1	0	1	0	0	0	1	0
lymphoma, malignant, multicentric		0	1	0	0	0	0	0	0
proliferation, fibro-osseous		2	9	4	5	10	2	1	6
	- minimal	2	6	3	4	8	2	0	5
	- mild	0	3	1	1	2	0	0	1
	- moderate	0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	0	2	0
within normal limits		46	17	35	16	30	22	42	19

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
bone, tibia		(0)	(4)	(0)	(1)	(0)	(0)	(0)	(4)
proliferation, fibro-osseous		0	4	0	1	0	0	0	4
- minimal		0	1	0	0	0	0	0	4
- mild		0	3	0	1	0	0	0	0
brain		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
bacterial colonies	- minimal	0	0	2	0	0	0	0	0
compression, ventral (pituitary tumor)		1	0	0	0	0	1	0	0
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	0	0	0	1	0	0
hemorrhage	- mild	1	0	0	0	0	0	0	0
infiltration, lymphoid, perivascular	- minimal	0	0	0	1	0	1	0	0
inflammation, perivascular	- minimal	0	0	1	0	0	0	0	0
inflammation, subacute		0	0	2	0	0	0	0	0
- minimal		0	0	1	0	0	0	0	0
- moderate		0	0	1	0	0	0	0	0
leukocytosis, vascular	- mild	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	1	1	0	0	0	1
mineralization, focal	- minimal	10	13	5	6	8	8	8	4
necrosis		1	0	2	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
- moderate		0	0	1	0	0	0	0	0
oligodendroglioma, malignant, primary		1	0	0	0	0	0	0	0
brain		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
within normal limits		37	13	36	13	32	15	42	20
cavity, abdominal		(0)	(0)	(1)	(0)	(0)	(0)	(3)	(0)
adhesion	- moderate	0	0	0	0	0	0	2	0
bacterial colonies	- mild	0	0	0	0	0	0	1	0
inflammation, subacute	- moderate	0	0	1	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	0	1	0
cavity, thoracic		(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
diaphragm		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
within normal limits		0	0	0	0	0	0	1	0
ears		(2)	(0)	(2)	(0)	(0)	(0)	(0)	(1)
erosion/ulcer		1	0	1	0	0	0	0	1
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
- moderate		0	0	0	0	0	0	0	1
exudate, epidermal surface		1	0	1	0	0	0	0	1
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	1	0	0	0	0	1
ears		(2)	(0)	(2)	(0)	(0)	(0)	(0)	(1)
hyperplasia, epithelial cell		2	0	1	0	0	0	0	1
- minimal		1	0	1	0	0	0	0	0
- mild		1	0	0	0	0	0	0	1
inflammation, subacute		1	0	0	0	0	0	0	1
- minimal		1	0	0	0	0	0	0	0
- moderate		0	0	0	0	0	0	0	1
within normal limits		0	0	1	0	0	0	0	0
esophagus		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
degeneration, myofiber	- minimal	0	0	0	0	0	1	0	0
inflammation, subacute	- mild	0	0	0	1	0	0	0	0
within normal limits		49	26	43	20	40	24	50	25
eyes		(49)	(25)	(43)	(21)	(40)	(25)	(50)	(25)
cataract		1	0	0	2	1	1	0	0
- mild		0	0	0	2	0	1	0	0
- moderate		1	0	0	0	1	0	0	0
degeneration/atrophy, retina, bilateral	- mild	1	0	0	0	0	1	0	0
degeneration/atrophy, retina, unilateral		0	2	1	3	0	0	0	0
- minimal		0	1	0	2	0	0	0	0
- mild		0	1	1	0	0	0	0	0
- moderate		0	0	0	1	0	0	0	0
erosion/ulcer, corneal	- moderate	0	0	0	0	0	0	1	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
eyes		(49)	(25)	(43)	(21)	(40)	(25)	(50)	(25)
fold/rosette, retinal	- minimal	0	0	0	0	1	0	0	0
hemorrhage	- mild	0	0	0	1	0	0	0	0
inflammation, acute	- mild	2	0	0	1	0	1	1	0
keratopathy		7	4	1	7	2	7	5	8
	- minimal	7	3	0	2	2	6	5	7
	- mild	0	1	1	4	0	1	0	1
	- moderate	0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	0	0
phthisis bulbi	- mild	0	0	0	1	0	0	0	1
within normal limits		39	19	40	12	35	17	44	16
eyes, optic nerves		(45)	(25)	(39)	(21)	(39)	(25)	(43)	(24)
inflammation, subacute	- mild	0	0	1	0	0	0	0	0
within normal limits		45	25	38	21	39	25	43	24
gallbladder		(48)	(25)	(42)	(21)	(40)	(23)	(49)	(25)
adhesion/inflammation/fibrosis, capsule	- mild	0	0	0	0	0	0	1	0
infiltration, lymphocytic		3	8	0	10	0	6	1	5
	- minimal	2	8	0	10	0	6	1	5
	- mild	1	0	0	0	0	0	0	0
inflammation, subacute	- mild	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	2	1	1	1	0	0	0
polyarteritis	- mild	0	0	1	0	0	0	0	0
gallbladder		(48)	(25)	(42)	(21)	(40)	(23)	(49)	(25)
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		44	15	40	10	39	17	46	20
harderian glands		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenoma, benign, primary		1	1	1	1	1	1	2	2
atrophy		1	0	1	0	0	0	0	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	1	0	0	0	0	0	0	0
hyperplasia, focal		0	2	1	2	0	2	0	2
	- minimal	0	1	1	1	0	0	0	0
	- mild	0	1	0	0	0	1	0	0
	- moderate	0	0	0	1	0	1	0	2
infiltration, lymphocytic		11	11	17	13	12	17	14	13
	- minimal	11	11	17	12	12	17	14	12
	- mild	0	0	0	1	0	0	0	1
inflammation, subacute		0	0	0	1	1	0	0	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		0	2	0	0	2	0	1	0
thrombus	- mild	0	0	1	0	0	0	0	0
within normal limits		36	11	25	8	24	6	34	9
heart		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenocarcinoma, malignant, secondary		0	0	1	0	0	0	0	0
amyloid		2	0	4	3	2	0	2	1
	- minimal	1	0	2	3	2	0	1	1
	- mild	1	0	2	0	0	0	1	0
bacterial colonies		3	0	0	0	0	0	3	0
	- minimal	0	0	0	0	0	0	2	0
	- mild	2	0	0	0	0	0	0	0
	- moderate	0	0	0	0	0	0	1	0
	- severe	1	0	0	0	0	0	0	0
cardiomyopathy	- minimal	4	2	3	0	0	1	9	4
endocarditis, valvular vegetative	- severe	1	0	2	0	0	0	1	0
hemorrhage	- mild	1	0	1	0	0	0	0	0
infiltration, lymphocytic	- minimal	2	2	2	1	3	2	2	0
inflammation, acute		1	0	1	0	0	0	2	0
	- minimal	1	0	1	0	0	0	0	0
	- mild	0	0	0	0	0	0	1	0
	- moderate	0	0	0	0	0	0	1	0
inflammation, subacute		4	0	0	0	1	0	0	1
	- minimal	1	0	0	0	0	0	0	0
	- mild	3	0	0	0	0	0	0	1
	- severe	0	0	0	0	1	0	0	0
leukocytosis, vascular	- mild	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	3	1	0	6	1	2	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
heart		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
mineralization, myofiber		3	0	4	0	1	0	0	0
- minimal		2	0	3	0	1	0	0	0
- mild		1	0	1	0	0	0	0	0
necrosis		0	0	1	0	0	0	1	0
- minimal		0	0	1	0	0	0	0	0
- mild		0	0	0	0	0	0	1	0
polyarteritis		0	1	1	0	0	1	1	1
- minimal		0	1	1	0	0	1	1	0
- moderate		0	0	0	0	0	0	0	1
sarcoma, histiocytic, malignant, multicentric		2	0	1	0	1	1	2	0
thrombus		2	0	1	0	2	0	3	0
- mild		1	0	0	0	0	0	2	0
- moderate		0	0	1	0	1	0	0	0
- severe		1	0	0	0	1	0	1	0
within normal limits		32	18	25	17	25	19	29	18
joint, tibiofemoral		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
inflammation, chronic-active	- mild	0	1	0	0	0	0	0	0
within normal limits		49	25	43	21	40	25	50	25
kidneys		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenocarcinoma, malignant, secondary		1	0	0	0	0	0	0	0
adhesion/inflammation/fibrosis, capsule	- mild	1	0	0	0	0	0	1	0
kidneys		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		8	1	7	6	4	2	4	2
- minimal		1	0	2	3	0	0	1	1
- mild		4	1	2	2	1	2	0	0
- moderate		1	0	0	1	1	0	0	0
- severe		2	0	3	0	2	0	3	1
bacterial colonies		1	0	0	0	0	0	1	0
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	0	0	0	0	1	0
cyst		5	3	5	3	4	4	4	1
- minimal		5	3	2	3	4	3	2	1
- mild		0	0	3	0	0	0	2	0
- moderate		0	0	0	0	0	1	0	0
dilatation, tubular	- mild	0	1	0	0	0	0	0	0
hematopoiesis, extramedullary, increased	- mild	1	0	0	0	0	0	0	0
hyaline, droplets, increased		3	1	1	0	2	1	0	0
- minimal		0	1	0	0	0	0	0	0
- mild		2	0	0	0	1	1	0	0
- moderate		1	0	1	0	1	0	0	0
hydronephrosis, bilateral		5	1	5	0	0	1	1	0
- minimal		3	1	2	0	0	0	0	0
- mild		1	0	2	0	0	1	1	0
- moderate		1	0	1	0	0	0	0	0
kidneys		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
hydronephrosis, unilateral		2	1	2	1	2	0	0	1
- minimal		2	1	0	1	1	0	0	1
- mild		0	0	2	0	0	0	0	0
- severe		0	0	0	0	1	0	0	0
hyperplasia, tubular	- minimal	0	1	0	0	0	0	0	0
hypoplasia, unilateral	- moderate	0	0	1	0	0	0	0	0
infarct		0	2	5	0	0	1	2	0
- minimal		0	2	4	0	0	1	1	0
- mild		0	0	0	0	0	0	1	0
- moderate		0	0	1	0	0	0	0	0
infiltration, lymphocytic		41	20	34	20	32	20	35	24
- minimal		37	18	30	18	26	17	33	24
- mild		4	2	4	2	6	3	2	0
inflammation, acute		1	0	0	0	0	1	1	0
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	0	0	0	1	1	0
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		3	4	5	1	6	2	5	1
macrophages, pigmented	- minimal	0	0	1	0	2	0	0	0
mineralization, tubular	- minimal	1	0	0	0	0	1	2	1
necrosis, papillary	- moderate	0	0	0	0	0	1	1	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
kidneys		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
nephropathy, chronic progressive		18	12	8	8	10	10	10	12
- minimal		8	9	2	7	3	9	6	11
- mild		2	3	0	1	2	1	0	1
- moderate		6	0	4	0	5	0	3	0
- severe		2	0	2	0	0	0	1	0
pigment, tubular		0	0	1	0	1	0	2	0
- minimal		0	0	0	0	1	0	1	0
- mild		0	0	1	0	0	0	1	0
- moderate		0	0	0	0	0	0	0	0
- severe		0	0	0	0	0	0	0	0
polyarteritis		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		1	0	1	0	1	1	1	0
within normal limits		3	1	2	0	1	2	6	0
lacrimal glands, exorbital		(49)	(25)	(42)	(21)	(40)	(25)	(50)	(25)
amyloid		0	0	3	0	0	0	0	0
- minimal		0	0	2	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
atrophy		1	0	0	1	1	1	1	0
- minimal		1	0	0	0	0	0	0	0
- mild		0	0	0	0	1	0	0	0
- moderate		0	0	0	0	0	1	0	0
- severe		0	0	0	1	0	0	1	0
lacrimal glands, exorbital		(49)	(25)	(42)	(21)	(40)	(25)	(50)	(25)
infiltration, lymphocytic		35	18	19	17	21	19	20	23
- minimal		27	16	14	14	14	16	15	20
- mild		8	2	5	3	6	3	5	3
- moderate		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	2	1	1	3	0	2	1
metaplasia, harderian		0	2	2	0	0	2	0	1
- minimal		0	2	1	0	0	2	0	1
- mild		0	0	1	0	0	0	0	0
within normal limits		14	5	18	3	16	5	28	1
large intestine, cecum		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		1	0	0	0	1	0	0	0
- mild		1	0	0	0	0	0	0	0
- moderate		0	0	0	0	1	0	0	0
dilatation, gland/lumen		1	0	0	0	0	0	0	0
- mild		1	0	0	0	0	0	0	0
edema		1	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
hemorrhage		0	0	1	0	0	0	0	0
- minimal		0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	1	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		46	26	41	20	39	25	48	25
large intestine, colon		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
carcinoma, bronchiolar alveolar, malignant, secondary		0	0	1	0	0	0	0	0
edema		1	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		47	26	41	21	39	25	50	25
large intestine, rectum		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
edema		1	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
leiomyoma, benign, primary		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	1	1	0	0	0
prolapse		0	0	0	0	0	0	1	0
- severe		0	0	0	0	0	0	1	0
within normal limits		47	26	43	20	39	25	49	25
liver		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenoma, hepatocellular, benign, primary		1	0	1	0	0	1	1	2
adhesion/inflammation/fibrosis, capsule		0	0	0	0	0	0	1	0
- moderate		0	0	0	0	0	0	1	0
amyloid		6	1	6	3	2	0	3	1
- minimal		2	1	4	1	0	0	1	0
- mild		2	0	2	2	2	0	2	1
- moderate		1	0	0	0	0	0	0	0
- severe		1	0	0	0	0	0	0	0
bacterial colonies		0	0	0	0	0	0	1	0

Summary of Microscopic Observations - FEMALE

		Terminal								
Tissue	Observation	Severity	0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
			DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined			49	26	43	21	40	25	50	25
liver			(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
	carcinoma, hepatocellular, malignant, primary		0	0	0	0	1	0	0	0
	fibrosis	- minimal	1	0	0	0	0	0	0	0
	focus of cellular alteration, basophilic	- minimal	0	0	0	0	0	1	0	0
	focus of cellular alteration, eosinophilic	- mild	0	0	0	1	0	0	0	0
	hemangiosarcoma, malignant, multicentric		0	1	0	0	0	0	0	0
	hematopoiesis, extramedullary		3	1	4	0	3	0	7	0
		- minimal	1	0	2	0	2	0	5	0
		- mild	2	1	2	0	1	0	2	0
	hepatodiaphragmatic nodule	- no grade	0	0	1	0	0	0	0	0
	infiltration, mononuclear cell		21	19	20	19	13	21	13	21
		- minimal	21	18	15	16	12	21	13	18
		- mild	0	1	5	3	1	0	0	3
	leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
	leukocytosis, sinusoidal		1	0	2	0	0	0	0	0
		- mild	0	0	2	0	0	0	0	0
		- moderate	1	0	0	0	0	0	0	0
	lymphoma, malignant, multicentric		2	4	3	1	6	1	4	1
	necrosis, focal		4	1	3	0	2	1	2	1
		- minimal	2	1	1	0	1	1	1	1
		- mild	1	0	1	0	1	0	1	0
		- moderate	0	0	1	0	0	0	0	0
		- severe	1	0	0	0	0	0	0	0
	sarcoma, histiocytic, malignant, multicentric		3	1	2	1	2	1	1	1
liver			(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
	schwannoma, malignant, secondary		0	0	0	0	0	0	1	0
	vacuolation, centrilobular		0	0	2	0	0	0	0	0
		- minimal	0	0	1	0	0	0	0	0
		- mild	0	0	1	0	0	0	0	0
	within normal limits		18	2	13	0	13	2	22	3
lung			(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
	adenocarcinoma, malignant, secondary		2	0	1	0	0	0	2	0
	adenoma, bronchiolar alveolar, benign, primary		2	1	3	3	6	2	1	4
	adhesion/inflammation/fibrosis, pleural		3	0	1	0	1	0	2	2
		- minimal	0	0	0	0	0	0	2	2
		- mild	2	0	1	0	0	0	0	0
		- moderate	1	0	0	0	0	0	0	0
		- severe	0	0	0	0	1	0	0	0
	bacterial colonies		2	0	0	0	0	0	3	0
		- minimal	0	0	0	0	0	0	1	0
		- mild	1	0	0	0	0	0	0	0
		- moderate	1	0	0	0	0	0	1	0
		- severe	0	0	0	0	0	0	1	0
	carcinoma, bronchiolar alveolar, malignant, primary		0	2	4	0	1	2	2	0
lung			(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
	congestion, chronic passive		1	1	8	0	3	0	3	0
		- minimal	1	0	0	0	0	0	1	0
		- mild	0	1	3	0	3	0	2	0
		- moderate	0	0	4	0	0	0	0	0
		- severe	0	0	1	0	0	0	0	0
	edema	- mild	0	0	1	0	0	0	1	0
	fibrosis	- mild	0	0	0	0	0	0	1	0
	granulosa cell tumor, malignant, secondary		0	0	0	1	0	0	0	0
	hemorrhage		6	0	2	0	9	0	2	1
		- minimal	1	0	1	0	4	0	1	1
		- mild	4	0	0	0	4	0	0	0
		- moderate	1	0	1	0	1	0	1	0
	histiocytosis, alveolar		4	4	1	2	2	6	2	8
		- minimal	1	4	1	2	2	4	1	7
		- mild	3	0	0	0	0	2	1	1
	hyperplasia, bronchiolar-alveolar		2	3	1	3	4	0	0	3
		- minimal	0	0	1	0	0	0	0	0
		- mild	2	2	0	2	4	0	0	2
		- moderate	0	1	0	1	0	0	0	0
		- severe	0	0	0	0	0	0	0	1
	infiltration, lymphocytic		3	2	0	1	2	2	0	2
		- minimal	1	1	0	1	2	1	0	2
		- mild	2	1	0	0	0	0	0	0
		- moderate	0	0	0	0	0	1	0	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
lung		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
inflammation, acute		0	0	0	0	0	0	2	0
- mild		0	0	0	0	0	0	1	0
- moderate		0	0	0	0	0	0	1	0
inflammation, subacute		2	0	0	0	0	0	0	1
- minimal		1	0	0	0	0	0	0	0
- mild		1	0	0	0	0	0	0	1
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
leukocytosis, vascular		1	0	1	0	0	0	2	0
- mild		1	0	1	0	0	0	1	0
- moderate		0	0	0	0	0	0	1	0
luteoma, malignant, secondary		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		2	4	3	1	6	3	2	1
macrophages, pigmented alveolar	- mild	0	0	0	0	0	0	0	1
pneumonitis, uremic		2	0	1	0	1	0	2	0
- minimal		0	0	1	0	0	0	0	0
- mild		1	0	0	0	1	0	2	0
- moderate		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		2	0	2	0	2	1	2	0
sarcoma, stromal, malignant, secondary		0	0	0	1	0	0	0	0
within normal limits		30	13	24	13	11	14	27	12
lymph node, axillary		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
hyperplasia, lymphocyte/plasmacyte	- mild	0	0	0	0	0	0	1	0
lymph node, hepatic		(0)	(0)	(1)	(0)	(0)	(0)	(0)	(2)
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		0	0	0	0	0	0	0	2
lymph node, iliac		(2)	(1)	(2)	(2)	(4)	(1)	(1)	(1)
hematopoiesis, extramedullary	- severe	0	1	0	0	0	0	0	0
hyperplasia, lymphocyte/plasmacyte	- moderate	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	2	0	1	0	0	0
necrosis	- mild	0	0	0	0	0	0	1	0
within normal limits		2	0	0	2	3	1	0	1
lymph node, inguinal		(2)	(1)	(1)	(1)	(0)	(0)	(1)	(2)
hyperplasia, lymphocyte/plasmacyte	- moderate	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	1	1	1	0	0	1	1
within normal limits		0	0	0	0	0	0	0	1
lymph node, mandibular		(48)	(24)	(39)	(20)	(40)	(25)	(48)	(22)
amyloid	- minimal	0	0	1	0	0	0	0	0
lymph node, mandibular		(48)	(24)	(39)	(20)	(40)	(25)	(48)	(22)
erythrocytosis/erythrophagocytosis, sinus		1	1	4	0	0	2	2	0
- minimal		1	1	2	0	0	2	1	0
- mild		0	0	1	0	0	0	1	0
- moderate		0	0	1	0	0	0	0	0
hematopoiesis, extramedullary		0	1	0	0	0	0	1	0
- minimal		0	1	0	0	0	0	0	0
- mild		0	0	0	0	0	0	1	0
hyperplasia, lymphocyte/plasmacyte		2	0	1	2	0	1	0	0
- minimal		0	0	0	0	0	1	0	0
- mild		2	0	0	1	0	0	0	0
- moderate		0	0	0	1	0	0	0	0
- severe		0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		3	2	5	2	6	1	4	1
polyarteritis	- mild	1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		40	20	28	17	34	21	41	21
lymph node, mediastinal		(0)	(3)	(3)	(0)	(3)	(2)	(5)	(0)
adenocarcinoma, malignant, secondary		0	0	0	0	0	0	2	0
erythrocytosis/erythrophagocytosis, sinus		0	0	0	0	1	0	1	0
- mild		0	0	0	0	0	0	1	0
- moderate		0	0	0	0	1	0	0	0
hyperplasia, generalized lymphoid	- minimal	0	0	0	0	0	0	1	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
lymph node, mediastinal		(0)	(3)	(3)	(0)	(3)	(2)	(5)	(0)
inflammation, subacute	- severe	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	1	2	0	1	1	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		0	2	1	0	1	1	0	0
lymph node, mesenteric		(49)	(26)	(43)	(21)	(38)	(25)	(48)	(25)
adenocarcinoma, malignant, secondary		0	0	1	0	0	0	0	0
amyloid	- minimal	1	0	7	0	2	0	3	1
angiectasis		1	1	0	1	0	0	1	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	1	1	0	1	0	0	0	0
dilatation, sinus		0	0	0	1	0	0	0	1
	- minimal	0	0	0	0	0	0	0	1
	- mild	0	0	0	1	0	0	0	0
erythrocytosis/erythrophagocytosis, sinus		14	11	9	5	13	7	20	8
	- minimal	8	9	3	3	9	7	12	7
	- mild	6	2	5	2	3	0	6	1
	- moderate	0	0	1	0	1	0	2	0
hematopoiesis, extramedullary		4	6	1	3	1	5	1	4
	- minimal	0	1	0	2	0	2	0	3
	- mild	3	4	1	0	1	2	0	1
	- moderate	1	1	0	1	0	1	1	0
hemorrhage	- mild	0	0	1	0	0	0	0	0
lymph node, mesenteric		(49)	(26)	(43)	(21)	(38)	(25)	(48)	(25)
hyperplasia, generalized lymphoid		0	0	0	2	0	0	0	0
	- mild	0	0	0	1	0	0	0	0
	- moderate	0	0	0	1	0	0	0	0
hyperplasia, lymphocyte/plasmacyte	- mild	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		3	3	3	1	5	1	4	3
polyarteritis	- minimal	0	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		2	0	0	0	0	0	1	0
within normal limits		28	9	25	12	18	16	21	13
lymph node, renal		(1)	(1)	(1)	(0)	(2)	(0)	(0)	(0)
erythrocytosis/erythrophagocytosis, sinus	- mild	1	0	0	0	0	0	0	0
hematopoiesis, extramedullary	- severe	0	1	0	0	0	0	0	0
hyperplasia, lymphocyte/plasmacyte	- mild	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	2	0	0	0
mammary gland		(48)	(26)	(42)	(21)	(40)	(25)	(50)	(25)
adenocarcinoma, malignant, primary		2	0	1	0	1	0	3	1
amyloid	- minimal	0	0	1	0	0	0	0	0
edema	- mild	0	0	0	0	0	0	1	0
hyperplasia, lobular		6	4	7	6	9	3	7	1
	- minimal	6	3	5	5	8	3	4	1
	- mild	0	1	2	1	1	0	2	0
	- moderate	0	0	0	0	0	0	1	0
mammary gland		(48)	(26)	(42)	(21)	(40)	(25)	(50)	(25)
infiltration, lymphocytic	- mild	1	0	0	0	0	0	0	0
inflammation, subacute	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		1	0	1	1	3	2	1	0
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		39	22	34	14	27	21	40	23
mediastinum		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
inflammation, subacute	- moderate	0	0	0	0	0	0	0	1
necrosis	- moderate	0	0	0	0	0	0	0	1
mesentery/peritoneum		(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	1	0	0
multicentric neoplasm		(9)	(6)	(9)	(4)	(11)	(4)	(10)	(5)
hemangiosarcoma, malignant, multicentric		0	1	0	0	0	0	0	0
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		4	4	6	3	8	3	8	4
sarcoma, histiocytic, malignant, multicentric		5	1	3	1	2	1	2	1

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
nerve, sciatic		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
degeneration, axonal/myelin		22	19	14	20	16	17	18	21
- minimal		18	19	12	20	15	15	17	20
- mild		3	0	2	0	1	2	1	1
- moderate		1	0	0	0	0	0	0	0
infiltration, lymphocytic	- minimal	0	0	0	0	0	0	0	1
lymphoma, malignant, multicentric		0	1	1	0	2	0	0	1
within normal limits		27	6	28	1	22	8	32	4
ovaries		(49)	(26)	(42)	(21)	(40)	(25)	(50)	(25)
abscess		1	0	1	0	1	0	2	0
- moderate		0	0	1	0	0	0	0	0
- severe		1	0	0	0	1	0	2	0
adenocarcinoma, malignant, primary		0	0	1	0	0	0	0	0
adenocarcinoma, malignant, secondary		1	0	0	0	0	0	0	0
adhesion/inflammation/fibrosis, capsule	- moderate	0	0	0	0	0	0	1	0
amyloid		5	0	3	0	4	0	4	0
- minimal		0	0	0	0	1	0	0	0
- mild		2	0	1	0	1	0	1	0
- moderate		1	0	0	0	2	0	1	0
- severe		2	0	2	0	0	0	2	0
angiectasis		0	1	0	0	1	0	0	0
- mild		0	1	0	0	0	0	0	0
- moderate		0	0	0	0	1	0	0	0
ovaries		(49)	(26)	(42)	(21)	(40)	(25)	(50)	(25)
cyst		27	26	23	19	27	23	27	24
- minimal		4	4	2	0	3	6	7	3
- mild		5	7	10	9	11	8	9	5
- moderate		14	10	7	9	11	6	9	12
- severe		4	5	4	1	2	3	2	4
cystadenoma, benign, primary		2	0	0	0	0	0	0	0
granulosa cell tumor, benign, primary		0	2	1	0	1	0	0	1
granulosa cell tumor, malignant, primary		1	0	0	1	0	0	0	0
hyperplasia, cystic/papillary	- mild	0	1	0	0	0	0	0	0
hyperplasia, sex-cord/stromal		0	3	0	0	0	0	2	1
- minimal		0	0	0	0	0	0	1	1
- mild		0	3	0	0	0	0	0	0
- moderate		0	0	0	0	0	0	1	0
- minimal		0	0	1	0	0	0	0	0
luteoma, malignant, primary		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		2	1	5	1	5	1	4	1
mineralization	- minimal	0	0	0	0	1	0	0	0
polyarteritis		1	1	1	0	2	2	0	0
- minimal		0	0	1	0	1	2	0	0
- mild		1	1	0	0	0	0	0	0
- moderate		0	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		2	0	1	0	1	0	1	0
ovaries		(49)	(26)	(42)	(21)	(40)	(25)	(50)	(25)
within normal limits		18	0	11	2	5	2	14	0
oviducts		(48)	(26)	(41)	(21)	(40)	(24)	(48)	(24)
within normal limits		48	26	41	21	40	24	48	24
pancreas		(49)	(26)	(43)	(21)	(39)	(25)	(50)	(25)
adenoma, islet cell, benign, primary		1	0	0	0	0	0	0	1
adhesion/inflammation/fibrosis, capsule	- mild	0	0	0	0	0	0	2	0
amyloid		0	0	3	0	1	0	0	1
- minimal		0	0	2	0	1	0	0	1
- mild		0	0	1	0	0	0	0	0
atrophy, acinar		1	3	0	0	0	0	1	1
- minimal		1	2	0	0	0	0	1	1
- severe		0	1	0	0	0	0	0	0
edema		1	0	2	0	2	0	0	0
- minimal		1	0	1	0	2	0	0	0
- mild		0	0	1	0	0	0	0	0
infiltration, lymphocytic		4	10	9	8	10	13	6	16
- minimal		4	10	9	8	10	13	6	15
- mild		0	0	0	0	0	0	0	1
inflammation, subacute	- mild	0	0	0	0	0	0	0	1
ischemic/atrophic lobe	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		3	1	2	1	2	0	3	1

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
pancreas		(49)	(26)	(43)	(21)	(39)	(25)	(50)	(25)
polyarteritis	- mild	0	1	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		2	0	0	0	0	0	1	0
within normal limits		37	12	28	12	24	12	37	6
parathyroid glands		(27)	(17)	(16)	(11)	(24)	(19)	(29)	(17)
amyloid		3	0	3	1	2	0	1	1
	- minimal	3	0	2	1	1	0	0	1
	- mild	0	0	1	0	1	0	1	0
infiltration, lymphocytic	- minimal	2	0	0	0	0	0	0	0
within normal limits		22	17	13	10	22	19	28	16
peyers patch		(45)	(23)	(37)	(20)	(39)	(25)	(45)	(25)
hyperplasia, lymphoid	- mild	0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		0	2	2	1	1	0	2	1
within normal limits		45	21	35	18	38	25	43	24
pituitary gland		(48)	(26)	(41)	(21)	(39)	(25)	(47)	(25)
adenoma, pars distalis, benign, primary		1	0	1	1	0	1	1	0
adenoma, pars intermedia, benign, primary		0	0	0	0	0	0	0	1
cyst	- minimal	0	0	1	0	0	0	0	1
fibrosis	- minimal	0	0	0	0	1	0	0	0
pituitary gland		(48)	(26)	(41)	(21)	(39)	(25)	(47)	(25)
hyperplasia, focal, pars distalis		1	2	1	1	0	2	0	1
	- minimal	0	2	0	1	0	1	0	0
	- mild	1	0	1	0	0	1	0	0
	- moderate	0	0	0	0	0	0	0	1
infiltration, lymphocytic	- minimal	0	0	0	1	0	1	0	0
inflammation, subacute	- mild	0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	1	0	0	0	0	0	0
within normal limits		46	23	37	18	38	21	46	22
salivary gland, mandibular		(48)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		0	0	3	0	0	0	0	0
	- minimal	0	0	2	0	0	0	0	0
	- mild	0	0	1	0	0	0	0	0
atrophy	- moderate	0	1	0	0	0	0	0	0
infiltration, lymphocytic		12	5	3	7	7	8	4	9
	- minimal	11	4	3	7	7	8	4	9
	- mild	1	1	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	2	1	1	2	0	2	2
polyarteritis	- mild	0	0	0	0	0	1	0	0
within normal limits		36	19	36	13	31	16	44	14
salivary gland, parotid		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		3	0	8	2	3	0	3	1
	- minimal	2	0	1	0	0	0	0	0
	- mild	0	0	3	1	2	0	2	0
	- moderate	1	0	2	1	1	0	1	1
	- severe	0	0	2	0	0	0	0	0
atrophy		2	1	0	0	1	0	0	0
	- minimal	0	1	0	0	0	0	0	0
	- mild	1	0	0	0	1	0	0	0
	- moderate	1	0	0	0	0	0	0	0
hemorrhage	- moderate	0	0	1	0	0	0	0	0
hypertrophy, basophilic focal	- minimal	0	0	1	0	0	0	0	0
infiltration, lymphocytic		6	1	1	4	3	4	1	4
	- minimal	5	1	1	4	3	4	1	4
	- mild	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	2	0	0	2	0	0	0
within normal limits		38	22	32	15	32	21	46	20
salivary gland, sublingual		(48)	(26)	(42)	(21)	(40)	(25)	(49)	(25)
amyloid	- minimal	0	0	3	0	0	0	0	0
infiltration, lymphocytic	- minimal	0	0	1	0	2	1	0	0
lymphoma, malignant, multicentric		0	1	0	1	1	0	0	0
within normal limits		48	25	38	20	37	24	49	25

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
skeletal muscle		(1)	(0)	(2)	(0)	(3)	(0)	(2)	(0)
inflammation, subacute	- mild	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	2	0	2	0	2	0
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
skeletal muscle, biceps femoris		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
degeneration/regeneration, myofiber	- mild	0	0	0	0	1	0	0	0
infiltration, lymphocytic	- minimal	1	0	0	0	0	0	0	0
inflammation, subacute	- minimal	0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	1
mineralization, myofiber	- minimal	1	0	0	0	0	0	0	0
within normal limits		47	26	42	20	39	25	50	24
skin		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
alopecia/hypotrichosis	- mild	1	0	0	0	0	0	1	0
erosion/ulcer		3	1	1	1	1	0	2	3
	- mild	0	1	1	0	1	0	2	2
	- moderate	2	0	0	1	0	0	0	1
	- severe	1	0	0	0	0	0	0	0
exudate, epidermal surface		4	1	0	0	2	0	2	2
	- minimal	1	0	0	0	1	0	0	0
	- mild	1	1	0	0	1	0	1	2
	- moderate	2	0	0	0	0	0	1	0
hyperkeratosis	- mild	1	0	0	0	0	0	0	0
skin		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
hyperplasia, epidermal		4	1	1	0	3	0	1	2
	- minimal	0	1	0	0	0	0	0	2
	- mild	3	0	1	0	2	0	1	0
	- moderate	1	0	0	0	1	0	0	0
inflammation, chronic		1	0	1	0	0	0	1	0
	- mild	0	0	1	0	0	0	1	0
	- moderate	1	0	0	0	0	0	0	0
inflammation, subacute		2	1	0	1	3	0	1	3
	- minimal	0	0	0	0	1	0	0	0
	- mild	1	1	0	0	2	0	1	2
	- moderate	1	0	0	1	0	0	0	1
keratoacanthoma, benign, primary		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
metaplasia, osseous	- mild	1	0	0	0	0	0	0	0
within normal limits		44	25	41	20	35	25	47	22
skin, subcutis		(3)	(0)	(5)	(0)	(4)	(0)	(4)	(0)
abscess	- severe	0	0	1	0	1	0	0	0
edema		2	0	1	0	2	0	1	0
	- minimal	1	0	0	0	1	0	0	0
	- mild	1	0	1	0	1	0	1	0
erosion/ulcer	- mild	0	0	1	0	0	0	0	0
fibrosarcoma, malignant, primary		0	0	0	0	0	0	1	0
skin, subcutis		(3)	(0)	(5)	(0)	(4)	(0)	(4)	(0)
fibrous histiocytoma, malignant, primary		0	0	0	0	0	0	1	0
hemorrhage		1	0	1	0	1	0	0	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	1	0	1	0	0	0	0	0
inflammation, subacute	- mild	0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		0	0	1	0	0	0	1	0
small intestine, duodenum		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		5	1	8	5	3	2	5	1
	- minimal	1	1	3	5	0	2	0	0
	- mild	4	0	4	0	3	0	4	1
	- moderate	0	0	1	0	0	0	1	0
hemorrhage	- mild	0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	2	0	2	0	1	1
polyarteritis	- minimal	0	0	1	0	0	0	0	0
within normal limits		44	25	32	16	35	23	44	23

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
small intestine, ileum		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		11	5	11	9	12	5	12	4
- minimal		0	0	0	0	0	0	0	1
- mild		1	3	2	2	4	3	5	0
- moderate		7	2	7	7	7	2	5	3
- severe		3	0	2	0	1	0	2	0
lymphoma, malignant, multicentric		0	1	1	1	3	0	1	1
within normal limits		38	20	31	11	26	20	37	20
small intestine, jejunum		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenocarcinoma, malignant, primary		0	0	0	0	0	0	0	1
amyloid		5	2	8	5	3	2	4	1
- minimal		1	1	0	2	0	1	0	0
- mild		2	1	7	3	2	1	1	1
- moderate		2	0	1	0	1	0	2	0
- severe		0	0	0	0	0	0	1	0
hemorrhage		0	0	1	0	0	0	0	0
intussusception	- no grade	0	0	0	0	0	0	2	0
lymphoma, malignant, multicentric		0	1	0	0	0	0	1	0
within normal limits		44	23	34	16	37	23	44	23
spinal cord, cervical		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
hemorrhage	- mild	0	0	1	0	0	0	0	0
inflammation, subacute	- mild	0	0	1	0	0	0	0	0
spinal cord, cervical		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
within normal limits		49	26	41	21	40	25	50	25
spinal cord, lumbar		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
astrocytoma, benign, primary		1	0	0	0	0	0	0	0
inflammation, embolic	- mild	0	0	1	0	0	0	0	0
within normal limits		48	26	42	21	40	25	50	25
spinal cord, thoracic		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
inflammation, subacute	- minimal	0	0	1	0	0	0	0	0
within normal limits		49	26	42	21	40	25	50	25
spleen		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		3	0	5	1	2	0	3	0
- minimal		1	0	1	1	0	0	0	0
- mild		2	0	1	0	1	0	2	0
- moderate		0	0	1	0	1	0	0	0
- severe		0	0	2	0	0	0	1	0
angiectasis	- mild	0	0	0	0	0	0	0	1
depletion, lymphoid		3	0	4	0	2	0	5	0
- minimal		0	0	0	0	1	0	1	0
- mild		3	0	4	0	0	0	2	0
- moderate		0	0	0	0	1	0	2	0
spleen		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
hematopoiesis, extramedullary, increased		23	6	14	9	16	3	28	7
- minimal		8	2	4	7	5	3	8	6
- mild		12	1	7	2	10	0	11	1
- moderate		2	3	3	0	0	0	8	0
- severe		1	0	0	0	1	0	1	0
hyperplasia, generalized lymphoid		1	0	0	5	0	3	0	3
- minimal		0	0	0	3	0	3	0	2
- mild		1	0	0	2	0	0	0	1
hyperplasia, reactive red pulp/stromal	- moderate	0	0	0	0	0	1	0	0
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		4	4	5	2	7	2	4	2
necrosis, lymphoid	- minimal	1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		18	17	17	6	13	17	13	15
stomach, glandular		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		1	0	5	0	0	0	0	0
- minimal		1	0	4	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
erosion/ulcer		0	0	1	0	1	0	0	1
- minimal		0	0	1	0	0	0	0	1
- mild		0	0	0	0	1	0	0	0
foreign material	- minimal	0	0	1	0	0	0	0	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
stomach, glandular		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
granulosa cell tumor, malignant, secondary		1	0	0	0	0	0	0	0
inflammation, chronic-active	- minimal	0	0	1	0	0	0	0	0
inflammation, subacute		1	0	0	0	0	0	0	1
	- minimal	1	0	0	0	0	0	0	0
	- mild	0	0	0	0	0	0	0	1
lymphoma, malignant, multicentric		1	0	0	1	2	1	1	1
mineralization	- minimal	1	0	0	0	0	0	0	0
polyarteritis	- minimal	0	0	0	0	0	0	0	1
sarcoma, histiocytic, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		43	26	36	20	37	24	49	23
stomach, nonglandular		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid	- minimal	1	0	1	0	0	0	0	0
edema	- minimal	0	0	0	0	1	0	0	0
erosion/ulcer	- mild	0	0	0	0	1	0	0	0
hyperplasia, epithelial, nonglandular	- minimal	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	0	1	0	0	0	0
papilloma, squamous cell, benign, primary		1	0	0	0	0	0	0	0
within normal limits		47	26	42	20	38	25	50	25
tail		(0)	(1)	(2)	(1)	(2)	(0)	(2)	(0)
cyst, keratin	- mild	0	1	0	1	1	0	2	0
erosion/ulcer	- moderate	0	0	0	0	1	0	0	0
tail		(0)	(1)	(2)	(1)	(2)	(0)	(2)	(0)
hyperostosis	- mild	0	0	1	0	0	0	0	0
within normal limits		0	0	1	0	0	0	0	0
thymus gland		(47)	(25)	(39)	(20)	(34)	(24)	(47)	(24)
adenocarcinoma, malignant, secondary		0	0	1	0	0	0	0	0
amyloid	- minimal	0	0	1	0	0	0	0	0
angiectasis	- mild	0	0	0	0	1	0	0	0
bacterial colonies		1	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	1	0
	- moderate	1	0	0	0	0	0	0	0
cyst		2	0	0	0	1	0	0	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	1	0	0	0	1	0	0	0
depletion, lymphoid		39	21	32	19	28	22	39	21
	- minimal	0	0	0	0	0	0	1	0
	- mild	3	3	0	2	4	6	4	4
	- moderate	13	11	10	13	12	10	17	12
	- severe	23	7	22	4	12	6	17	5
hemorrhage	- mild	0	0	0	1	0	0	0	0
hyperplasia, lymphoid		6	6	1	9	3	6	6	6
	- minimal	0	5	0	5	0	3	0	1
	- mild	4	1	1	4	3	3	6	4
	- moderate	2	0	0	0	0	0	0	1
thymus gland		(47)	(25)	(39)	(20)	(34)	(24)	(47)	(24)
inflammation, acute		1	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	1	0
	- moderate	1	0	0	0	0	0	0	0
inflammation, chronic	- mild	0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		4	4	4	1	6	2	7	3
mineralization, vascular	- moderate	0	0	0	0	1	0	0	0
necrosis, lymphoid		2	0	1	0	2	0	0	0
	- minimal	0	0	0	0	2	0	0	0
	- mild	1	0	0	0	0	0	0	0
	- severe	1	0	1	0	0	0	0	0
polyarteritis		1	0	1	0	1	1	2	1
	- mild	1	0	1	0	1	0	2	1
	- moderate	0	0	0	0	0	1	0	0
sarcoma, histiocytic, malignant, multicentric		2	0	0	0	1	1	0	0
within normal limits		1	0	1	0	0	0	1	0
thyroid gland		(48)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenoma, c-cell, benign, primary		0	0	0	0	0	0	0	1
adenoma, follicular cell, benign, primary		0	0	0	0	0	0	0	1

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
thyroid gland		(48)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		4	1	6	1	3	0	4	1
- minimal		3	1	2	1	0	0	2	1
- mild		1	0	3	0	1	0	2	0
- moderate		0	0	0	0	2	0	0	0
- severe		0	0	1	0	0	0	0	0
infiltration, lymphocytic		1	3	2	1	4	3	3	3
- minimal		1	3	2	1	3	3	3	3
- mild		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	1	1	0	2	0	1	0
within normal limits		43	21	34	19	32	22	43	19
tongue		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid	- minimal	1	0	1	0	0	0	0	0
infiltration, lymphocytic	- minimal	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	0	0
polyarteritis	- mild	0	0	0	0	1	0	0	0
within normal limits		47	26	42	21	38	25	50	25
trachea		(48)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
exudate, luminal	- mild	1	0	0	0	0	0	0	0
within normal limits		47	26	43	21	40	25	50	25
ureters		(42)	(26)	(41)	(21)	(38)	(25)	(46)	(25)
dilatation		1	0	1	0	0	0	1	0
- minimal		1	0	0	0	0	0	1	0
- mild		0	0	1	0	0	0	0	0
infiltration, lymphocytic	- minimal	0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	1	4	1	3	0	1	0
within normal limits		41	25	35	20	35	25	44	25
urinary bladder		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
dilatation	- mild	1	0	1	0	0	0	0	0
hyperplasia, simple transitional cell	- minimal	0	1	0	0	0	0	0	0
infiltration, lymphocytic		24	13	18	11	17	16	15	13
- minimal		24	13	18	11	17	16	15	12
- mild		0	0	0	0	0	0	0	1
lymphoma, malignant, multicentric		2	2	3	2	3	1	2	1
within normal limits		22	11	21	8	20	8	33	11
uterus with cervix		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
adenocarcinoma, malignant, primary		1	0	0	0	0	0	0	0
adenocarcinoma, malignant, secondary		0	0	1	0	0	0	0	0
adenoma, benign, primary		1	0	0	0	0	0	0	0
alteration, decidual	- mild	0	0	0	0	1	0	0	0
uterus with cervix		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
amyloid		1	0	0	0	0	0	0	1
- minimal		1	0	0	0	0	0	0	0
- moderate		0	0	0	0	0	0	0	1
angiectasis		4	2	1	2	4	0	10	1
- mild		3	2	1	2	1	0	4	0
- moderate		1	0	0	0	1	0	2	1
- severe		0	0	0	0	2	0	4	0
bacterial colonies		0	0	0	0	0	0	1	0
dilatation, gland/lumen	- moderate	0	0	0	0	0	0	0	1
exudate, luminal	- mild	0	0	1	0	1	0	0	0
hemorrhage	- severe	0	0	1	0	0	0	0	0
hyperplasia, cervical fibromuscular		0	2	0	1	0	0	0	1
- mild		0	1	0	0	0	0	0	1
- moderate		0	0	0	1	0	0	0	0
- severe		0	1	0	0	0	0	0	0
hyperplasia, cystic endometrial		36	25	28	21	35	24	38	24
- minimal		20	0	15	2	14	5	12	4
- mild		11	13	6	11	17	14	15	10
- moderate		5	11	3	7	3	5	9	6
- severe		0	1	4	1	1	0	2	4
hyperplasia, granular cell	- mild	0	1	0	0	0	0	0	0

(b) (4)

Study Number 900-062

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
uterus with cervix		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
hyperplasia, stromal		0	0	0	2	0	1	1	0
- mild		0	0	0	2	0	1	0	0
- severe		0	0	0	0	0	0	1	0
inflammation, subacute		0	2	0	0	1	0	1	1
- mild		0	1	0	0	0	0	0	0
- moderate		0	1	0	0	0	0	1	1
- severe		0	0	0	0	1	0	0	0
leiomyoma, benign, primary		3	0	0	2	1	2	3	3
leiomyosarcoma, malignant, primary		0	0	0	0	0	0	0	1
lymphoma, malignant, multicentric		2	1	0	2	3	0	3	1
polyarteritis		2	2	2	0	3	1	0	1
- minimal		2	0	0	0	0	1	0	0
- mild		0	2	2	0	2	0	0	1
- moderate		0	0	0	0	1	0	0	0
polyp, stromal, benign, primary		1	4	4	3	3	7	3	5
sarcoma, histiocytic, malignant, multicentric		3	1	2	0	1	1	0	1
sarcoma, stromal, malignant, primary		0	1	3	2	2	3	0	0
schwannoma, malignant, primary		0	0	0	0	0	2	1	0
within normal limits		7	1	11	0	4	0	7	0
vagina		(49)	(26)	(42)	(21)	(40)	(25)	(50)	(25)
cyst	- mild	1	0	0	0	0	0	0	0
inflammation, acute	- mild	0	0	0	0	0	1	0	0
lymphoma, malignant, multicentric		0	0	0	1	2	0	0	0
polyarteritis	- minimal	0	0	0	0	0	0	0	1
sarcoma, stromal, malignant, secondary		0	0	0	1	0	0	0	0
within normal limits		48	26	42	19	38	24	50	24

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Appendix B

Tabulated histopath findings in male SD rats treated with lorcaserin

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
adipose tissue		(1)	(0)	(4)	(0)	(4)	(1)	(3)	(0)
atrophy		1	0	3	0	4	0	1	0
	- mild	0	0	3	0	2	0	0	0
	- moderate	1	0	0	0	2	0	1	0
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
hibernoma, malignant, primary		0	0	0	0	0	0	1	0
inflammation, chronic	- severe	0	0	1	0	0	0	0	0
lipoma, benign, primary		0	0	0	0	0	0	1	0
within normal limits		0	0	0	0	0	1	0	0
adipose tissue, brown		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
hibernoma, malignant, primary		0	0	0	0	0	0	1	0
adrenal glands		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, cortical, benign, primary		0	0	0	0	0	1	1	0
angiectasis/cystic degeneration, focal cortical		8	5	14	6	16	9	28	2
	- minimal	5	4	9	5	10	6	16	1
	- mild	2	1	3	1	5	3	11	1
	- moderate	0	0	2	0	1	0	0	0
	- severe	1	0	0	0	0	0	1	0
atrophy, cortical	- moderate	0	1	0	0	0	0	0	0
bacterial colonies	- minimal	1	0	0	0	0	0	0	0
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
Number of Animals Examined		43	22	49	16	45	20	71	4
adrenal glands		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hematopoiesis, extramedullary		2	0	9	0	5	3	15	0
	- minimal	2	0	4	0	4	2	11	0
	- mild	0	0	4	0	1	1	4	0
	- moderate	0	0	1	0	0	0	0	0
hyperplasia, focal cortical		9	6	8	3	13	12	18	2
	- minimal	7	6	4	2	8	4	12	2
	- mild	1	0	4	1	4	7	3	0
	- moderate	1	0	0	0	1	1	2	0
	- severe	0	0	0	0	0	0	1	0
hyperplasia, focal medullary		10	6	21	7	13	12	12	1
	- minimal	2	1	10	1	1	4	4	1
	- mild	5	3	7	2	7	4	8	0
	- moderate	1	1	4	4	4	3	0	0
	- severe	2	1	0	0	1	1	0	0
hypertrophy, diffuse		3	0	6	0	1	0	15	0
	- minimal	2	0	6	0	0	0	10	0
	- mild	1	0	0	0	1	0	5	0
hypertrophy, focal cortical		16	14	20	14	23	19	23	3
	- minimal	13	11	14	9	18	12	15	2
	- mild	2	3	5	4	5	7	8	1
	- moderate	1	0	1	1	0	0	0	0
infiltration, lymphocytic	- minimal	1	0	0	0	0	0	3	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0
adrenal glands		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	1	0	0	0	1	0
mineralization	- minimal	0	0	0	0	0	0	1	0
osteosarcoma, malignant, secondary		0	0	0	0	1	0	0	0
pheochromocytoma, benign, primary		3	3	2	1	2	2	1	0
pheochromocytoma, malignant, primary		0	3	0	1	0	0	0	0
pigment	- minimal	0	0	0	0	0	1	0	0
polyarteritis		0	0	3	0	0	0	0	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	0	0	2	0	0	0	0	0

DOS- Died or euthanized on study
SNC- Scheduled necropsy
()- Number observed

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
renal mesenchymal tumor, malignant, secondary sarcoma, histiocytic, malignant, multicentric thrombus		1	0	0	0	0	0	0	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	1	0	0	0	0	0
vacuolation		18	9	22	10	21	12	31	1
	- minimal	12	6	15	9	15	8	26	1
	- mild	5	3	6	1	5	3	3	0
	- moderate	1	0	1	0	1	0	2	0
	- severe	0	0	0	0	0	1	0	0
within normal limits		8	1	5	0	4	0	8	0
aorta		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hibernoma, malignant, primary infiltration, mononuclear cell leukemia, large granular lymphocyte, malignant, multicentric	- minimal	1	0	0	0	0	0	0	0
		0	0	1	0	0	0	0	0
		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric mineralization		1	0	0	0	0	0	0	0
	- minimal	3	0	5	0	0	0	1	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	2	0	2	0	0	0	1	0
	- severe	0	0	2	0	0	0	0	0
		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric within normal limits		0	0	1	0	0	0	0	0
		38	22	42	16	45	20	68	4
bile duct, extrahepatic dilatation		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
	- severe	0	0	0	0	0	0	1	0
bone marrow, femur atrophy		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
		0	0	2	0	1	1	24	0
	- minimal	0	0	1	0	0	0	2	0
	- mild	0	0	1	0	0	1	4	0
	- moderate	0	0	0	0	1	0	4	0
	- severe	0	0	0	0	0	0	14	0
depletion, mixed		2	0	0	0	0	0	1	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	1	0	0	0	0	0	1	0
bone marrow, femur hyperplasia, granulocytic		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
		8	6	15	11	20	14	34	2
	- minimal	1	1	1	4	1	0	6	1
	- mild	5	5	10	4	12	7	14	1
	- moderate	2	0	4	3	7	7	14	0
increased adipocytes		24	13	20	4	17	8	11	1
	- minimal	14	6	15	3	13	6	5	0
	- mild	10	7	5	1	4	2	6	1
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric sarcoma, histiocytic, malignant, multicentric schwannoma, malignant, primary within normal limits		2	0	0	0	1	0	0	0
		0	0	1	0	2	0	0	0
		0	0	0	0	0	0	1	0
		7	3	11	1	8	0	6	1
bone marrow, sternum atrophy		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
		1	0	0	0	1	2	23	0
	- minimal	0	0	0	0	0	1	3	0
	- mild	1	0	0	0	0	1	7	0
	- moderate	0	0	0	0	1	0	11	0
	- severe	0	0	0	0	0	0	2	0
depletion, mixed		1	0	1	0	0	0	2	0
	- minimal	1	0	0	0	0	0	1	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	0	0	0	0	0	0	1	0

DOS- Died or euthanized on study
SNC- Scheduled necropsy
()- Number observed

The incidence of brain astrocytoma in the HD male #4111 was reclassified as moderate infarct. Thus the total number of astrocytoma was reduced from 9 to 8 in the HD male rats in the amended report.

Summary of Microscopic Observations - MALE

Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
bone marrow, sternum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hyperplasia, granulocytic		9	1	18	7	18	14	32	1
	- minimal	4	0	3	3	5	1	4	1
	- mild	4	1	12	4	8	12	24	0
	- moderate	1	0	3	0	5	1	4	0
increased adipocytes		11	4	5	1	5	2	4	1
	- minimal	10	4	4	1	4	2	3	1
	- mild	1	0	1	0	1	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	1	0	0	0
macrophages, pigmented	- minimal	0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	1	0	0	0
within normal limits		21	17	24	8	20	4	13	2
bone, femur		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration/necrosis, cartilage	- mild	0	0	0	0	1	0	0	0
fibrous osteodystrophy		0	0	2	0	0	0	1	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	0	0	1	0	0	0	1	0
hyperostosis		0	0	1	0	1	0	6	0
	- minimal	0	0	0	0	1	0	3	0
	- mild	0	0	1	0	0	0	3	0
lymphoma, malignant, multicentric		1	0	0	0	1	0	0	0
within normal limits		42	22	46	16	43	20	64	4
bone, sternum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration/necrosis, cartilage		1	0	0	0	0	1	3	0
	- minimal	1	0	0	0	0	1	2	0
	- mild	0	0	0	0	0	0	1	0
fibrous osteodystrophy		0	0	2	0	0	0	1	0
	- minimal	0	0	2	0	0	0	0	0
	- mild	0	0	0	0	0	0	1	0
hyperostosis		0	0	1	0	0	0	6	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	1	0	0	0	4	0
	- moderate	0	0	0	0	0	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		41	22	45	16	44	19	63	4
brain		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
astrocytoma, malignant, primary		1	0	0	0	4	0	8	1
bacterial colonies	- minimal	0	0	0	0	1	0	0	0
compression, ventral (pituitary tumor)		5	0	5	1	2	0	1	0
	- minimal	0	0	0	0	1	0	0	0
	- mild	2	0	3	1	1	0	1	0
	- moderate	3	0	2	0	0	0	0	0
brain		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
gliosis		1	0	1	0	2	0	4	0
	- minimal	0	0	1	0	1	0	2	0
	- mild	0	0	0	0	1	0	1	0
	- moderate	1	0	0	0	0	0	1	0
granular cell tumor, benign, primary		0	0	0	0	0	1	1	0
hemorrhage	- minimal	1	0	0	0	0	0	0	0
hydrocephalus		9	0	5	1	5	0	9	0
	- minimal	1	0	3	0	3	0	6	0
	- mild	7	0	2	1	2	0	3	0
	- moderate	1	0	0	0	0	0	0	0
inflammation, embolic	- mild	0	0	0	0	1	0	0	0
inflammation, subacute	- mild	0	0	0	0	1	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mineralization, focal		0	0	0	1	1	0	8	1
	- minimal	0	0	0	1	0	0	7	0
	- mild	0	0	0	0	1	0	1	1
mineralization, vascular	- minimal	0	0	0	0	1	0	0	0
mixed glioma, malignant, primary		0	0	0	0	0	0	1	0
necrosis	- minimal	0	0	1	0	0	0	0	0
oligodendroglioma, malignant, primary		1	0	0	0	0	0	0	0
polyarteritis	- minimal	0	0	0	0	1	0	0	0
reticulosis, malignant, primary		0	0	0	1	0	0	1	0

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
brain		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
within normal limits		31	22	43	13	31	19	45	2
cavity, abdominal		(0)	(0)	(1)	(0)	(2)	(0)	(4)	(0)
hibernoma, malignant, primary		0	0	0	0	0	0	1	0
mesothelioma, malignant, primary		0	0	1	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	1	0	1	0
sarcoma, undifferentiated, malignant, primary		0	0	0	0	1	0	0	0
schwannoma, malignant, primary		0	0	0	0	0	0	1	0
cavity, cranial		(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
within normal limits		0	1	0	0	0	0	0	0
cavity, thoracic		(0)	(1)	(0)	(0)	(0)	(0)	(2)	(0)
hibernoma, malignant, primary		0	0	0	0	0	0	1	0
schwannoma, malignant, primary		0	0	0	0	0	0	1	0
within normal limits		0	1	0	0	0	0	0	0
chordae tendineae, left		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hypertrophy		0	0	4	0	3	2	0	0
- minimal		0	0	3	0	3	2	0	0
- mild		0	0	1	0	0	0	0	0
within normal limits		43	22	45	16	42	18	71	4
chordae tendineae, right		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hypertrophy		0	0	2	0	3	2	1	0
- minimal		0	0	1	0	3	2	1	0
- mild		0	0	1	0	0	0	0	0
within normal limits		43	22	47	16	42	18	70	4
coagulating glands		(43)	(22)	(48)	(16)	(45)	(20)	(71)	(4)
atrophy		3	1	4	1	4	1	2	0
- minimal		1	0	0	0	2	1	1	0
- mild		1	1	4	1	1	0	1	0
- moderate		1	0	0	0	1	0	0	0
cyst, epidermal inclusion		0	0	1	0	0	0	0	0
depletion, secretory		4	1	4	2	5	1	5	0
- minimal		0	0	0	0	0	0	1	0
- mild		2	0	3	1	4	1	3	0
- moderate		0	1	1	1	1	0	1	0
- severe		2	0	0	0	0	0	0	0
dilatation		0	0	0	0	0	0	1	0
hyperplasia		0	0	0	0	0	0	1	0
inflammation, acute		0	0	1	0	0	0	0	0
inflammation, chronic		0	0	0	0	1	0	0	0
coagulating glands		(43)	(22)	(48)	(16)	(45)	(20)	(71)	(4)
inflammation, chronic-active		3	1	1	1	2	2	9	0
- minimal		0	0	0	0	0	0	1	0
- mild		1	0	0	0	1	0	1	0
- moderate		2	1	1	1	1	1	5	0
- severe		0	0	0	0	0	1	2	0
inflammation, subacute		2	0	2	0	1	0	3	0
- minimal		1	0	0	0	1	0	0	0
- mild		1	0	2	0	0	0	3	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	0	0
sarcoma, undifferentiated, malignant		0	0	0	0	1	0	0	0
within normal limits		32	20	38	13	36	17	50	4
endocardium		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
fibrosis		0	0	0	1	0	0	0	0
hyperplasia, endocardial		0	0	1	1	0	0	0	0
within normal limits		43	22	48	14	45	20	71	4
epididymides		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
granuloma, spermatic		0	1	0	0	1	0	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

(b) (4)
 Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE
 Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
epididymides		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hematopoiesis, extramedullary hyperplasia, mesothelial	- mild	0	0	0	0	1	0	0	0
	- minimal	0	0	1	0	2	3	1	0
	- mild	0	0	0	0	1	1	0	0
	- moderate	0	0	1	0	1	2	1	0
infiltration, lymphocytic		9	5	9	8	16	10	23	1
	- minimal	9	5	9	7	15	10	22	1
	- mild	0	0	0	1	0	0	1	0
	- moderate	0	0	0	0	1	0	0	0
inflammation, subacute	- mild	1	0	0	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
luminal cellular debris, bilateral		6	1	7	1	8	2	17	0
	- minimal	5	0	1	0	6	0	3	0
	- mild	1	1	5	1	1	1	14	0
	- moderate	0	0	1	0	1	1	0	0
luminal cellular debris, unilateral		4	0	1	1	0	0	2	0
	- minimal	2	0	1	0	0	0	1	0
	- mild	2	0	0	1	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	1	0	0	0	1	0
mineralization		0	0	2	0	0	0	1	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	0	0	1	0	0	0	1	0
epididymides		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
oligospermia/germ cell debris, bilateral		5	3	6	3	3	0	7	0
	- mild	0	1	1	0	0	0	1	0
	- moderate	0	0	0	0	2	0	2	0
	- severe	5	2	5	3	1	0	4	0
oligospermia/germ cell debris, unilateral		3	2	1	1	0	0	2	0
	- moderate	1	1	0	0	0	0	0	0
	- severe	2	1	1	1	0	0	2	0
polyarteritis		0	0	1	0	2	0	0	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	1	0
sarcoma, undifferentiated, malignant, secondary		0	0	0	0	1	0	0	0
spermatocoele	- minimal	0	0	0	0	1	0	0	0
thrombus	- minimal	0	0	0	0	0	0	1	0
vacuolar change		1	3	3	1	3	2	3	0
	- minimal	1	3	3	1	2	2	3	0
	- mild	0	0	0	0	1	0	0	0
vacuolation		29	18	40	15	37	20	67	4
	- minimal	28	18	37	14	28	18	7	1
	- mild	1	0	3	1	9	2	34	3
	- moderate	0	0	0	0	0	0	26	0
within normal limits		7	2	5	0	1	0	2	0
esophagus		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hyperkeratosis	- mild	0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	0	0
within normal limits		40	22	47	16	45	20	70	4
eyes		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
cataract		4	2	4	1	3	0	9	0
	- minimal	3	1	1	0	3	0	5	0
	- mild	1	0	3	0	0	0	4	0
	- moderate	0	1	0	0	0	0	0	0
	- severe	0	0	0	1	0	0	0	0
degeneration/atrophy, retina, bilateral		0	0	1	2	0	0	2	2
	- minimal	0	0	1	2	0	0	2	1
	- mild	0	0	0	0	0	0	0	1
degeneration/atrophy, retina, unilateral		0	1	0	0	1	2	1	0
	- minimal	0	1	0	0	0	2	1	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	1	0	0	0	0	0
erosion/ulcer, corneal		1	1	0	0	0	0	0	0
fold/rosette, retinal		1	0	0	0	0	0	0	0
	- minimal	1	0	0	0	0	0	0	0
	- severe	0	1	0	0	0	0	0	0

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
eyes		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
inflammation, acute		1	0	1	0	2	0	1	0
- minimal		1	0	1	0	0	0	0	0
- mild		0	0	0	0	2	0	1	0
inflammation, subacute		0	0	1	0	1	0	0	0
keratopathy		1	0	0	0	2	0	1	0
- minimal		0	0	0	0	1	0	0	0
- mild		1	0	0	0	1	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
phthisis bulbi	- moderate	1	0	0	0	0	0	0	0
pigment	- minimal	0	0	1	0	0	0	0	0
schwannoma, malignant, primary		0	0	0	0	0	0	1	0
within normal limits		35	19	41	14	36	18	57	2
eyes, optic nerves		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
infiltration, mononuclear cell	- mild	0	0	1	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		42	22	48	16	45	20	70	4
foot/feet		(0)	(1)	(1)	(0)	(0)	(0)	(0)	(0)
crust, serocellular	- mild	0	0	1	0	0	0	0	0
erosion/ulcer	- moderate	0	1	0	0	0	0	0	0
hyperplasia, epidermal	- mild	0	0	1	0	0	0	0	0
foot/feet		(0)	(1)	(1)	(0)	(0)	(0)	(0)	(0)
inflammation, subacute	- moderate	0	0	1	0	0	0	0	0
harderian glands		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
atrophy		1	2	2	0	1	0	1	0
- minimal		1	2	2	0	1	0	0	0
- mild		0	0	0	0	0	0	1	0
cyst, epidermal inclusion	- moderate	0	0	0	0	1	0	0	0
hyperplasia, focal		6	6	12	5	6	7	9	2
- minimal		3	6	5	4	4	5	5	2
- mild		3	0	6	1	2	2	4	0
- moderate		0	0	1	0	0	0	0	0
infiltration, lymphocytic		7	9	10	6	8	5	9	3
- minimal		6	7	9	4	8	5	9	3
- mild		0	2	0	2	0	0	0	0
- moderate		1	0	1	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
macrophages, pigmented	- mild	1	0	1	0	0	0	0	0
pigment, porphyrin		12	5	15	4	16	5	13	1
- minimal		6	4	12	4	11	4	6	1
- mild		6	1	3	0	5	1	7	0
within normal limits		24	8	22	5	20	7	44	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
heart		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
bacterial colonies		1	0	1	0	1	0	0	0
	- mild	1	0	0	0	0	0	0	0
	- moderate	0	0	1	0	1	0	0	0
cardiomyopathy		27	16	38	15	29	17	58	4
	- minimal	13	9	17	10	16	10	29	2
	- mild	13	7	20	5	13	7	28	2
	- moderate	1	0	1	0	0	0	0	0
	- severe	0	0	0	0	0	0	1	0
degeneration, myofiber	- mild	0	0	0	1	0	0	0	0
dilatation, ventricular/atrial	- moderate	1	0	0	0	0	0	0	0
endocarditis, valvular vegetative	- moderate	0	0	0	0	1	0	0	0
hypertrophy/hyperplasia, mesothelial cell	- minimal	1	0	0	0	2	0	0	0
inflammation, chronic		1	0	0	0	1	0	2	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	1	0	0	0	1	0	1	0
inflammation, embolic		0	0	2	0	2	0	0	0
	- minimal	0	0	1	0	1	0	0	0
	- mild	0	0	1	0	1	0	0	0
inflammation, subacute		1	0	1	1	0	0	3	0
	- minimal	0	0	0	1	0	0	3	0
	- mild	1	0	1	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	1	0
heart		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
macrophages, pigmented	- mild	0	0	0	0	1	0	0	0
mesothelioma, malignant, primary		0	1	1	0	0	0	0	0
mineralization, myofiber		2	0	3	0	1	0	0	0
	- minimal	0	0	1	0	1	0	0	0
	- mild	1	0	2	0	0	0	0	0
	- moderate	1	0	0	0	0	0	0	0
mineralization, vascular		5	0	5	1	1	0	3	0
	- minimal	1	0	1	1	1	0	2	0
	- mild	4	0	4	0	0	0	1	0
	- mild	0	0	1	0	0	0	0	0
necrosis		0	0	0	0	1	0	0	0
osteosarcoma, malignant, secondary		2	2	0	0	0	0	0	0
polyarteritis		2	1	0	0	0	0	0	0
	- minimal	0	1	0	0	0	0	0	0
	- mild	0	0	2	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	3	1	0	0	1	0
thrombus		0	0	1	0	0	0	0	0
	- minimal	0	0	2	1	0	0	1	0
	- mild	0	0	1	0	0	0	0	0
within normal limits		14	5	8	1	15	3	9	0
joint, tibiofemoral		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hyperplasia/hypertrophy, synovial		1	0	1	0	0	0	0	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	0	0	1	0	0	0	0	0
Number of Animals Examined		43	22	49	16	45	20	71	4
joint, tibiofemoral		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
inflammation, chronic		0	1	1	0	0	0	1	0
	- minimal	0	1	0	0	0	0	0	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	1	0	0	0
within normal limits		41	21	47	16	44	20	70	4

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
kidneys		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)		
abscess	- minimal	0	0	0	0	0	0	1	0		
adenoma, tubular cell, benign, primary		1	0	1	0	0	0	0	0		
bacterial colonies	- mild	0	0	1	0	1	0	1	0		
carcinoma, tubular cell, malignant, primary		0	0	0	0	0	0	1	0		
cyst		2	1	1	1	5	0	1	1		
	- minimal	0	0	0	0	0	0	1	0		
	- mild	2	1	0	1	2	0	0	0		
	- moderate	0	0	1	0	2	0	0	1		
	- severe	0	0	0	0	1	0	0	0		
dilatation, tubular		2	0	2	0	0	0	5	0		
	- minimal	0	0	0	0	0	0	3	0		
	- mild	1	0	2	0	0	0	2	0		
	- moderate	1	0	0	0	0	0	0	0		
hematopoiesis, extramedullary	- mild	0	0	0	0	1	0	0	0		
kidneys		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)		
hyaline, droplets, increased		0	0	3	0	2	0	0	0		
	- mild	0	0	0	0	2	0	0	0		
	- moderate	0	0	1	0	0	0	0	0		
	- severe	0	0	2	0	0	0	0	0		
hydronephrosis, bilateral		1	0	2	0	0	0	3	0		
	- minimal	0	0	0	0	0	0	1	0		
	- mild	1	0	1	0	0	0	2	0		
	- severe	0	0	1	0	0	0	0	0		
hydronephrosis, unilateral		1	0	4	0	1	0	3	0		
	- minimal	1	0	1	0	0	0	2	0		
	- mild	0	0	3	0	1	0	1	0		
hyperplasia, transitional cell		1	0	0	0	1	0	0	0		
	- minimal	0	0	0	0	1	0	0	0		
	- mild	1	0	0	0	0	0	0	0		
hyperplasia, tubular		1	1	0	1	1	0	0	0		
	- minimal	0	1	0	1	1	0	0	0		
	- mild	1	0	0	0	0	0	0	0		
increased adipocytes	- minimal	0	0	0	0	0	0	1	0		
infarct	- mild	0	0	0	0	1	0	0	0		
infiltration, lymphocytic	- minimal	2	0	0	0	2	0	1	0		
inflammation, chronic		0	1	2	0	0	0	0	0		
	- minimal	0	1	0	0	0	0	0	0		
	- mild	0	0	1	0	0	0	0	0		
	- moderate	0	0	1	0	0	0	0	0		
kidneys		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)		
inflammation, embolic		0	0	0	0	1	0	1	0		
	- mild	0	0	0	0	1	0	0	0		
	- moderate	0	0	0	0	0	0	1	0		
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0		
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0		
lipoma, benign, primary		0	0	0	0	0	0	1	0		
liposarcoma, malignant, secondary		0	0	1	0	0	0	0	0		
lymphoma, malignant, multicentric		2	0	0	0	1	0	0	0		
mineralization	- mild	0	0	0	0	0	0	1	0		
mineralization, pelvic		2	2	1	0	4	0	0	0		
	- minimal	1	1	0	0	1	0	0	0		
	- mild	1	1	1	0	3	0	0	0		
mineralization, tubular		4	0	4	0	2	0	4	0		
	- minimal	1	0	1	0	2	0	2	0		
	- mild	2	0	2	0	0	0	1	0		
	- moderate	1	0	1	0	0	0	1	0		
mineralization, vascular	- minimal	1	0	1	0	0	0	0	0		
necrosis, papillary	- mild	0	0	0	0	0	0	1	0		
nephropathy, chronic progressive		37	20	46	16	39	20	55	4		
	- minimal	10	3	17	3	6	4	20	1		
	- mild	16	11	17	10	25	11	30	3		
	- moderate	7	3	7	1	7	4	4	0		
	- severe	4	3	5	2	1	1	1	0		

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
kidneys		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
pigment, tubular		10	3	4	1	11	1	25	2
- minimal		9	3	4	1	10	1	20	2
- mild		1	0	0	0	1	0	5	0
pyelitis		2	1	2	1	2	1	2	0
- minimal		1	1	0	0	1	1	2	0
- mild		1	0	1	1	1	0	0	0
- moderate		0	0	1	0	0	0	0	0
pyelonephritis, bilateral		0	0	0	0	0	0	2	0
pyelonephritis, unilateral		1	1	0	0	1	0	2	0
- minimal		0	0	0	0	0	0	1	0
- mild		0	1	0	0	1	0	1	0
- moderate		1	0	0	0	0	0	0	0
renal mesenchymal tumor, malignant, primary		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	1	0	0	0
schwannoma, malignant, primary		0	0	0	0	1	0	0	0
thrombus		0	0	2	0	1	0	0	0
- minimal		0	0	0	0	1	0	0	0
- mild		0	0	2	0	0	0	0	0
- moderate		0	0	0	0	0	0	3	0
vacuolation, tubular		2	1	3	0	1	0	8	0
within normal limits									
lacrimal glands, exorbital		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
atrophy		1	2	2	1	1	0	3	0
- minimal		1	0	1	0	1	0	0	0
- mild		0	2	1	1	0	0	1	0
- moderate		0	0	0	0	0	0	2	0
infiltration, lymphocytic		18	12	23	15	18	13	30	2
- minimal		14	8	21	5	15	8	27	1
- mild		4	4	2	8	3	5	3	1
- moderate		0	0	0	2	0	0	0	0
inflammation, chronic-active		0	0	0	0	1	0	0	0
- severe		0	0	0	0	0	0	2	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	1	0	0	0
metaplasia, harderian		26	13	27	11	19	10	31	3
- minimal		5	5	12	3	7	2	7	2
- mild		13	6	14	6	10	7	12	1
- moderate		8	1	1	2	2	1	12	0
- severe		0	1	0	0	0	0	0	0
pigment, increased		0	0	0	1	0	0	1	0
vacuolation		0	0	1	0	1	0	0	0
- minimal		0	0	0	0	1	0	0	0
- mild		0	0	1	0	0	0	0	0
within normal limits		12	4	15	1	16	5	25	1
large intestine, cecum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
dilatation, gland/lumen		0	0	1	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
inflammation, acute		1	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
inflammation, granulomatous		0	1	0	0	0	0	0	0
- mild		0	1	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
polyarteritis		1	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	0	0
within normal limits		41	21	46	16	45	20	70	4
large intestine, colon		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
inflammation, embolic		0	0	1	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
leiomyoma, benign, primary		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	0	0
within normal limits		42	22	46	16	44	20	71	4
large intestine, rectum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hemorrhage		1	0	0	0	0	0	0	0
- mild		1	0	0	0	0	0	0	0
inflammation, embolic		0	0	1	0	0	0	0	0
- mild		0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
polyarteritis		1	0	0	0	0	0	0	0
- mild		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	0	0
sarcoma, undifferentiated, malignant, secondary		0	0	0	0	1	0	0	0
within normal limits		40	22	46	16	44	20	70	4

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

(b) (4)

Study Number 900-063

A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE

Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
liver		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, hepatocellular, benign, primary		1	0	0	1	1	1	6	0
angiectasis	- minimal	4	0	0	1	4	7	3	2
bacterial colonies	- minimal	2	0	0	0	0	0	0	0
carcinoma, hepatocellular, malignant, primary		1	0	3	0	2	1	4	0
cyst, biliary		0	0	0	0	0	0	1	1
	- minimal	0	0	0	0	0	0	0	1
	- mild	0	0	0	0	0	0	1	0
cyst, nos	- moderate	1	0	0	0	0	0	0	0
degeneration, cystic, focal		5	7	11	6	11	18	32	4
	- minimal	4	5	9	5	7	8	20	3
	- mild	1	2	2	1	3	9	10	1
	- moderate	0	0	0	0	1	1	2	0
fibrosis		1	1	0	0	0	0	2	0
	- minimal	0	1	0	0	0	0	1	0
	- mild	1	0	0	0	0	0	1	0
focus of cellular alteration, basophilic		8	2	9	3	15	17	34	3
	- minimal	6	2	7	1	8	9	16	0
	- mild	2	0	2	1	7	7	11	2
	- moderate	0	0	0	1	0	1	6	0
	- severe	0	0	0	0	0	0	1	1
focus of cellular alteration, clear		0	9	9	7	9	12	21	4
	- minimal	0	7	3	2	8	2	12	2
	- mild	0	1	4	3	1	4	8	0
	- moderate	0	1	2	1	0	2	1	1
	- severe	0	0	0	1	0	4	0	1
focus of cellular alteration, eosinophilic		1	3	6	4	8	18	30	3
	- minimal	1	1	4	3	5	4	11	0
	- mild	0	2	1	0	2	12	12	1
	- moderate	0	0	1	1	1	2	4	1
	- severe	0	0	0	0	0	0	3	1
focus of cellular alteration, mixed		0	0	0	0	0	0	3	0
	- minimal	0	0	0	0	0	0	1	0
	- moderate	0	0	0	0	0	0	2	0
hematopoiesis, extramedullary		6	2	15	7	13	10	36	1
	- minimal	4	2	10	7	10	9	18	1
	- mild	2	0	5	0	1	1	14	0
	- moderate	0	0	0	0	2	0	3	0
	- severe	0	0	0	0	0	0	1	0
hyperplasia, bile duct		17	14	26	8	16	16	33	3
	- minimal	11	10	10	4	5	6	20	1
	- mild	6	3	16	4	11	10	11	2
	- moderate	0	1	0	0	0	0	2	0
hypertrophy, hepatocyte, centrilobular		0	0	0	0	2	0	46	4
	- minimal	0	0	0	0	2	0	17	1
	- mild	0	0	0	0	0	0	22	3
	- moderate	0	0	0	0	0	0	6	0
	- severe	0	0	0	0	0	0	1	0
hypertrophy/hyperplasia, kupffer cell		1	0	5	0	1	0	8	0
	- minimal	1	0	5	0	0	0	6	0
	- mild	0	0	0	0	1	0	2	0
	- severe	0	0	0	0	1	0	0	0
infarct, focal		0	0	0	0	0	0	1	0
infiltration, lymphocytic	- minimal	0	0	0	0	0	0	1	0
infiltration, mononuclear cell		31	22	39	15	37	20	52	3
	- minimal	30	21	36	15	32	19	46	3
	- mild	1	1	2	0	5	1	6	0
	- moderate	0	0	1	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
necrosis		3	1	5	0	1	0	3	0
	- minimal	1	1	2	0	1	0	2	0
	- mild	1	0	2	0	0	0	1	0
	- moderate	1	0	1	0	0	0	0	0
necrosis, focal	- minimal	1	0	0	0	0	0	0	0

DCS - Died or euthanized on study

SNC - Scheduled necropsy

()- Number observed

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
liver		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
necrosis, hepatocytes, centrilobular		2	0	6	0	4	0	6	0
	- minimal	0	0	0	0	2	0	3	0
	- mild	0	0	2	0	0	0	1	0
	- moderate	2	0	3	0	2	0	1	0
	- severe	0	0	1	0	0	0	1	0
necrosis, individual hepatocyte pigment, increased kupffer cell		0	0	0	0	0	0	2	0
	- minimal	1	0	0	0	5	0	21	0
	- mild	1	0	0	0	4	0	17	0
	- moderate	0	0	0	0	1	0	4	0
polyarteritis		0	0	1	0	0	0	0	0
proliferation, intimal		0	0	0	0	0	1	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	3	0	2	0	0	0
sarcoma, undifferentiated, malignant, secondary		0	0	0	0	1	0	0	0
vacuolation, centrilobular		0	0	6	0	7	0	24	2
	- minimal	0	0	1	0	1	0	6	0
	- mild	0	0	2	0	5	0	14	2
	- moderate	0	0	2	0	1	0	3	0
	- severe	0	0	1	0	0	0	1	0
vacuolation, focal		0	0	0	1	0	0	0	0
vacuolation, hepatocellular		2	5	23	3	24	17	48	3
	- minimal	1	3	18	3	20	10	28	2
	- mild	0	2	5	0	4	6	17	1
	- moderate	1	0	0	0	0	1	3	0
vacuolation, periportal		3	3	8	3	5	1	8	0
	- minimal	1	2	4	1	2	1	5	0
	- mild	1	0	3	2	2	0	2	0
	- moderate	1	1	1	0	1	0	1	0
within normal limits		2	0	2	0	2	0	0	0
lung		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
carcinoma, hepatocellular, malignant, secondary		0	0	1	0	0	1	0	0
cholesterol clefts		0	1	0	0	3	0	5	0
edema		1	0	0	0	0	0	0	0
fibrosarcoma, malignant, secondary		0	0	0	0	0	0	1	0
hemorrhage		3	0	3	1	2	0	1	0
	- minimal	1	0	0	1	2	0	1	0
	- mild	2	0	2	0	0	0	0	0
	- moderate	0	0	1	0	0	0	0	0
hibernoma, malignant, primary		0	0	1	0	0	0	0	0
histiocytosis		0	0	1	0	0	0	0	0
histiocytosis, alveolar		10	5	14	2	10	9	48	4
	- minimal	6	5	11	2	10	6	18	2
	- mild	4	0	3	0	0	3	23	2
	- moderate	0	0	0	0	0	0	7	0
hyperplasia, type ii cell		0	0	0	0	1	2	1	0
	- mild	0	0	0	0	1	1	0	0
	- moderate	0	0	0	0	0	0	1	0
	- severe	0	0	0	0	0	1	0	0
infiltration, lymphocytic		0	0	0	0	1	0	0	0
inflammation, acute		3	0	1	0	0	0	1	0
	- minimal	1	0	1	0	0	0	0	0
	- mild	1	0	0	0	0	0	1	0
	- severe	1	0	0	0	0	0	0	0
inflammation, chronic		0	0	0	0	1	0	0	0
inflammation, embolic		0	0	1	0	0	0	0	0
inflammation, granulomatous		1	0	0	0	0	0	0	0
inflammation, subacute		1	0	0	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0
lipidosis, alveolar		0	0	0	0	0	0	6	1
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	4	1
	- severe	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	0	0
macrophages, pigmented		1	0	4	0	2	0	0	0
	- minimal	1	0	3	0	2	0	0	0
	- mild	0	0	1	0	0	0	0	0
mesothelioma, malignant, secondary		0	1	1	0	0	0	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
lung		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
mineralization		1	0	1	0	0	0	0	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	1	0	0	0	0	0	0	0
	- minimal	0	0	0	0	1	0	0	0
mineralization, vascular		0	0	0	0	1	0	0	0
osteosarcoma, malignant, secondary		0	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	2	0	0	0
schwannoma, malignant, secondary		0	0	0	0	0	0	1	0
thrombus	- mild	0	0	1	0	0	0	0	0
within normal limits		25	15	30	13	26	8	18	0
lymph node, axillary		(5)	(0)	(4)	(0)	(4)	(3)	(9)	(3)
erythrocytosis/erythrophagocytosis, sinus	- mild	0	0	0	0	1	0	1	0
histiocytosis, sinus	- mild	0	0	0	0	0	0	1	0
hyperplasia, lymphocyte/plasmacyte		0	0	1	0	1	1	1	0
	- moderate	0	0	1	0	0	1	1	0
	- severe	0	0	0	0	1	0	0	0
inflammation, acute	- mild	0	0	0	0	0	0	1	0
macrophages, pigmented	- mild	0	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		5	0	2	0	3	2	6	3
lymph node, cervical		(0)	(0)	(1)	(0)	(2)	(0)	(1)	(0)
hyperplasia, lymphocyte/plasmacyte	- mild	0	0	0	0	1	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	1	0	0	0
lymph node, cervical		(0)	(0)	(1)	(0)	(2)	(0)	(1)	(0)
within normal limits		0	0	1	0	0	0	0	0
lymph node, hepatic		(0)	(0)	(1)	(0)	(2)	(2)	(1)	(0)
hyperplasia, lymphocyte/plasmacyte		0	0	0	0	1	1	0	0
	- mild	0	0	0	0	0	1	0	0
	- moderate	0	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	1	0	0	0
within normal limits		0	0	1	0	0	1	1	0
lymph node, iliac		(0)	(1)	(0)	(0)	(3)	(1)	(3)	(0)
dilatation, sinus		0	1	0	0	3	0	0	0
	- mild	0	1	0	0	2	0	0	0
	- moderate	0	0	0	0	1	0	0	0
erythrocytosis/erythrophagocytosis, sinus	- mild	0	0	0	0	1	0	0	0
hemangiosarcoma, malignant, multicentric		0	1	0	0	0	0	0	0
hyperplasia, generalized lymphoid	- mild	0	0	0	0	0	0	1	0
hyperplasia, lymphocyte/plasmacyte		0	1	0	0	2	1	1	0
	- mild	0	1	0	0	1	0	0	0
	- moderate	0	0	0	0	1	1	0	0
	- severe	0	0	0	0	0	0	1	0
macrophages, pigmented	- minimal	0	0	0	0	1	0	0	0
within normal limits		0	0	0	0	0	0	1	0
lymph node, inguinal		(0)	(0)	(4)	(0)	(5)	(7)	(6)	(2)
carcinoma, squamous cell, malignant, secondary		0	0	0	0	0	0	1	0
hemangiosarcoma, malignant, multicentric		0	0	0	0	1	0	0	0
hyperplasia, lymphocyte/plasmacyte		0	0	1	0	1	3	2	0
	- mild	0	0	1	0	0	3	2	0
	- moderate	0	0	0	0	1	0	0	0
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
within normal limits		0	0	3	0	3	4	3	2
lymph node, mandibular		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
depletion, lymphoid	- minimal	0	0	0	0	0	0	2	0
erythrocytosis/erythrophagocytosis, sinus		1	0	3	1	3	0	1	0
	- minimal	1	0	1	1	3	0	0	0
	- mild	0	0	2	0	0	0	1	0
histiocytosis, sinus		0	0	0	0	0	0	2	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	1	0
hyperplasia, lymphocyte/plasmacyte		5	3	7	1	9	3	11	1
	- minimal	3	0	3	0	1	0	3	0
	- mild	1	3	3	1	4	3	7	1
	- moderate	1	0	1	0	2	0	1	0
	- severe	0	0	0	0	2	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	0	0

Summary of Microscopic Observations - MALE

Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
lymph node, mandibular		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
macrophages, pigmented		0	0	0	1	3	0	1	0
	- minimal	0	0	0	0	3	0	1	0
	- mild	0	0	0	1	0	0	0	0
sarcoma, histiocytic, malignant, multicentric within normal limits		0	0	0	0	2	0	0	0
		34	19	40	14	28	17	53	3
lymph node, mediastinal		(2)	(1)	(3)	(0)	(1)	(0)	(5)	(0)
erythrocytosis/erythrophagocytosis, sinus		1	1	0	0	0	0	2	0
	- mild	1	0	0	0	0	0	2	0
	- severe	0	1	0	0	0	0	0	0
lymphoma, malignant, multicentric macrophages, pigmented		0	0	0	0	0	0	1	0
	- minimal	1	1	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	1	0
	- severe	0	1	0	0	0	0	0	0
mesothelioma, malignant, secondary sarcoma, histiocytic, malignant, multicentric within normal limits		0	1	2	0	0	0	0	0
		0	0	1	0	1	0	0	0
		0	0	0	0	0	0	1	0
lymph node, mesenteric		(42)	(22)	(49)	(16)	(44)	(20)	(71)	(4)
carcinoma, rete testis, malignant, secondary depletion, lymphoid		0	0	0	0	0	0	1	0
	- minimal	1	0	3	0	1	0	8	0
	- mild	0	0	3	0	1	0	7	0
	- severe	1	0	0	0	0	0	1	0
lymph node, mesenteric		(42)	(22)	(49)	(16)	(44)	(20)	(71)	(4)
dilatation, sinusoidal	- mild	0	1	0	0	0	0	0	0
erythrocytosis/erythrophagocytosis, sinus		1	1	9	2	5	0	5	0
	- minimal	0	0	3	1	3	0	2	0
	- mild	0	1	4	1	2	0	2	0
	- moderate	1	0	1	0	0	0	1	0
	- severe	0	0	1	0	0	0	0	0
fibrosis	- moderate	0	0	0	0	0	0	1	0
hemangioma, benign, multicentric histiocytosis, sinus		0	0	1	0	0	0	0	0
	- minimal	1	0	1	0	2	1	9	0
	- mild	0	0	0	0	0	0	3	0
hyperplasia, lymphocyte/plasmacyte		1	0	1	0	0	0	0	0
	- mild	1	0	0	0	0	0	0	0
	- severe	0	0	1	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric macrophages, pigmented		2	0	0	0	1	0	0	0
	- minimal	6	8	18	7	19	8	33	1
	- mild	6	6	13	4	15	8	22	1
	- severe	0	2	5	3	4	0	11	0
mesothelioma, malignant, secondary mineralization, vascular	- mild	0	0	1	0	0	0	1	0
	- moderate	1	0	1	0	0	0	0	0
polyarteritis		0	0	1	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	2	0	0	0
lymph node, mesenteric		(42)	(22)	(49)	(16)	(44)	(20)	(71)	(4)
thrombus	- mild	0	0	1	0	0	0	0	0
within normal limits		30	14	25	9	19	12	25	3
lymph node, popliteal		(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
hyperplasia, lymphocyte/plasmacyte	- mild	0	0	0	0	1	0	0	0
lymph node, renal		(1)	(0)	(1)	(0)	(2)	(0)	(3)	(0)
abscess	- moderate	0	0	0	0	1	0	0	0
erythrocytosis/erythrophagocytosis, sinus	- moderate	1	0	0	0	0	0	0	0
hyperplasia, lymphocyte/plasmacyte		0	0	1	0	1	0	0	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	0	0	0	0	1	0	0	0
macrophages, pigmented	- mild	0	0	0	0	0	0	1	0
renal mesenchymal tumor, malignant, secondary within normal limits		1	0	0	0	0	0	0	0
		0	0	0	0	0	0	2	0
mammary gland		(41)	(22)	(49)	(16)	(44)	(20)	(70)	(4)
adenocarcinoma, malignant, primary feminization		0	0	0	0	1	1	2	0
	- minimal	20	16	31	11	28	17	43	4
	- mild	10	7	22	3	20	10	33	2
	- severe	10	9	9	8	8	7	10	2
fibroadenoma, benign, primary		0	0	1	0	3	1	5	1

DOS- Died or euthanized on study

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
mammary gland		(41)	(22)	(49)	(16)	(44)	(20)	(70)	(4)
galactocele		10	4	6	2	9	5	9	1
	- minimal	5	3	3	1	4	2	3	1
	- mild	4	0	3	1	4	2	5	0
	- moderate	1	1	0	0	1	1	1	0
hyperplasia, lobular		1	1	1	0	1	2	2	0
	- minimal	0	1	1	0	1	0	1	0
	- mild	1	0	0	0	0	2	0	0
	- moderate	0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		2	0	0	0	0	0	0	0
within normal limits		17	4	18	5	13	3	26	0
mesentery/peritoneum		(0)	(0)	(1)	(0)	(0)	(0)	(1)	(0)
granulation tissue	- severe	0	0	0	0	0	0	1	0
mesothelioma, malignant, secondary		0	0	1	0	0	0	0	0
multicentric neoplasm		(4)	(1)	(5)	(0)	(4)	(0)	(6)	(0)
hemangioma, benign, multicentric		0	0	1	0	0	0	1	0
hemangiosarcoma, malignant, multicentric		1	1	0	0	1	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	1	0	1	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	4	0	2	0	1	0
nerve, sciatic		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration, axonal/myelin		29	20	33	16	27	19	55	4
	- minimal	21	9	24	7	17	10	33	1
	- mild	8	11	9	9	8	9	22	3
	- moderate	0	0	0	0	2	0	0	0
infiltration, mononuclear cell	- minimal	0	0	0	0	1	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mineralization		1	0	1	0	1	0	3	0
	- minimal	0	0	1	0	1	0	3	0
	- moderate	1	0	0	0	0	0	0	0
within normal limits		14	2	15	0	18	1	15	0
pancreas		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, islet cell, benign, primary		2	3	2	0	2	0	6	0
alteration, basophilic focal		2	3	3	2	3	6	11	0
	- minimal	1	3	2	2	1	6	10	0
	- mild	1	0	1	0	2	0	1	0
atrophy, acinar		3	7	1	3	6	1	6	1
	- minimal	3	6	0	2	5	1	2	0
	- mild	0	1	1	0	1	0	3	0
	- moderate	0	0	0	1	0	0	0	1
	- severe	0	0	0	0	0	0	1	0
carcinoma, islet cell, malignant, primary		1	0	0	0	0	0	0	0
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
cyst	- minimal	0	0	0	0	1	0	2	0
depletion, secretory		0	0	7	0	2	0	9	0
	- minimal	0	0	2	0	1	0	4	0
	- mild	0	0	5	0	1	0	5	0
	- moderate	0	0	0	0	0	0	0	0
	- severe	0	0	0	0	0	0	0	0
fibrosis	- minimal	0	0	0	0	1	0	0	0
hyperplasia, acinar cell, focal		1	0	1	2	0	1	4	0
	- minimal	0	0	0	1	0	1	3	0
	- mild	0	0	1	1	0	0	1	0
	- moderate	1	0	0	0	0	0	0	0
hyperplasia, islet cell		2	0	0	2	2	2	1	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	1	0	0	1	1	1	0	0
	- moderate	1	0	0	1	0	0	0	0
	- severe	0	0	0	0	1	1	0	0
infiltration, mononuclear cell		8	6	6	4	4	5	3	0
	- minimal	8	6	6	4	3	5	3	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	0	0	0	0	0	0
inflammation, peritoneal	- minimal	0	0	1	0	0	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	0	0
mesothelioma, malignant, secondary		0	0	1	0	0	0	1	0

(b) (4)
 Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE
 Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
pancreas		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
mineralization, vascular		2	0	0	0	0	0	0	0
- minimal		1	0	0	0	0	0	0	0
- mild		1	0	0	0	0	0	0	0
necrosis, single cell pigment		1	0	0	0	0	0	0	0
- minimal		13	7	8	8	11	4	10	1
- mild		12	6	8	7	9	4	9	1
- moderate		1	1	0	1	1	0	1	0
- severe		0	0	0	0	1	0	0	0
polyarteritis		1	1	1	1	0	1	0	0
- minimal		0	0	1	1	0	0	0	0
- mild		0	0	0	0	0	1	0	0
- moderate		0	1	0	0	0	0	0	0
- severe		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric within normal limits		0	0	2	0	1	0	1	0
		19	6	26	3	25	7	32	3
parathyroid glands		(37)	(18)	(38)	(12)	(39)	(15)	(59)	(4)
adenoma, benign, primary cyst		1	0	0	0	0	0	0	0
- mild		0	1	0	0	0	0	1	0
hyperplasia, diffuse		4	1	8	0	1	0	1	0
- minimal		3	1	2	0	0	0	1	0
- mild		1	0	3	0	1	0	0	0
- moderate		0	0	3	0	0	0	0	0
hyperplasia, focal		2	3	7	3	7	2	5	2
- minimal		2	2	5	1	6	0	4	1
- mild		0	1	1	2	1	2	1	1
- severe		0	0	1	0	0	0	0	0
- minimal		0	1	0	0	0	0	1	0
infiltration, lymphocytic lymphoma, malignant, multicentric within normal limits		1	0	0	0	0	0	0	0
		29	14	23	9	31	13	51	2
peyers patch		(43)	(22)	(49)	(16)	(44)	(20)	(71)	(4)
depletion, lymphoid		1	0	2	0	1	0	1	0
- minimal		1	0	1	0	1	0	1	0
- mild		0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric secretory product, increased within normal limits		1	0	0	0	1	0	0	0
- mild		0	0	0	0	0	0	1	0
		41	22	47	16	42	20	69	4
pituitary gland		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, pars distalis, benign, primary		21	11	15	7	15	7	14	1
adenoma, pars intermedia, benign, primary cholesterol clefts		0	0	0	1	0	0	1	0
- mild		0	0	0	0	1	0	0	0
cyst		5	3	3	0	2	5	2	1
- minimal		3	0	1	0	0	1	0	0
- mild		0	1	2	0	1	3	0	1
- moderate		2	2	0	0	1	1	2	0
hyperplasia, pars distalis		10	6	16	7	14	7	26	3
- minimal		3	1	4	1	3	3	9	1
- mild		4	3	6	3	6	1	9	1
- moderate		2	2	5	1	4	1	5	1
- severe		1	0	1	2	1	2	3	0
leukemia, malignant, multicentric lymphoma, malignant, multicentric mineralization		0	0	0	0	1	0	0	0
- mild		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric within normal limits		0	0	0	0	0	0	0	1
		8	4	17	1	16	5	29	0
preputial glands		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
inflammation, chronic-active		0	0	0	0	0	0	1	0
- mild		0	0	0	0	0	0	1	0
prostate gland		(43)	(22)	(48)	(16)	(44)	(20)	(71)	(4)
atrophy		33	19	33	15	35	17	57	4
- minimal		5	2	8	1	3	0	7	0
- mild		20	7	21	11	29	15	31	3
- moderate		8	10	4	3	3	2	19	1
hyperplasia		0	0	0	0	0	2	0	0
infiltration, lymphocytic		2	0	3	0	3	2	1	0
- minimal		2	0	3	0	3	1	1	0
- mild		0	0	0	0	0	1	0	0

(b) (4)

Study Number 900-063

A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
prostate gland		(43)	(22)	(48)	(16)	(44)	(20)	(71)	(4)
inflammation, acute		0	0	1	0	0	1	0	0
	- moderate	0	0	0	0	0	1	0	0
	- severe	0	0	1	0	0	0	0	0
inflammation, chronic		1	2	0	0	2	0	1	0
	- minimal	1	1	0	0	2	0	1	0
	- moderate	0	1	0	0	0	0	0	0
inflammation, chronic-active		6	1	9	3	5	5	17	1
	- minimal	0	0	0	2	0	0	2	0
	- mild	3	0	1	0	2	3	5	1
	- moderate	2	1	7	1	2	1	8	0
	- severe	1	0	1	0	1	1	2	0
inflammation, subacute		0	0	2	0	0	0	2	0
	- mild	0	0	0	0	0	0	2	0
	- moderate	0	0	2	0	0	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	0	0
secretory product, increased	- mild	0	0	0	0	0	0	1	0
within normal limits		5	1	4	0	6	1	1	0
salivary gland, mandibular		(43)	(22)	(49)	(16)	(44)	(20)	(71)	(4)
atrophy	- minimal	0	0	0	0	2	0	0	0
infiltration, lymphocytic	- minimal	1	0	0	0	1	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		41	22	48	16	41	20	71	4
salivary gland, parotid		(43)	(22)	(49)	(16)	(45)	(20)	(70)	(4)
atrophy		9	1	17	1	13	1	28	0
	- minimal	3	1	5	1	5	1	11	0
	- mild	5	0	12	0	7	0	14	0
	- moderate	1	0	0	0	1	0	3	0
infiltration, lymphocytic	- minimal	0	0	1	0	1	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mineralization	- minimal	0	0	1	0	0	0	0	0
osteosarcoma, malignant, secondary		0	0	1	0	0	0	0	0
within normal limits		34	21	31	15	31	19	42	4
salivary gland, sublingual		(43)	(22)	(49)	(15)	(43)	(20)	(71)	(4)
atrophy	- minimal	0	0	1	0	0	0	0	0
cyst	- minimal	0	0	1	0	0	0	0	0
hypertrophy, basophilic focal	- mild	0	0	0	0	1	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
mineralization, vascular	- minimal	0	0	1	0	0	0	0	0
within normal limits		42	22	46	15	42	20	71	4
seminal vesicles		(43)	(22)	(48)	(16)	(45)	(20)	(71)	(4)
abscess	- moderate	1	0	0	0	0	0	0	0
atrophy		8	1	10	2	12	2	25	0
	- minimal	1	0	2	1	4	1	11	0
	- mild	6	1	8	1	8	1	14	0
	- moderate	1	0	0	0	0	0	0	0
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
depletion, secretory		8	1	11	2	7	1	15	0
	- minimal	0	0	0	0	1	0	2	0
	- mild	1	0	3	0	1	0	4	0
	- moderate	5	0	8	0	5	1	9	0
	- severe	2	1	0	2	0	0	0	0
dilatation	- mild	0	0	1	0	0	0	2	0
hematopoiesis, extramedullary	- minimal	0	0	0	0	1	0	0	0
hemorrhage	- minimal	0	0	0	0	0	0	1	0
hyperplasia, epithelial cell	- minimal	0	0	0	0	2	0	0	0
infiltration, lymphocytic	- mild	1	0	0	0	0	0	0	0
inflammation, acute	- mild	0	0	1	0	0	0	0	0
inflammation, chronic	- minimal	0	0	0	1	0	0	0	0
inflammation, chronic-active		1	0	1	0	0	2	5	0
	- minimal	0	0	1	0	0	0	3	0
	- mild	1	0	0	0	0	1	1	0
	- moderate	0	0	0	0	0	1	1	0

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
seminal vesicles		(43)	(22)	(48)	(16)	(45)	(20)	(71)	(4)
inflammation, subacute		0	0	1	0	0	0	2	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	1	0	0	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	1	0
secretory product, increased within normal limits	- moderate	0	1	0	0	0	0	1	0
		30	20	35	14	29	17	37	4
skeletal muscle		(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
osteosarcoma, malignant, primary		0	0	0	0	1	0	0	0
skeletal muscle, biceps femoris		(40)	(22)	(47)	(16)	(45)	(20)	(71)	(4)
atrophy		5	2	6	6	8	8	18	2
	- minimal	3	1	6	5	4	7	9	2
	- mild	2	1	0	1	4	1	9	0
degeneration, myofiber		1	0	6	0	4	2	19	0
	- minimal	1	0	5	0	4	2	14	0
	- mild	0	0	1	0	0	0	4	0
	- moderate	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	2	0	0
inflammation, chronic		0	0	0	0	0	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mineralization, myofiber		1	0	1	0	0	0	0	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric within normal limits		0	0	1	0	0	0	0	0
		31	20	34	10	33	9	37	2
skeletal muscle, gastrocnemius		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
atrophy	- minimal	0	0	0	0	0	0	1	0
skin		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
abscess		1	0	1	0	2	1	0	0
	- moderate	1	0	1	0	0	0	0	0
	- severe	0	0	0	0	2	1	0	0
alopecia/hypotrichosis	- minimal	0	0	0	0	1	0	0	0
atrophy		1	0	5	0	5	0	9	0
	- minimal	1	0	3	0	5	0	7	0
	- mild	0	0	2	0	0	0	2	0
carcinoma, squamous cell, malignant, primary crust, serocellular		0	0	0	0	3	1	5	0
	- minimal	0	0	0	0	2	0	1	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	0	0	1	0	0	0
cyst, epidermal inclusion		0	1	1	0	1	0	3	0
	- mild	0	0	0	0	1	0	1	0
	- moderate	0	1	0	0	0	0	1	0
	- severe	0	0	1	0	0	0	1	0
edema		0	0	0	0	0	0	3	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	1	0
	- moderate	0	0	0	0	0	0	1	0
erosion/ulcer	- mild	0	0	0	0	0	0	1	0
fibrosis	- mild	0	0	1	0	0	0	0	0
hemorrhage	- moderate	0	0	0	0	0	0	1	0
hyperkeratosis		1	1	2	1	4	0	5	0
	- minimal	0	1	2	1	4	0	3	0
	- mild	1	0	0	0	0	0	2	0
hyperplasia, epidermal		0	0	1	0	4	1	1	0
	- minimal	0	0	0	0	1	0	0	0
	- mild	0	0	1	0	3	0	1	0
	- severe	0	0	0	0	0	1	0	0
infiltration, mononuclear cell		0	0	0	0	0	0	3	0
	- minimal	0	0	0	0	0	0	2	0
	- mild	0	0	0	0	0	0	1	0
inflammation, chronic-active	- mild	0	0	0	0	0	0	1	0
inflammation, subacute	- mild	0	0	0	0	1	0	0	0
keratoacanthoma, benign, primary		1	0	0	1	0	0	2	0

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
skin		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
papilloma, squamous cell, benign, primary		0	1	0	0	1	0	2	0
within normal limits		38	19	40	15	29	17	47	4
skin, subcutis		(7)	(0)	(13)	(1)	(6)	(9)	(22)	(3)
fibroma, benign, primary		3	0	7	0	4	7	14	3
fibrosarcoma, malignant, primary		1	0	0	0	0	0	0	0
fibrous histiocytoma, malignant, primary		0	0	1	0	0	0	2	0
hemangiosarcoma, malignant, multicentric		1	0	0	0	1	0	0	0
inflammation, acute	- moderate	0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
lipoma, benign, primary		0	0	0	1	0	1	0	0
liposarcoma, malignant, primary		0	0	1	0	0	0	0	0
lymphangiosarcoma, malignant, primary		1	0	0	0	0	0	0	0
necrosis	- severe	0	0	0	0	0	1	0	0
osteosarcoma, malignant, primary		0	0	1	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	0	0
sarcoma, undifferentiated, malignant, primary		0	0	1	0	0	0	1	0
schwannoma, malignant, primary		0	0	0	0	1	0	5	0
within normal limits		1	0	0	0	0	0	0	0
small intestine, duodenum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	1	0
sarcoma, undifferentiated, malignant, secondary		0	0	0	0	1	0	0	0
within normal limits		43	22	48	16	44	20	69	4
small intestine, ileum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
intussusception	- mild	1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	0	0
within normal limits		42	22	47	16	45	20	71	4
small intestine, jejunum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
diverticulum	- moderate	1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	0	0
within normal limits		42	22	47	16	45	20	70	4
spinal cord, cervical		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration, axonal/myelin		1	3	2	3	7	5	5	2
- minimal		1	1	2	2	7	5	5	2
- mild		0	2	0	1	0	0	0	0
gliosis	- minimal	0	0	0	0	0	0	1	0
mineralization	- minimal	0	0	0	0	0	1	0	0
within normal limits		42	19	47	13	38	14	65	2
spinal cord, lumbar		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
cyst	- mild	0	0	0	0	1	0	0	0
degeneration, axonal/myelin		11	16	17	12	21	18	42	4
- minimal		10	14	17	10	18	16	35	3
- mild		0	2	0	2	3	2	7	1
- severe		1	0	0	0	0	0	0	0
gliosis	- minimal	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	0	0	1	0	0	0
within normal limits		32	6	32	4	23	2	29	0
spinal cord, thoracic		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration, axonal/myelin		5	8	5	2	8	6	12	3
- minimal		5	6	5	2	7	6	12	3
- mild		0	2	0	0	1	0	0	0
leukemia, malignant, multicentric		0	0	0	0	1	0	0	0
mineralization, focal	- minimal	0	0	1	0	0	0	0	0
within normal limits		38	14	43	14	37	14	59	1
spleen		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adhesion/inflammation/fibrosis, capsule		0	0	1	0	0	0	1	0
- minimal		0	0	1	0	0	0	0	0
- mild		0	0	0	0	0	0	1	0
angiectasis	- moderate	0	0	0	0	0	0	1	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

(b) (4)
 Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
spleen		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
atrophy		2	0	3	0	2	0	3	0
	- minimal	0	0	3	0	0	0	2	0
	- mild	2	0	0	0	2	0	1	0
bacterial colonies	- minimal	1	0	0	0	0	0	0	0
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
depletion, lymphoid		7	0	14	0	10	0	27	0
	- minimal	3	0	6	0	4	0	10	0
	- mild	3	0	7	0	6	0	12	0
	- moderate	1	0	1	0	0	0	5	0
fibrosarcoma, malignant, primary		0	0	0	0	0	0	1	0
hemangioma, benign, multicentric		0	0	0	0	0	0	1	0
hemangioma, benign, primary		0	0	0	0	1	0	0	0
hemangiosarcoma, malignant, multicentric		0	0	0	0	0	0	1	0
hematopoiesis, extramedullary, increased		11	5	24	7	31	13	59	1
	- minimal	3	5	8	4	14	5	17	0
	- mild	5	0	8	3	15	7	31	1
	- moderate	3	0	7	0	1	1	10	0
	- severe	0	0	1	0	1	0	1	0
hyperplasia, lymphocyte/plasmacyte	- mild	0	0	1	0	0	0	0	0
hyperplasia, reactive red pulp/stromal		0	0	1	0	2	1	10	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	1	0	1	0	4	0
	- moderate	0	0	0	0	1	0	3	0
	- severe	0	0	0	0	0	1	2	0
infarct	- moderate	0	0	1	0	0	0	0	0
leiomyosarcoma, malignant, primary		0	0	0	0	0	0	1	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	0	0
macrophages, pigmented		16	3	10	4	25	6	47	2
	- minimal	11	3	8	4	12	3	18	1
	- mild	5	0	2	0	13	3	24	1
	- moderate	0	0	0	0	0	0	5	0
mesothelioma, malignant, secondary		0	0	1	0	0	0	1	0
necrosis	- minimal	1	0	0	0	0	0	0	0
polyarteritis		1	0	0	0	0	1	0	0
	- minimal	0	0	0	0	0	1	0	0
	- mild	1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	2	0	0	0
within normal limits		15	16	14	6	3	3	1	2
stomach, glandular		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
bacterial colonies	- moderate	0	0	1	0	0	0	0	0
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
dilatation, gland/lumen		8	3	5	6	10	8	23	0
	- minimal	6	3	5	6	9	6	19	0
	- mild	2	0	0	0	1	2	4	0
erosion/ulcer		0	0	4	0	2	0	1	0
	- minimal	0	0	1	0	2	0	0	0
	- mild	0	0	2	0	0	0	1	0
	- severe	0	0	1	0	0	0	0	0
fibrosis	- minimal	0	0	0	0	1	0	0	0
giant cells	- mild	1	0	0	0	0	0	0	0
hemorrhage	- minimal	0	0	0	0	1	0	0	0
inflammation, chronic	- minimal	0	1	0	0	0	0	0	0
inflammation, embolic		0	0	1	0	1	0	0	0
	- minimal	0	0	0	0	1	0	0	0
	- moderate	0	0	1	0	0	0	0	0
	- mild	0	0	1	0	0	0	0	0
inflammation, peritoneal		0	0	0	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	2	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
mineralization		2	0	5	0	0	0	1	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	5	0	0	0	0	0
	- moderate	2	0	0	0	0	0	0	0
polyarteritis	- minimal	1	0	0	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	2	0	0	0	1	0
within normal limits		32	19	37	10	33	12	43	4

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
stomach, nonglandular		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
cyst, keratin	- mild	0	0	0	0	0	1	0	0
edema	- mild	0	0	0	0	0	0	1	0
erosion/ulcer		3	0	1	0	1	0	2	0
	- mild	1	0	0	0	0	0	0	0
	- moderate	2	0	1	0	1	0	2	0
hyperplasia, epithelial, nonglandular	- mild	3	0	0	0	1	0	2	0
inflammation, subacute		1	0	0	0	1	0	1	0
	- minimal	0	0	0	0	1	0	0	0
	- mild	1	0	0	0	0	0	1	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
papilloma, squamous cell, benign, primary		1	0	1	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		36	22	46	16	42	19	65	4
tail		(0)	(0)	(0)	(1)	(1)	(2)	(1)	(0)
cyst, epidermal inclusion	- mild	0	0	0	0	0	1	0	0
erosion/ulcer	- moderate	0	0	0	0	1	0	1	0
hyperkeratosis	- severe	0	0	0	1	0	0	0	0
inflammation, chronic	- mild	0	0	0	0	0	1	0	0
inflammation, chronic-active	- mild	0	0	0	0	0	1	0	0
testes		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, interstitial cell, benign, primary		0	1	1	2	1	1	2	0
carcinoma, rete testis, malignant, primary		0	0	0	0	0	0	1	0
degeneration, spermatocyte/spermatid, bilateral	- minimal	0	0	0	0	1	0	3	0
degeneration, spermatocyte/spermatid, unilateral	- minimal	0	0	0	0	0	0	1	0
degeneration/atrophy, seminiferous tubules, bilateral		7	4	8	4	2	1	10	0
	- minimal	1	2	1	0	0	0	4	0
	- mild	1	0	0	1	2	1	0	0
	- moderate	0	0	2	1	0	0	1	0
	- severe	5	2	5	2	0	0	5	0
degeneration/atrophy, seminiferous tubules, unilateral		3	3	3	1	1	1	12	1
	- minimal	0	0	2	0	1	1	9	0
	- mild	0	0	0	0	0	0	1	1
	- moderate	1	1	0	0	0	0	0	0
	- severe	2	2	1	1	0	0	2	0
hyperplasia, interstitial cell		0	0	1	2	1	0	2	0
	- minimal	0	0	0	1	0	0	0	0
	- mild	0	0	1	0	1	0	1	0
	- moderate	0	0	0	1	0	0	0	0
	- severe	0	0	0	0	0	0	1	0
hyperplasia, mesothelial	- minimal	0	0	0	0	1	0	0	0
infiltration, lymphocytic		0	0	1	1	0	0	0	0
	- minimal	0	0	0	1	0	0	0	0
	- mild	0	0	1	0	0	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mesothelioma, malignant, secondary		0	0	1	0	0	0	1	0
mineralization	- mild	1	0	0	0	0	0	0	0
polyarteritis		7	6	3	2	1	1	0	0
	- minimal	2	1	1	0	0	1	0	0
	- mild	3	2	2	1	0	0	0	0
	- moderate	2	3	0	1	1	0	0	0
vacuolation, sertoli cell, bilateral	- minimal	0	0	0	0	0	0	1	0
within normal limits		28	12	34	8	38	17	42	3
thymus gland		(41)	(22)	(47)	(15)	(41)	(20)	(63)	(4)
carcinoma, rete testis, malignant, secondary		0	0	0	0	0	0	1	0
cyst		1	0	1	0	0	1	2	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	0	0	1	0	0	1	2	0
depletion, lymphoid		33	22	42	14	32	19	56	4
	- minimal	4	1	3	4	1	4	1	0
	- mild	18	14	24	6	19	13	23	3
	- moderate	7	6	8	4	8	2	23	1
	- severe	4	1	7	0	4	0	9	0
ectopic parathyroid	- minimal	0	0	0	0	0	1	0	0
fibrosarcoma, malignant, primary		0	0	0	0	0	0	2	0
hyperplasia, epithelial cell	- mild	0	1	0	0	0	0	0	0
hyperplasia, lymphoid	- mild	0	0	0	0	1	0	0	0
karyomegaly	- moderate	0	0	1	0	0	0	0	0

Summary of Microscopic Observations - MALE

Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
thymus gland		(41)	(22)	(47)	(15)	(41)	(20)	(63)	(4)
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	1	0	1	0
mineralization, vascular		1	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	1	0
	- moderate	1	0	0	0	0	0	0	0
	- mild	0	0	0	0	1	0	0	0
necrosis, lymphoid		0	0	0	0	1	0	0	0
osteosarcoma, malignant, secondary		0	0	0	0	1	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	1	0	1	0	0	0
sarcoma, undifferentiated, malignant, secondary		0	0	0	0	1	0	0	0
schwannoma, malignant, secondary		0	0	0	0	0	0	2	0
thrombus	- mild	0	0	1	0	0	0	1	0
within normal limits		6	0	4	1	4	1	2	0
thyroid gland		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, c-cell, benign, primary		4	2	2	0	3	6	3	1
adenoma, follicular cell, benign, primary		0	0	4	1	4	0	8	0
carcinoma, c-cell, malignant, primary		1	0	0	0	0	0	0	0
carcinoma, follicular cell, malignant, primary		0	1	0	1	1	1	1	1
cyst, follicular		1	0	1	0	3	1	9	2
	- minimal	1	0	1	0	2	1	6	0
	- mild	0	0	0	0	1	0	1	2
	- moderate	0	0	0	0	0	0	2	0
hyperplasia, c-cell, diffuse		2	7	4	9	8	9	14	2
	- minimal	2	6	4	7	5	5	10	1
	- mild	0	1	0	2	3	4	4	1
hyperplasia, c-cell, focal		6	7	8	2	6	7	6	1
	- minimal	2	5	4	1	2	2	3	1
	- mild	2	1	3	1	4	4	1	0
	- moderate	1	1	1	0	0	0	1	0
	- severe	1	0	0	0	0	1	1	0
hyperplasia, follicular cell		0	0	5	1	7	7	13	0
	- minimal	0	0	0	1	1	2	2	0
	- mild	0	0	1	0	1	2	2	0
	- moderate	0	0	2	0	3	3	6	0
	- severe	0	0	2	0	2	0	3	0
hypertrophy, follicular cell	- minimal	0	0	0	0	0	0	2	0
infiltration, lymphocytic	- minimal	1	1	0	0	1	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		2	0	0	0	0	0	0	0
within normal limits		29	6	29	6	21	4	35	0
tongue		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hyperkeratosis	- mild	1	0	0	0	0	0	0	0
infiltration, lymphocytic	- minimal	1	1	0	0	0	0	0	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
mineralization, vascular		1	0	1	0	0	0	0	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	1	0	0	0	0	0	0	0
polyarteritis	- mild	0	0	0	1	0	0	0	0
within normal limits		38	21	48	15	45	20	71	4
trachea		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration/necrosis, respiratory epithelium		6	0	1	0	1	0	16	0
	- minimal	3	0	0	0	0	0	1	0
	- mild	3	0	1	0	0	0	9	0
	- moderate	0	0	0	0	1	0	6	0
fibrosarcoma, malignant, secondary		0	0	0	0	0	0	1	0
hyperplasia, respiratory mucosa	- mild	1	0	0	0	0	0	0	0
hypertrophy, cartilage	- minimal	1	0	0	0	0	0	0	0
infiltration, lymphocytic	- minimal	1	1	1	0	1	0	1	0
inflammation, acute	- minimal	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		33	21	47	16	43	20	53	4

(b) (4)

Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - MALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
ureters		(43)	(19)	(49)	(16)	(45)	(20)	(71)	(4)
dilatation		15	2	18	5	11	6	20	1
- minimal		4	0	6	2	5	1	1	0
- mild		9	2	8	3	5	5	12	1
- moderate		2	0	4	0	1	0	6	0
- severe		0	0	0	0	0	0	1	0
- minimal		0	0	1	0	0	0	0	0
infiltration, lymphocytic		2	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	1	0
mesothelioma, malignant, secondary		0	0	2	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	0	0	0
within normal limits		27	17	30	11	34	14	50	3
urinary bladder		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
dilatation		4	0	2	0	0	0	5	1
- mild		3	0	2	0	0	0	4	1
- moderate		1	0	0	0	0	0	1	0
erosion/ulcer		0	0	1	0	0	0	0	0
exudate, luminal		0	0	0	0	0	1	0	0
hematopoiesis, extramedullary		0	0	0	0	1	0	0	0
hyperplasia, papillary/nodular transitional cell		0	0	1	0	0	0	0	0
hyperplasia, simple transitional cell		0	0	3	0	1	0	4	0
- minimal		0	0	2	0	0	0	2	0
- mild		0	0	1	0	0	0	2	0
- moderate		0	0	0	0	1	0	0	0
- minimal		1	0	0	0	1	1	0	0
infiltration, lymphocytic		0	0	0	0	0	1	0	0
inflammation, acute		4	0	3	0	1	1	13	0
inflammation, subacute		0	0	0	0	0	1	4	0
- mild		4	0	2	0	1	0	5	0
- moderate		0	0	1	0	0	0	3	0
- severe		0	0	0	0	0	0	1	0
leiomyosarcoma, malignant, primary		0	0	0	0	1	0	0	0
leukemia, large granular lymphocyte, malignant, multicentric		0	0	0	0	0	0	1	0
leukemia, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
macrophages, pigmented		0	0	0	0	0	0	1	0
- mild		0	0	0	0	0	0	1	0
mesothelioma, malignant, secondary		0	0	0	0	0	0	1	0
sarcoma, histiocytic, malignant, multicentric		0	0	3	0	0	0	0	0
sarcoma, undifferentiated, malignant, primary		0	0	0	0	1	0	0	0
within normal limits		35	22	40	16	41	16	51	3
valve, aortic		(35)	(22)	(42)	(13)	(42)	(17)	(61)	(2)
endocardiosis, valvular		0	0	1	0	2	1	1	0
- minimal		0	0	1	0	1	0	2	0
hypertrophy		0	0	1	0	1	0	2	0
- minimal		0	0	1	0	1	0	2	0
within normal limits		35	22	40	13	39	16	58	2
valve, left atrioventricular		(38)	(20)	(47)	(14)	(40)	(19)	(68)	(4)
endocardiosis, valvular		0	0	1	1	2	0	3	0
- minimal		0	0	1	1	2	0	3	0
hypertrophy		1	0	0	0	3	0	2	0
- minimal		1	0	0	0	3	0	2	0
valve, left atrioventricular		(38)	(20)	(47)	(14)	(40)	(19)	(68)	(4)
within normal limits		37	20	46	13	35	19	63	4
valve, pulmonic		(41)	(21)	(39)	(9)	(33)	(16)	(57)	(3)
hypertrophy		1	0	0	0	1	1	0	0
- minimal		1	0	0	0	1	1	0	0
within normal limits		40	21	39	9	32	15	57	3
valve, right atrioventricular		(42)	(22)	(48)	(16)	(45)	(20)	(71)	(4)
endocardiosis, valvular		0	0	0	0	0	2	0	0
- minimal		0	0	0	0	0	2	0	0
hypertrophy		1	0	1	0	0	0	0	0
- minimal		1	0	1	0	0	0	0	0
within normal limits		41	22	47	16	45	18	71	4
zymbal's gland		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
carcinoma, zymbal's gland, malignant, primary		0	0	0	0	0	0	1	0

DOS- Died or euthanized on study

SNC- Scheduled necropsy

(-) - Number observed

Tabulated histopath findings in female SD treated with lorcaserin

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
adipose tissue		(0)	(0)	(2)	(0)	(0)	(0)	(0)	(0)
inflammation, acute	- moderate	0	0	1	0	0	0	0	0
inflammation, chronic	- mild	0	0	1	0	0	0	0	0
adipose tissue, brown, interscapular		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
hibernoma, malignant, primary		0	0	0	0	0	0	1	0
adrenal glands		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, cortical, benign, primary		0	0	0	0	1	0	1	0
angiectasis/cystic degeneration, focal cortical		39	21	49	10	56	5	69	0
	- minimal	0	2	2	1	5	0	6	0
	- mild	23	11	33	5	29	4	38	0
	- moderate	13	5	9	4	18	1	21	0
	- severe	3	3	5	0	4	0	4	0
carcinoma, cortical, malignant, primary		0	0	1	1	0	0	0	0
hematopoiesis, extramedullary		2	0	4	0	8	0	13	0
	- minimal	2	0	4	0	4	0	5	0
	- mild	0	0	0	0	3	0	8	0
	- moderate	0	0	0	0	1	0	0	0
hyperplasia, focal cortical	- moderate	1	0	0	0	1	0	0	0
hyperplasia, focal medullary		3	0	2	1	3	0	2	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	2	0	2	1	3	0	1	0
	- moderate	1	0	0	0	0	0	0	0
hypertrophy, focal cortical		2	2	3	0	4	0	2	0
	- minimal	0	0	1	0	2	0	0	0
	- mild	2	1	1	0	2	0	2	0
	- moderate	0	1	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
mineralization	- mild	0	0	0	0	0	0	1	0
pheochromocytoma, benign, primary		1	1	1	0	1	0	0	0
pheochromocytoma, malignant, primary		0	0	0	1	1	0	0	0
within normal limits		2	1	3	0	2	0	5	0
aorta		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
mineralization		5	0	0	0	1	0	0	0
	- mild	2	0	0	0	0	0	0	0
	- moderate	3	0	0	0	1	0	0	0
necrosis	- severe	0	0	0	0	1	0	0	0
within normal limits		37	23	53	12	58	5	75	0
bone marrow, femur		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
angiectasis	- moderate	0	0	1	0	0	0	0	0
atrophy		2	0	1	0	2	0	15	0
	- minimal	1	0	0	0	0	0	1	0
	- mild	1	0	1	0	1	0	5	0
	- moderate	0	0	0	0	0	0	5	0
	- severe	0	0	0	0	1	0	4	0
cyst		0	0	1	0	1	0	0	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	0	0	0	0	1	0	0	0
hyperplasia, granulocytic		10	4	18	3	31	0	41	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	3	4	4	3	10	0	9	0
	- moderate	7	0	12	0	19	0	32	0
	- severe	0	0	1	0	2	0	0	0
hyperplasia, mixed		9	0	11	3	9	1	14	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	5	0	6	3	7	1	8	0
	- moderate	3	0	5	0	2	0	6	0
leukemia, granulocytic, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	1	0	0
within normal limits		21	19	20	6	19	3	9	0
bone marrow, sternum		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
angiectasis	- moderate	0	0	1	0	0	0	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

Summary of Microscopic Observations - FEMALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
bone marrow, sternum		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
atrophy		1	0	0	0	1	0	11	0
	- minimal	0	0	0	0	0	0	4	0
	- mild	1	0	0	0	0	0	5	0
	- moderate	0	0	0	0	1	0	1	0
	- severe	0	0	0	0	0	0	1	0
hyperplasia, granulocytic		10	2	17	1	29	0	42	0
	- mild	7	2	14	1	23	0	34	0
	- moderate	3	0	3	0	6	0	8	0
hyperplasia, mixed		9	2	10	5	11	1	13	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	8	2	9	5	11	1	13	0
	- moderate	0	0	1	0	0	0	0	0
leukemia, granulocytic, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		22	19	24	6	19	4	11	0
bone, femur		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
cyst		1	4	3	0	2	0	2	0
	- minimal	0	2	0	0	0	0	0	0
	- mild	1	2	3	0	2	0	2	0
hyperostosis		2	2	3	0	2	0	15	0
	- minimal	1	2	2	0	0	0	6	0
	- mild	1	0	1	0	2	0	9	0
within normal limits		39	18	48	12	56	5	58	0
bone, sternum		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration/necrosis, cartilage		1	2	7	1	4	1	1	0
	- minimal	0	0	4	1	3	1	0	0
	- mild	1	2	3	0	1	0	1	0
hyperostosis		1	1	3	0	3	0	10	0
	- minimal	1	1	2	0	2	0	1	0
	- mild	0	0	1	0	1	0	9	0
within normal limits		40	20	44	11	54	4	64	0
brain		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
astrocytoma, malignant, primary		0	0	2	0	0	0	1	0
carcinoma, malignant, secondary		0	0	2	0	0	0	0	0
compression, ventral (pituitary tumor)		14	4	13	5	10	0	8	0
	- minimal	0	0	0	0	2	0	0	0
	- mild	5	3	8	5	3	0	8	0
	- moderate	9	1	5	0	4	0	0	0
	- severe	0	0	0	0	1	0	0	0
gliosis		0	0	0	0	0	0	1	0
hemorrhage		0	0	2	0	1	0	1	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	1	0	1	0	0	0
	- moderate	0	0	1	0	0	0	0	0
hydrocephalus		0	0	1	0	0	0	0	0
inflammation, acute		0	0	1	0	0	0	0	0
mixed glioma, malignant, primary		0	0	0	0	1	0	0	0
necrosis		0	0	0	0	2	0	0	0
within normal limits		28	19	35	7	46	5	67	0
cavity, abdominal		(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
carcinoma, malignant, secondary		0	0	0	1	0	0	0	0
cavity, cranial		(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
hemorrhage		1	0	0	0	0	0	0	0
hyperplasia, mesothelial		1	0	0	0	0	0	0	0
chordae tendineae, left		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hypertrophy		0	1	2	0	0	0	1	0
within normal limits		42	22	51	12	60	5	74	0
chordae tendineae, right		(41)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hypertrophy		0	0	2	0	1	0	0	0
	- minimal	0	0	2	0	0	0	0	0
	- mild	0	0	0	0	1	0	0	0
within normal limits		41	23	51	12	59	5	75	0
endocardium		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
inflammation, chronic-active		0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0

DOS- Died or euthanized on study
SNC- Scheduled necropsy

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
endocardium		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
within normal limits		42	23	51	12	60	5	75	0
esophagus		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
within normal limits		42	23	53	12	60	5	75	0
eyes		(42)	(23)	(53)	(12)	(60)	(5)	(74)	(0)
cataract		7	0	10	0	10	0	11	0
- minimal		3	0	6	0	3	0	9	0
- mild		4	0	3	0	7	0	2	0
- moderate		0	0	1	0	0	0	0	0
inflammation, acute		2	0	0	0	0	0	0	0
inflammation, subacute		0	0	0	0	1	0	1	0
- minimal		0	0	0	0	1	0	0	0
- mild		0	0	0	0	0	0	1	0
keratopathy		2	9	9	4	5	1	2	0
- minimal		2	2	3	1	0	0	0	0
- mild		0	5	6	1	5	1	2	0
- moderate		0	2	0	2	0	0	0	0
phthisis bulbi		0	0	0	0	1	0	0	0
within normal limits		31	14	35	8	44	4	60	0
eyes, optic nerves		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
within normal limits		42	23	53	12	60	5	75	0
harderian glands		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
atrophy		16	8	20	6	19	4	21	0
- minimal		9	6	14	3	15	3	16	0
- mild		7	2	6	2	4	1	5	0
- moderate		0	0	0	1	0	0	0	0
infiltration, lymphocytic		8	3	7	3	6	1	14	0
inflammation, acute		0	0	1	0	1	0	0	0
inflammation, granulomatous		0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		18	12	25	3	35	1	45	0
heart		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
abscess		0	0	1	0	0	0	0	0
bacterial colonies		0	0	1	0	0	0	0	0
cardiomyopathy		25	18	27	9	34	4	36	0
- minimal		13	13	19	7	23	3	22	0
- mild		12	5	8	2	10	1	14	0
- moderate		0	0	0	0	1	0	0	0
infarct		0	0	0	0	0	0	1	0
inflammation, chronic		0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
mineralization, myofiber		0	0	0	0	1	0	0	0
mineralization, vascular		3	0	0	0	2	0	0	0
- minimal		0	0	0	0	1	0	0	0
- moderate		3	0	0	0	1	0	0	0
within normal limits		17	5	24	3	26	1	38	0
joint, tibiofemoral		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration/necrosis, cartilage		2	0	5	0	3	0	10	0
- minimal		1	0	4	0	0	0	4	0
- mild		1	0	1	0	3	0	6	0
within normal limits		40	23	48	12	57	5	65	0
kidneys		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, tubular cell, benign, primary		0	0	1	0	0	0	0	0
arteritis		0	1	0	0	0	0	0	0
carcinoma, malignant, secondary		0	0	0	0	0	0	1	0
carcinoma, transitional cell, malignant, primary		0	0	0	0	0	1	0	0
carcinoma, tubular cell, malignant, primary		0	0	0	0	0	0	1	0
cholesterol clefts		0	1	0	0	0	0	0	0
cyst		0	1	2	0	2	0	1	0
- minimal		0	0	1	0	0	0	0	0
- mild		0	1	1	0	2	0	1	0
dilatation, tubular		1	0	0	0	0	0	3	0
- minimal		1	0	0	0	0	0	1	0
- mild		0	0	0	0	0	0	1	0
- moderate		0	0	0	0	0	0	1	0
hematopoiesis, extramedullary		0	0	0	0	0	0	1	0
hydronephrosis, bilateral		1	0	0	0	0	0	0	0

Summary of Microscopic Observations - FEMALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
kidneys		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hydronephrosis, unilateral		2	2	1	0	2	0	4	0
- minimal		1	0	1	0	1	0	2	0
- mild		1	2	0	0	1	0	2	0
hyperplasia, transitional cell		15	12	26	9	26	3	19	0
- minimal		10	9	16	6	16	2	12	0
- mild		5	3	9	3	10	1	6	0
- moderate		0	0	1	0	0	0	1	0
hyperplasia, tubular	- minimal	0	0	1	0	0	0	0	0
hypertrophy, tubular		0	0	0	0	0	0	3	0
- minimal		0	0	0	0	0	0	2	0
- mild		0	0	0	0	0	0	1	0
infiltration, lymphocytic		0	0	3	1	2	0	9	0
- minimal		0	0	2	0	0	0	8	0
- mild		0	0	1	1	2	0	1	0
- moderate		0	0	1	0	0	0	0	0
inflammation, acute		0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
mineralization	- minimal	2	0	0	0	3	0	0	0
mineralization, pelvic		25	15	36	8	28	2	24	0
- minimal		17	10	25	6	20	1	19	0
- mild		8	3	11	2	8	1	4	0
- moderate		0	2	0	0	0	0	1	0
mineralization, tubular		2	3	2	0	3	1	1	0
- minimal		2	3	2	0	2	1	1	0
- mild		0	0	0	0	1	0	0	0
nephropathy, chronic progressive		18	16	18	10	26	3	28	0
- minimal		8	10	10	8	18	2	20	0
- mild		6	6	8	1	7	1	8	0
- moderate		3	0	0	1	0	0	0	0
- severe		1	0	0	0	1	0	0	0
pigment, tubular		22	15	17	6	28	5	48	0
- minimal		16	13	17	5	23	4	31	0
- mild		6	2	0	1	5	1	17	0
pyelitis		1	1	1	2	0	0	0	0
- minimal		1	1	1	1	0	0	0	0
- moderate		0	0	0	1	0	0	0	0
pyelonephritis, bilateral		0	1	1	0	2	0	2	0
- minimal		0	0	0	0	1	0	0	0
- mild		0	1	0	0	0	0	0	0
- moderate		0	0	1	0	1	0	2	0
pyelonephritis, unilateral	- mild	0	0	0	1	0	0	1	0
vacuolation, tubular		0	0	0	0	0	0	2	0
- mild		0	0	0	0	0	0	1	0
- moderate		0	0	0	0	0	0	1	0
within normal limits		2	0	4	0	6	0	9	0
lacrimal glands, exorbital		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
atrophy		1	2	1	0	4	0	2	0
- mild		1	2	1	0	3	0	2	0
- moderate		0	0	0	0	1	0	0	0
infiltration, lymphocytic		1	5	2	1	4	3	1	0
- minimal		0	4	2	1	4	2	1	0
- mild		1	1	0	0	0	1	0	0
inflammation, acute	- minimal	0	0	1	0	0	0	0	0
vacuolation		0	5	0	0	0	0	0	0
- minimal		0	2	0	0	0	0	0	0
- mild		0	3	0	0	0	0	0	0
within normal limits		40	11	49	11	52	2	72	0
large intestine, cecum		(41)	(23)	(53)	(12)	(59)	(5)	(74)	(0)
inflammation, granulomatous	- mild	0	0	0	1	0	0	0	0
within normal limits		41	23	53	11	59	5	74	0
large intestine, colon		(42)	(23)	(53)	(12)	(60)	(5)	(74)	(0)
arteriopathy	- mild	0	1	0	0	0	0	0	0
dilatation, gland/lumen	- mild	0	0	0	0	0	0	2	0
within normal limits		42	22	53	12	60	5	72	0
large intestine, rectum		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
arteriopathy	- mild	0	1	0	0	0	0	0	0

(b) (4)
 Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE

		Terminal		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0		
large intestine, rectum		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)		
dilatation, gland/lumen		0	3	0	3	1	2	0	0		
	- minimal	0	0	0	0	0	1	0	0		
	- mild	0	3	0	3	1	1	0	0		
within normal limits		42	19	53	9	59	3	75	0		
liver		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)		
angiectasis		0	3	2	2	3	0	1	0		
	- minimal	0	3	1	1	1	0	1	0		
	- mild	0	0	1	1	1	0	0	0		
	- moderate	0	0	0	0	1	0	0	0		
atrophy	- mild	0	0	0	0	0	0	1	0		
carcinoma, hepatocellular, malignant, primary		0	1	0	0	0	0	1	0		
carcinoma, malignant, secondary		0	0	0	1	0	0	1	0		
cyst	- mild	1	1	0	0	0	0	0	0		
degeneration, cystic, focal		0	1	0	0	1	0	2	0		
	- minimal	0	1	0	0	0	0	0	0		
	- mild	0	0	0	0	1	0	2	0		
fibrosis		10	3	10	0	6	1	2	0		
	- minimal	7	2	8	0	4	0	2	0		
	- mild	3	1	2	0	2	1	0	0		
focus of cellular alteration, basophilic		3	3	9	1	17	4	19	0		
	- minimal	0	1	4	1	9	1	11	0		
	- mild	3	0	4	0	8	3	7	0		
	- moderate	0	2	1	0	0	0	1	0		
focus of cellular alteration, clear		1	2	1	0	2	3	3	0		
	- minimal	1	0	0	0	2	0	2	0		
	- mild	0	2	1	0	0	3	1	0		
focus of cellular alteration, eosinophilic		0	0	7	4	17	2	39	0		
	- minimal	0	0	2	0	7	1	16	0		
	- mild	0	0	3	4	7	0	15	0		
	- moderate	0	0	2	0	3	1	8	0		
hematopoiesis, extramedullary		13	2	17	2	27	0	54	0		
	- minimal	12	2	16	2	19	0	37	0		
	- mild	1	0	1	0	8	0	16	0		
	- moderate	0	0	0	0	0	0	1	0		
hyperplasia, bile duct		18	8	18	3	10	1	7	0		
	- minimal	14	5	10	2	5	0	7	0		
	- mild	4	3	8	1	5	1	0	0		
hypertrophy, hepatocyte, centrilobular		0	1	0	0	2	0	25	0		
	- minimal	0	0	0	0	2	0	16	0		
	- mild	0	1	0	0	0	0	8	0		
	- moderate	0	0	0	0	0	0	1	0		
hypertrophy, hepatocyte, periportal		2	2	2	0	0	0	4	0		
	- minimal	0	0	0	0	0	0	2	0		
	- mild	2	2	2	0	0	0	2	0		
	- minimal	1	0	0	0	0	0	0	0		
hypertrophy/hyperplasia, kupffer cell											
infiltration, mononuclear cell		32	21	33	10	28	5	51	0		
	- minimal	32	20	32	10	25	5	48	0		
	- mild	0	1	1	0	3	0	3	0		
inflammation, acute		1	0	3	0	2	0	3	0		
	- minimal	0	0	3	0	0	0	2	0		
	- mild	1	0	0	0	2	0	1	0		
inflammation, bile ducts	- minimal	0	0	1	0	0	0	0	0		
inflammation, chronic	- minimal	0	0	0	0	0	0	1	0		
leukemia, granulocytic, malignant, multicentric		1	0	0	0	0	0	0	0		
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0		
macrophages, pigmented	- minimal	0	0	0	0	0	0	1	0		
necrosis		2	0	5	0	12	0	16	0		
	- minimal	0	0	1	0	6	0	4	0		
	- mild	2	0	2	0	5	0	10	0		
	- moderate	0	0	2	0	1	0	2	0		
pigment, increased kupffer cell		5	2	8	2	8	0	23	0		
	- minimal	4	1	5	2	5	0	18	0		
	- mild	1	1	3	0	3	0	5	0		
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	1	0	0		
vacuolation, hepatocellular		6	8	16	4	5	0	13	0		
	- minimal	2	7	5	2	1	0	9	0		
	- mild	3	1	11	2	4	0	4	0		
	- moderate	1	0	0	0	0	0	0	0		
within normal limits		5	1	6	0	6	0	1	0		

(b) (4) Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
lung		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
carcinoma, bronchiolar alveolar, malignant, primary		0	0	0	0	1	0	0	0
carcinoma, malignant, secondary		0	0	2	2	9	0	6	0
carcinosarcoma, malignant, secondary		0	0	0	0	0	0	1	0
cholesterol clefts		4	3	2	0	3	1	2	0
	- minimal	2	2	2	0	3	1	1	0
	- mild	2	1	0	0	0	0	1	0
hemangiosarcoma, malignant, multicentric		0	0	0	0	1	0	0	0
hemorrhage	- minimal	0	0	0	0	1	0	0	0
histiocytosis, alveolar		7	6	15	5	28	3	47	0
	- minimal	6	6	11	4	17	3	22	0
	- mild	1	0	4	1	10	0	21	0
	- moderate	0	0	0	0	1	0	4	0
infiltration, lymphocytic	- minimal	0	2	0	2	1	0	0	0
inflammation, acute	- moderate	0	0	0	0	1	0	0	0
inflammation, chronic		0	0	0	0	1	0	2	0
	- minimal	0	0	0	0	0	0	2	0
	- moderate	0	0	0	0	1	0	0	0
inflammation, granulomatous		1	1	0	0	2	0	1	0
	- minimal	0	0	0	0	1	0	1	0
	- mild	1	0	0	0	1	0	0	0
	- moderate	0	1	0	0	0	0	0	0
lipidosis, alveolar		0	0	0	0	3	0	24	0
	- mild	0	0	0	0	2	0	14	0
	- moderate	0	0	0	0	1	0	6	0
	- severe	0	0	0	0	0	0	4	0
macrophages, pigmented	- minimal	0	0	0	0	1	0	0	0
metaplasia, osseous	- minimal	0	0	1	0	0	0	0	0
mineralization		2	0	0	0	1	0	0	0
	- minimal	2	0	0	0	0	0	0	0
	- moderate	0	0	0	0	1	0	0	0
mineralization, vascular		1	1	1	0	0	0	0	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	0	1	1	0	0	0	0	0
within normal limits		30	12	33	4	20	2	26	0
lymph node, axillary		(15)	(6)	(33)	(8)	(43)	(4)	(50)	(0)
hyperplasia, lymphocyte/plasmacyte		9	1	14	0	27	0	40	0
	- minimal	1	0	1	0	1	0	3	0
	- mild	2	1	4	0	10	0	12	0
	- moderate	3	0	7	0	14	0	21	0
	- severe	3	0	2	0	2	0	4	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
macrophages, pigmented		8	5	19	1	16	1	10	0
	- minimal	2	3	11	1	13	1	8	0
	- mild	6	2	8	0	3	0	2	0
within normal limits		4	1	8	7	10	3	8	0
lymph node, cervical		(1)	(1)	(2)	(0)	(0)	(0)	(3)	(0)
macrophages, pigmented	- minimal	0	0	2	0	0	0	0	0
within normal limits		1	1	0	0	0	0	3	0
lymph node, hepatic		(0)	(0)	(0)	(0)	(0)	(0)	(2)	(0)
hyperplasia, lymphocyte/plasmacyte	- moderate	0	0	0	0	0	0	1	0
macrophages, pigmented	- mild	0	0	0	0	0	0	2	0
lymph node, iliac		(0)	(2)	(2)	(0)	(2)	(0)	(1)	(0)
hyperplasia, lymphocyte/plasmacyte		0	1	2	0	1	0	1	0
	- moderate	0	1	0	0	0	0	1	0
	- severe	0	0	2	0	1	0	0	0
macrophages, pigmented	- minimal	0	1	0	0	1	0	0	0
within normal limits		0	1	0	0	0	0	0	0
lymph node, inguinal		(9)	(4)	(14)	(1)	(17)	(3)	(19)	(0)
carcinoma, malignant, secondary		0	0	0	0	1	0	0	0
hyperplasia, lymphocyte/plasmacyte		1	0	4	0	8	0	8	0
	- minimal	1	0	0	0	1	0	1	0
	- mild	0	0	3	0	2	0	4	0
	- moderate	0	0	1	0	5	0	2	0
	- severe	0	0	0	0	0	0	1	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
lymph node, inguinal		(9)	(4)	(14)	(1)	(17)	(3)	(19)	(0)
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
macrophages, pigmented		1	2	6	0	8	1	3	0
- minimal		0	1	2	0	2	1	3	0
- mild		1	1	4	0	6	0	0	0
within normal limits		7	2	4	1	5	2	11	0
lymph node, mandibular		(41)	(23)	(53)	(12)	(59)	(5)	(75)	(0)
hyperplasia, lymphocyte/plasmacyte		12	1	21	6	33	0	45	0
- minimal		2	0	3	1	4	0	6	0
- mild		4	1	14	3	22	0	27	0
- moderate		5	0	4	2	7	0	12	0
- severe		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
macrophages, pigmented		20	12	32	8	35	2	54	0
- minimal		18	12	29	8	31	2	46	0
- mild		2	0	3	0	4	0	8	0
within normal limits		15	10	11	0	10	3	10	0
lymph node, mediastinal		(0)	(0)	(1)	(0)	(2)	(0)	(0)	(0)
macrophages, pigmented		0	0	0	0	2	0	0	0
- minimal		0	0	0	0	1	0	0	0
- mild		0	0	0	0	1	0	0	0
within normal limits		0	0	1	0	0	0	0	0
lymph node, mesenteric		(41)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hyperplasia, lymphocyte/plasmacyte		0	0	0	0	0	0	3	0
inflammation, granulomatous		0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
macrophages, pigmented		40	22	52	12	58	5	74	0
- minimal		28	15	28	4	30	1	11	0
- mild		11	7	24	8	28	4	60	0
- moderate		1	0	0	0	0	0	3	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	1	0	0
within normal limits		1	1	0	0	2	0	1	0
lymph node, renal		(0)	(0)	(1)	(2)	(1)	(0)	(1)	(0)
hyperplasia, lymphocyte/plasmacyte		0	0	0	0	1	0	0	0
macrophages, pigmented		0	0	1	1	0	0	1	0
- minimal		0	0	1	0	0	0	0	0
- mild		0	0	0	1	0	0	1	0
within normal limits		0	0	0	1	0	0	0	0
mammary gland		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenocarcinoma, malignant, primary		18	10	28	6	34	1	60	0
area normal, no mammary tissue present		0	0	0	0	0	0	1	0
carcinosarcoma, malignant, primary		0	0	0	0	0	0	1	0
fibroadenoma, benign, primary		14	6	36	11	48	5	45	0
galactocele		8	10	10	4	3	4	1	0
- minimal		0	2	2	2	1	1	0	0
- mild		8	8	8	2	2	3	1	0
hemangiosarcoma, malignant, multicentric		0	0	0	0	1	0	0	0
hemorrhage		0	0	0	1	0	0	0	0
hyperplasia, lobular		12	12	19	4	18	5	27	0
- minimal		1	5	2	1	0	3	2	0
- mild		9	6	15	3	14	0	19	0
- moderate		2	1	2	0	4	2	6	0
hyperplasia, lobular with atypia		14	4	17	1	26	0	22	0
- mild		5	3	8	0	8	0	10	0
- moderate		9	1	9	1	18	0	12	0
within normal limits		2	3	2	0	1	0	0	0
mediastinum		(0)	(0)	(0)	(1)	(0)	(0)	(1)	(0)
carcinoma, malignant, secondary		0	0	0	1	0	0	1	0
mesentery/peritoneum		(0)	(0)	(1)	(1)	(0)	(0)	(0)	(0)
carcinoma, malignant, secondary		0	0	0	1	0	0	0	0
sarcoma, undifferentiated, malignant, primary		0	0	1	0	0	0	0	0
multicentric neoplasm		(2)	(0)	(3)	(0)	(5)	(1)	(0)	(0)
hemangioma, benign, multicentric		0	0	0	0	1	0	0	0
hemangiosarcoma, malignant, multicentric		0	0	0	0	3	0	0	0

(b) (4)
Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE
Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
multicentric neoplasm		(2)	(0)	(3)	(0)	(5)	(1)	(0)	(0)
leukemia, granulocytic, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		1	0	3	0	2	0	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	1	0	0
nerve, sciatic		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration, axonal/myelin		11	17	15	11	11	4	12	0
- minimal		8	5	13	7	8	1	9	0
- mild		3	12	2	4	3	3	3	0
infiltration, mononuclear cell	- minimal	0	0	0	0	0	0	1	0
within normal limits		31	6	38	1	49	1	62	0
ovaries		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
corpus luteum absent	- no grade	35	19	46	12	43	4	57	0
corpus luteum decreased	- no grade	3	0	3	0	7	0	7	0
cyst		11	11	10	5	13	1	23	0
- minimal		5	5	3	1	3	0	3	0
- mild		6	5	6	3	6	1	15	0
- moderate		0	1	1	1	4	0	5	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
sarcoma, undifferentiated, malignant, primary		0	0	0	0	0	0	1	0
sex-cord/stromal tumor, benign, primary		0	0	0	1	0	0	0	0
within normal limits		4	2	5	0	10	1	13	0
within normal limits		42	23	53	12	60	5	75	0
pancreas		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, islet cell, benign, primary		0	1	2	1	0	0	1	0
alteration, basophilic focal		2	4	1	3	1	1	0	0
- minimal		1	3	1	1	1	1	0	0
- mild		1	1	0	2	0	0	0	0
arteriopathy	- minimal	0	1	0	0	0	0	0	0
atrophy, acinar		4	0	3	3	3	0	2	0
- minimal		2	0	2	1	1	0	1	0
- mild		2	0	1	1	2	0	1	0
- moderate		0	0	0	1	0	0	0	0
depletion, secretory		0	0	0	0	2	0	0	0
- minimal		0	0	0	0	1	0	0	0
- mild		0	0	0	0	1	0	0	0
fibrosis	- minimal	0	0	0	0	1	0	0	0
hyperplasia, acinar cell, focal		3	0	1	0	4	0	4	0
- minimal		2	0	1	0	2	0	2	0
- mild		1	0	0	0	2	0	2	0
- minimal		0	0	0	0	1	0	1	0
hyperplasia, islet cell	- minimal	0	0	0	0	1	0	1	0
infiltration, eosinophilic	- moderate	0	0	1	0	0	0	0	0
infiltration, mononuclear cell		1	1	2	2	3	1	1	0
- minimal		0	1	2	2	3	1	1	0
- mild		1	0	0	0	0	0	0	0
inflammation, acute	- minimal	0	0	1	0	0	0	0	0
inflammation, chronic-active	- severe	0	0	1	0	0	0	0	0
lipomatosis		0	2	0	1	0	0	0	0
- minimal		0	1	0	1	0	0	0	0
- mild		0	1	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
mineralization, vascular	- mild	1	0	0	0	1	0	0	0
necrosis	- mild	0	0	1	0	0	0	0	0
necrosis, single cell	- minimal	0	0	1	0	0	0	0	0
pigment	- minimal	3	3	0	0	0	0	0	0
within normal limits		34	13	42	2	47	4	67	0
parathyroid glands		(34)	(22)	(44)	(10)	(55)	(5)	(67)	(0)
adenoma, benign, primary		0	0	0	0	0	0	1	0
hyperplasia, diffuse		3	0	2	0	2	0	2	0
- minimal		1	0	1	0	0	0	2	0
- mild		1	0	1	0	2	0	0	0
- moderate		1	0	0	0	0	0	0	0
hyperplasia, focal		1	0	2	0	0	1	0	0
- minimal		1	0	0	0	0	1	0	0
- mild		0	0	2	0	0	0	0	0
within normal limits		30	22	40	10	53	4	64	0

DOS- Died or euthanized on study
SNC- Scheduled necropsy
()- Number observed

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
peyers patch		(40)	(23)	(53)	(12)	(58)	(5)	(71)	(0)
inflammation, granulomatous	- mild	0	0	0	1	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	1	0	0	0
macrophages, pigmented		7	5	11	3	11	1	18	0
	- minimal	5	5	10	3	11	1	10	0
	- mild	2	0	1	0	0	0	8	0
within normal limits		33	18	41	8	46	4	53	0
pituitary gland		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, pars distalis, benign, primary		31	19	36	10	28	3	20	0
carcinoma, pars distalis, malignant, primary		0	0	2	0	0	0	0	0
cyst	- minimal	0	0	0	0	0	0	2	0
hematopoiesis, extramedullary		0	0	0	0	2	0	1	0
	- minimal	0	0	0	0	1	0	0	0
	- moderate	0	0	0	0	1	0	0	0
	- severe	0	0	0	0	0	0	1	0
hypertrophy/hyperplasia		10	4	14	2	27	2	46	0
	- minimal	3	3	2	1	4	1	15	0
	- mild	5	1	11	1	19	1	28	0
	- moderate	2	0	1	0	4	0	3	0
within normal limits		1	0	1	0	5	0	9	0
salivary gland, mandibular		(41)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
atrophy	- minimal	0	0	0	0	1	0	0	0
infiltration, lymphocytic	- minimal	0	0	0	0	1	0	0	0
within normal limits		41	23	53	12	58	5	75	0
salivary gland, parotid		(42)	(23)	(53)	(12)	(58)	(5)	(72)	(0)
adenocarcinoma, malignant, secondary		0	0	0	0	1	0	0	0
atrophy		5	1	9	0	13	0	10	0
	- minimal	1	0	3	0	2	0	0	0
	- mild	4	1	6	0	7	0	9	0
	- moderate	0	0	0	0	2	0	1	0
	- severe	0	0	0	0	2	0	0	0
hyperplasia, focal	- minimal	0	0	1	0	1	0	0	0
hypertrophy, diffuse		1	4	6	2	1	0	1	0
	- minimal	1	1	3	2	0	0	1	0
	- mild	0	3	3	0	1	0	0	0
infiltration, lymphocytic	- minimal	1	0	0	1	0	0	0	0
inflammation, acute	- minimal	0	0	1	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
mineralization	- minimal	1	0	0	0	0	0	0	0
within normal limits		34	18	35	9	43	5	61	0
salivary gland, sublingual		(40)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
within normal limits		40	23	53	12	60	5	75	0
skeletal muscle, biceps femoris		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration, myofiber		1	0	1	0	3	0	5	0
	- minimal	1	0	1	0	2	0	2	0
	- mild	0	0	0	0	1	0	3	0
degeneration/regeneration, myofiber		0	0	0	0	0	1	2	0
	- minimal	0	0	0	0	0	1	1	0
	- mild	0	0	0	0	0	0	1	0
infiltration, mononuclear cell		1	0	1	0	0	1	0	0
	- minimal	0	0	1	0	0	1	0	0
	- mild	1	0	0	0	0	0	0	0
within normal limits		40	23	51	12	57	3	68	0
skin		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, sebaceous cell, benign, primary		0	0	1	0	0	0	0	0
carcinoma, zymbals gland, malignant, primary		0	0	0	0	1	0	0	0
crust, serocellular	- mild	0	0	0	0	1	0	0	0
exudate, epidermal surface	- mild	0	0	1	0	0	0	0	0
hemangiosarcoma, malignant, multicentric		0	0	0	0	1	0	0	0
infiltration, mononuclear cell	- moderate	0	0	1	0	0	0	0	0
lymphangioma, benign, primary		0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		1	0	1	0	1	0	0	0
within normal limits		41	23	50	12	57	5	74	0
skin, subcutis		(0)	(0)	(3)	(0)	(1)	(0)	(5)	(0)
fibroma, benign, primary		0	0	3	0	0	0	1	0

(b) (4)
 Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE
 Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
skin, subcutis		(0)	(0)	(3)	(0)	(1)	(0)	(5)	(0)
fibrosarcoma, malignant, primary		0	0	0	0	0	0	2	0
within normal limits		0	0	0	0	1	0	2	0
small intestine, duodenum		(42)	(23)	(53)	(12)	(59)	(5)	(74)	(0)
within normal limits		42	23	53	12	59	5	74	0
small intestine, ileum		(42)	(23)	(53)	(12)	(60)	(5)	(73)	(0)
arteriopathy	- mild	0	1	0	0	0	0	0	0
dilatation, gland/lumen	- mild	0	1	0	1	0	0	1	0
erosion/ulcer	- moderate	0	0	1	0	0	0	0	0
hypertrophy/hyperplasia	- severe	0	0	0	0	0	1	0	0
inflammation, acute	- moderate	0	0	1	0	0	0	0	0
within normal limits		42	21	52	11	60	4	72	0
small intestine, jejunum		(42)	(23)	(53)	(12)	(60)	(5)	(74)	(0)
dilatation, gland/lumen	- mild	0	1	0	0	1	0	0	0
erosion/ulcer	- moderate	0	0	1	0	0	0	0	0
within normal limits		42	22	52	12	59	5	74	0
spinal cord, cervical		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration, axonal/myelin		8	8	8	2	2	0	4	0
within normal limits		34	15	45	10	58	5	71	0
- minimal		7	8	8	2	2	0	4	0
- mild		1	0	0	0	0	0	0	0
spinal cord, lumbar		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration, axonal/myelin		14	19	17	11	20	5	12	0
within normal limits		28	4	36	1	40	0	63	0
- minimal		10	11	13	4	14	1	6	0
- mild		4	6	4	7	6	3	6	0
- moderate		0	2	0	0	0	1	0	0
spinal cord, thoracic		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration, axonal/myelin	- minimal	3	0	5	1	4	0	3	0
within normal limits		39	23	48	11	56	5	72	0
spleen		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
abscess	- moderate	0	0	0	0	0	0	1	0
cyst, capsule	- mild	0	0	0	0	0	0	1	0
depletion, lymphoid	- moderate	0	0	0	0	0	0	1	0
hematopoiesis, extramedullary, increased		14	7	28	9	39	3	61	0
hyperplasia, reactive red pulp/stromal	- minimal	0	1	0	0	1	0	1	0
inflammation, acute	- mild	12	5	25	9	22	3	30	0
leukemia, granulocytic, malignant, multicentric	- moderate	1	1	1	0	8	0	19	0
lymphoma, malignant, multicentric	- severe	1	0	2	0	8	0	11	0
macrophages, pigmented		0	0	0	0	2	0	2	0
sarcoma, histiocytic, malignant, multicentric	- mild	0	0	0	0	2	0	1	0
sarcoma, undifferentiated, malignant, primary	- moderate	0	0	0	0	0	0	1	0
within normal limits	- minimal	0	0	1	0	0	0	0	0
- mild		1	0	0	0	0	0	0	0
- moderate		34	20	47	10	54	4	72	0
- severe		3	0	4	0	3	0	3	0
- severe		18	18	27	9	33	3	42	0
- severe		12	2	15	1	17	1	26	0
- severe		1	0	1	0	1	0	1	0
- severe		0	0	0	0	0	1	0	0
- severe		0	0	0	0	0	0	1	0
- severe		4	1	2	0	0	0	0	0
stomach, glandular		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
erosion/ulcer	- severe	0	0	1	0	0	0	0	0
mineralization		3	0	0	0	1	0	0	0
within normal limits	- mild	2	0	0	0	0	0	0	0
- moderate		1	0	0	0	1	0	0	0
- moderate		0	0	1	0	0	0	0	0
- moderate		39	23	52	12	59	5	75	0
stomach, nonglandular		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
erosion/ulcer	- moderate	3	0	0	0	0	0	0	0
inflammation, subacute	- mild	1	0	0	0	0	0	0	0
mineralization	- moderate	0	0	0	0	1	0	0	0
within normal limits		39	23	53	12	59	5	75	0

(b) (4)

Study Number 900-063

A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE

Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
tail		(2)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
erosion/ulcer	- severe	1	0	0	0	0	0	0	0
inflammation, acute	- moderate	1	0	0	0	0	0	0	0
inflammation, hair follicle/epidermis	- moderate	1	0	0	0	0	0	0	0
thymus gland		(39)	(21)	(49)	(10)	(54)	(5)	(70)	(0)
cyst		3	8	5	4	1	2	3	0
	- minimal	0	2	2	2	0	1	2	0
	- mild	2	6	1	2	0	1	1	0
	- moderate	1	0	2	0	1	0	0	0
depletion, lymphoid		36	20	45	9	53	4	68	0
	- minimal	1	0	1	0	2	0	0	0
	- mild	4	6	7	2	3	0	8	0
	- moderate	24	13	23	5	35	4	33	0
	- severe	7	1	14	2	13	0	27	0
hyperplasia, lymphoid		1	4	2	2	5	2	3	0
	- minimal	0	0	0	0	1	0	2	0
	- mild	1	4	2	1	3	2	0	0
	- moderate	0	0	0	1	1	0	0	0
	- severe	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	2	0	0	0	0	0
macrophages, pigmented		21	12	23	7	38	2	62	0
	- minimal	15	11	15	7	27	2	18	0
	- mild	6	1	8	0	11	0	44	0
thymoma, benign, primary		1	0	0	0	0	0	0	0
within normal limits		2	0	1	0	0	1	1	0
thyroid gland		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, c-cell, benign, primary		1	2	3	0	1	0	5	0
adenoma, follicular cell, benign, primary		1	1	2	0	2	2	3	0
carcinoma, c-cell, malignant, primary		0	0	0	0	0	1	0	0
cyst, follicular		1	0	0	1	1	1	1	0
	- minimal	1	0	0	0	1	0	0	0
	- mild	0	0	0	1	0	0	1	0
	- moderate	0	0	0	0	0	1	0	0
cyst, ultimobranchial		1	0	4	0	4	0	8	0
	- minimal	1	0	3	0	4	0	7	0
	- mild	0	0	1	0	0	0	1	0
hyperplasia, c-cell, diffuse		2	13	7	5	8	3	1	0
	- minimal	0	10	5	4	5	2	0	0
	- mild	2	3	2	1	2	1	1	0
	- moderate	0	0	0	0	1	0	0	0
hyperplasia, c-cell, focal		3	2	2	2	5	2	6	0
	- minimal	2	0	0	2	3	1	1	0
	- mild	1	2	2	0	2	1	2	0
	- moderate	0	0	0	0	0	0	3	0
hyperplasia, follicular cell		1	0	1	1	2	0	2	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	1	0	1	1	2	0	1	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		33	8	34	4	39	0	52	0
tongue		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
inflammation, subacute	- mild	0	0	1	0	0	0	0	0
mineralization, vascular	- mild	1	0	0	0	0	0	0	0
within normal limits		41	23	52	12	60	5	75	0
trachea		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
infiltration, lymphocytic	- mild	0	2	0	0	0	0	0	0
within normal limits		42	21	53	12	60	5	75	0
ureters		(40)	(23)	(53)	(12)	(55)	(5)	(72)	(0)
dilatation	- moderate	0	0	0	1	0	1	0	0
hyperplasia, transitional cell		0	0	1	1	0	1	3	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	1	1	0	0	0	0
	- moderate	0	0	0	0	0	1	2	0
infiltration, lymphocytic	- moderate	0	0	0	1	0	0	2	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
within normal limits		40	23	51	10	55	4	69	0

Summary of Microscopic Observations - FEMALE
 Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
urinary bladder		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
dilatation		0	0	1	1	0	0	3	0
- mild		0	0	1	0	0	0	3	0
- moderate		0	0	0	1	0	0	0	0
hyperplasia, papillary/nodular transitional cell		0	0	0	1	0	0	1	0
- mild		0	0	0	1	0	0	0	0
- moderate		0	0	0	0	0	0	1	0
hyperplasia, simple transitional cell		0	0	0	0	1	0	1	0
infiltration, lymphocytic		1	0	0	0	1	0	2	0
- mild		0	0	0	0	1	0	0	0
- moderate		1	0	0	0	0	0	2	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
papilloma, transitional cell, benign, primary		0	0	1	1	1	0	0	0
within normal limits		41	23	50	10	58	5	70	0
uterus with cervix		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenocarcinoma, malignant, primary		1	0	0	0	0	0	0	0
alteration, decidual	- moderate	0	0	1	0	0	0	0	0
cyst		10	12	21	6	19	1	30	0
- minimal		7	8	10	1	14	1	16	0
- mild		3	4	9	4	5	0	14	0
- moderate		0	0	2	1	0	0	0	0
- mild		0	0	0	0	1	0	0	0
fibrosis		0	1	1	0	0	0	0	0
granular cell tumor, benign, primary		0	0	0	0	1	0	0	0
granulation tissue	- moderate	0	0	0	0	1	0	0	0
hemangioma, benign, multicentric		0	0	0	0	0	0	1	0
hemangioma, benign, primary		0	0	0	0	0	0	0	0
hemangiosarcoma, malignant, multicentric		1	0	0	0	1	0	0	0
hyperplasia, stromal		1	1	0	1	0	1	0	0
- mild		0	1	0	1	0	0	0	0
- moderate		1	0	0	0	0	0	0	0
- severe		0	0	0	0	0	1	0	0
inflammation, acute	- moderate	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
metaplasia, squamous		3	0	0	0	0	0	2	0
- minimal		1	0	0	0	0	0	1	0
- mild		2	0	0	0	0	0	1	0
- severe		0	0	0	0	1	0	0	0
necrosis		0	0	0	0	2	1	3	0
polyp, glandular, benign, primary		1	0	0	0	1	0	2	0
polyp, stromal, benign, primary		0	0	0	0	0	1	0	0
sarcoma, histiocytic, malignant, multicentric		0	0	0	0	0	1	0	0
within normal limits		27	11	29	6	34	1	40	0
vagina		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenocarcinoma, malignant, primary		0	1	0	0	0	0	0	0
atrophy		15	3	18	4	22	0	37	0
- mild		15	2	18	4	19	0	32	0
- moderate		0	1	0	0	3	0	5	0
diestrus		20	16	28	7	24	4	21	0
estrus		6	2	5	1	9	0	11	0
fibrosis	- moderate	0	0	0	0	1	0	0	0
granular cell tumor, benign, primary		1	0	0	0	0	0	0	0
hyperplasia, stromal	- mild	0	1	0	1	0	0	1	0
inflammation, acute	- moderate	0	0	0	0	1	0	0	0
lymphoma, malignant, multicentric		0	0	1	0	0	0	0	0
metestrus		0	1	0	0	0	0	1	0
necrosis	- severe	0	0	0	0	1	0	0	0
polyp, benign, primary		0	0	0	0	1	0	1	0
proestrus		1	0	1	0	3	0	3	0
schwannoma, malignant, primary		0	0	0	0	0	1	0	0
within normal limits		0	0	1	0	0	0	0	0
valve, aortic		(38)	(22)	(45)	(12)	(49)	(3)	(67)	(0)
endocardiosis, valvular	- minimal	0	0	1	0	0	0	0	0
hypertrophy	- minimal	0	0	0	0	1	0	0	0
within normal limits		38	22	44	12	48	3	67	0
valve, left atrioventricular		(41)	(22)	(51)	(12)	(54)	(5)	(68)	(0)
cyst		0	0	1	0	1	0	0	0
- minimal		0	0	1	0	0	0	0	0
- mild		0	0	0	0	1	0	0	0
- moderate		0	0	0	0	0	0	0	0
endocardiosis, valvular	- minimal	0	0	1	0	0	0	2	0

Summary of Microscopic Observations - FEMALE

Terminal

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
valve, left atrioventricular		(41)	(22)	(51)	(12)	(54)	(5)	(68)	(0)
hypertrophy	- minimal	0	0	0	0	1	0	1	0
within normal limits		41	22	49	12	53	5	65	0
valve, pulmonic		(34)	(13)	(48)	(9)	(47)	(4)	(56)	(0)
hypertrophy	- minimal	0	0	1	0	0	0	0	0
within normal limits		34	13	47	9	47	4	56	0
valve, right atrioventricular		(41)	(23)	(52)	(12)	(58)	(5)	(73)	(0)
cyst	- minimal	0	1	0	0	1	0	0	0
endocardiosis, valvular	- moderate	0	0	1	0	0	0	0	0
within normal limits		41	22	51	12	57	5	73	0

DOS- Died or euthanized on study

SNC- Scheduled necropsy

()- Number observed

Appendix C

(b) (4) **Historical control Neoplastic Data in Male Sprague Dawley Rats
between 12/26/2002 and 1/15/2007**

(b) (4)
**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65

Protocol Tissues

Adipose Tissue, White, Inguinal	(0)*	(70)	(0)*	(1)*	(0)*	(0)*
Lipoma, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Adrenal Glands	(50)	(69)	(60)	(65)	(65)	(65)
Adenoma, cortical, benign, primary						
Inc.	0	2	0	0	2	0
% Inc.	0.0	2.9	0.0	0.0	3.1	0.0
Carcinoma, cortical, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Pheochromocytoma, benign, primary						
Inc.	2	3	4	3	12	6
% Inc.	4.0	4.3	6.7	4.6	18.5	9.2
Pheochromocytoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Brain	(50)	(70)	(60)	(65)	(65)	(65)
Astrocytoma, malignant, primary						
Inc.	2	0	2	1	1	2
% Inc.	4.0	0.0	3.3	1.5	1.5	3.1
Ependymoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Granular cell tumor, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Hemangiosarcoma, malignant, primary						
Inc.	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	1.5	0.0	0.0
Meningioma, malignant, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Coagulating Glands	(1)*	(69)	(7)*	(0)*	(0)*	(3)*
Adenoma, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0

Inc. - Incidence
% - Percent
() - Total number examined
* - Non-Protocol Tissue

(b) (4)

**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

Adipose Tissue, White, Inguinal	(0)*	(0)*	(1)*	(0)*	(0)*
Lipoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Adrenal Glands	(60)	(60)	(60)	(60)	(60)
Adenoma, cortical, benign, primary					
Inc.	2	2	0	1	0
% Inc.	3.3	3.3	0.0	1.7	0.0
Carcinoma, cortical, malignant, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Pheochromocytoma, benign, primary					
Inc.	10	10	10	5	12
% Inc.	16.7	16.7	16.7	8.3	20.0
Pheochromocytoma, malignant, primary					
Inc.	0	0	1	1	1
% Inc.	0.0	0.0	1.7	1.7	1.7
Brain	(60)	(60)	(60)	(60)	(60)
Astrocytoma, malignant, primary					
Inc.	2	3	3	1	1
% Inc.	3.3	5.0	5.0	1.7	1.7
Ependymoma, malignant, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Granular cell tumor, benign, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Hemangiosarcoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Meningioma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Coagulating Glands	(59)	(0)*	(60)	(3)*	(60)
Adenoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

Study	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>D</u>	<u>F</u>
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65

Protocol Tissues

	(50)	(70)	(60)	(65)	(65)	(65)
Heart	(50)	(70)	(60)	(65)	(65)	(65)
Fibroma, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Injection Site, Right Flank	(50)	(0)*	(0)*	(65)	(65)	(0)*
Fibrosarcoma, malignant, primary						
Inc.	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0
Kidneys	(50)	(70)	(60)	(65)	(65)	(65)
Carcinoma, tubular cell, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Lipoma, benign, primary						
Inc.	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	1.5
Liposarcoma, malignant, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Renal mesenchymal tumor, malignant, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Liver	(50)	(70)	(60)	(65)	(65)	(65)
Adenoma, hepatocellular, benign, primary						
Inc.	1	4	1	0	2	2
% Inc.	2.0	5.7	1.7	0.0	3.1	3.1
Carcinoma, hepatocellular, malignant, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Lung	(50)	(70)	(59)	(65)	(65)	(65)
Adenoma, bronchiolar alveolar, benign, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Lymph Node, Mesenteric	(50)	(68)	(60)	(64)	(65)	(65)
Hemangioma, benign, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Lymphangiosarcoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Mammary Gland	(2)*	(69)	(1)*	(2)*	(5)*	(10)*
Adenoma, benign, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Fibroadenoma, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

Heart	(60)	(60)	(60)	(60)	(60)
Fibroma, benign, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Injection Site, Right Flank	(60)	(0)*	(0)*	(0)*	(0)*
Fibrosarcoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Kidneys	(60)	(60)	(60)	(60)	(60)
Carcinoma, tubular cell, malignant, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Lipoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Liposarcoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Renal mesenchymal tumor, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Liver	(60)	(60)	(60)	(60)	(60)
Adenoma, hepatocellular, benign, primary					
Inc.	1	0	1	0	1
% Inc.	1.7	0.0	1.7	0.0	1.7
Carcinoma, hepatocellular, malignant, primary					
Inc.	1	0	1	0	0
% Inc.	1.7	0.0	1.7	0.0	0.0
Lung	(60)	(60)	(60)	(60)	(60)
Adenoma, bronchiolar alveolar, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Lymph Node, Mesenteric	(60)	(59)	(60)	(59)	(60)
Hemangioma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Lymphangiosarcoma, malignant, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Mammary Gland	(0)*	(6)*	(7)*	(5)*	(1)*
Adenoma, benign, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0
Fibroadenoma, benign, primary					
Inc.	0	2	1	2	0
% Inc.	0.0	3.3	1.7	3.3	0.0

Study	A	B	C	D	D	E
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65

	Protocol Tissues					
Multicentric Neoplasm	(1)	(0)	(1)	(4)	(2)	(2)
Hemangiosarcoma, malignant, multicentric						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Lymphoma, malignant, multicentric						
Inc.	0	0	1	2	1	1
% Inc.	0.0	0.0	1.7	3.1	1.5	1.5
Sarcoma, histiocytic, malignant, multicentric						
Inc.	1	0	0	2	1	1
% Inc.	2.0	0.0	0.0	3.1	1.5	1.5
Nose, Level A	(0)*	(70)	(60)	(0)	(0)	(0)*
Fibrosarcoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Nose, Level B	(0)*	(70)	(60)	(0)	(0)	(0)*
Carcinoma, squamous cell, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Nose, Level C	(0)*	(70)	(60)	(0)	(0)	(0)*
Olfactory neuroblastoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Schwannoma, malignant, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Nose, Level D	(0)*	(70)	(60)	(0)	(0)	(0)*
Adenoma, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Pancreas	(50)	(70)	(60)	(65)	(65)	(65)
Adenoma, acinar cell, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Adenoma, acinar-islet cell, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Adenoma, islet cell, benign, primary						
Inc.	1	2	2	3	2	4
% Inc.	2.0	2.9	3.3	4.6	3.1	6.2
Carcinoma, islet cell, malignant, primary						
Inc.	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	1.5	0.0	0.0
Parathyroid Glands	(37)	(57)	(47)	(51)	(46)	(57)
Adenoma, benign, primary						
Inc.	0	1	0	1	4	0
% Inc.	0.0	1.4	0.0	1.5	6.2	0.0

Inc. - Incidence
 % - Percent
 () - Total number examined
 * - Non-Protocol Tissue

Study	<u>G</u>	<u>H</u>	<u>I</u>	<u>J</u>	<u>K</u>
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

	Protocol Tissues				
Multicentric Neoplasm	(1)	(3)	(3)	(6)	(6)
Hemangiosarcoma, malignant, multicentric					
Inc.	0	1	0	2	1
% Inc.	0.0	1.7	0.0	3.3	1.7
Lymphoma, malignant, multicentric					
Inc.	0	1	1	2	4
% Inc.	0.0	1.7	1.7	3.3	6.7
Sarcoma, histiocytic, malignant, multicentric					
Inc.	1	1	2	2	1
% Inc.	1.7	1.7	3.3	3.3	1.7
Nose, Level A	(60)	(0)*	(60)	(59)	(60)
Fibrosarcoma, malignant, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0
Nose, Level B	(60)	(0)*	(60)	(59)	(60)
Carcinoma, squamous cell, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Nose, Level C	(60)	(0)*	(59)	(58)	(60)
Olfactory neuroblastoma, malignant, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0
Schwannoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Nose, Level D	(60)	(0)*	(59)	(59)	(60)
Adenoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Pancreas	(60)	(60)	(60)	(60)	(60)
Adenoma, acinar cell, benign, primary					
Inc.	0	0	0	0	3
% Inc.	0.0	0.0	0.0	0.0	5.0
Adenoma, acinar-islet cell, benign, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Adenoma, islet cell, benign, primary					
Inc.	1	4	4	5	5
% Inc.	1.7	6.7	6.7	8.3	8.3
Carcinoma, islet cell, malignant, primary					
Inc.	0	0	1	1	3
% Inc.	0.0	0.0	1.7	1.7	5.0
Parathyroid Glands	(55)	(53)	(54)	(58)	(58)
Adenoma, benign, primary					
Inc.	3	1	0	2	0
% Inc.	5.0	1.7	0.0	3.3	0.0

Historical Control Neoplastic Data
 Male Sprague Dawley Rat
 2 Year Studies
 12/02 to 03/08
 (b)
 (4)

**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65

Protocol Tissues

	(50)	(68)	(56)	(64)	(64)	(65)
Pituitary Gland						
Adenoma, pars distalis, benign, primary						
Inc.	27	42	40	36	29	36
% Inc.	54.0	60.0	66.7	55.4	44.6	55.4
Adenoma, pars intermedia, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, pars distalis, malignant, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, pars intermedia, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Preputial Glands	(0)*	(63)	(0)*	(52)	(50)	(0)*
Papilloma, squamous cell, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Skin	(50)	(70)	(60)	(65)	(65)	(65)
Adenoma, basal cell, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Adenoma, sebaceous cell, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, sebaceous cell, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, squamous cell, malignant, primary						
Inc.	0	0	1	0	1	0
% Inc.	0.0	0.0	1.7	0.0	1.5	0.0
Fibroma, benign, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Hair follicle tumor, benign, primary						
Inc.	0	0	0	0	0	0
Skin (continued)	(50)	(70)	(60)	(65)	(65)	(65)
Hemangioma, benign, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Keratoacanthoma, benign, primary						
Inc.	0	0	0	0	0	5
% Inc.	0.0	0.0	0.0	0.0	0.0	7.7
Papilloma, squamous cell, benign, primary						
Inc.	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0
Schwannoma, malignant, primary						
Inc.	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	1.5

(b) (4)

**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

	(59)	(60)	(57)	(59)	(59)
Pituitary Gland					
Adenoma, pars distalis, benign, primary					
Inc.	40	31	35	39	37
% Inc.	66.7	51.7	58.3	65.0	61.7
Adenoma, pars intermedia, benign, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Carcinoma, pars distalis, malignant, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0
Carcinoma, pars intermedia, malignant, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0
Preputial Glands	(60)	(2)*	(58)	(60)	(59)
Papilloma, squamous cell, benign, primary					
Inc.	0	0	1	0	1
% Inc.	0.0	0.0	1.7	0.0	1.7
Skin	(60)	(60)	(60)	(60)	(60)
Adenoma, basal cell, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Adenoma, sebaceous cell, benign, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Carcinoma, malignant, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Carcinoma, sebaceous cell, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Carcinoma, squamous cell, malignant, primary					
Inc.	0	0	0	1	1
% Inc.	0.0	0.0	0.0	1.7	1.7
Fibroma, benign, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Hair follicle tumor, benign, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Skin (continued)	(60)	(60)	(60)	(60)	(60)
Hemangioma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Keratoacanthoma, benign, primary					
Inc.	0	0	4	1	2
% Inc.	0.0	0.0	6.7	1.7	3.3
Papilloma, squamous cell, benign, primary					
Inc.	0	0	2	1	1
% Inc.	0.0	0.0	3.3	1.7	1.7
Schwannoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65

Protocol Tissues

	(4)	(5)	(2)	(8)	(9)	(5)
Skin, Subcutis	(4)	(5)	(2)	(8)	(9)	(5)
Carcinoma, squamous cell, malignant, primary						
Inc.	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	1.5
Fibroma, benign, primary						
Inc.	2	1	0	3	2	1
% Inc.	4.0	1.4	0.0	4.6	3.1	1.5
Fibrosarcoma, malignant, primary						
Inc.	0	0	1	0	1	0
% Inc.	0.0	0.0	1.7	0.0	1.5	0.0
Skin, Subcutis (continued)	(4)	(5)	(2)	(8)	(9)	(5)
Fibrous histiocytoma, malignant, primary						
Inc.	1	1	0	0	0	0
% Inc.	2.0	1.4	0.0	0.0	0.0	0.0
Hemangiosarcoma, malignant, primary						
Inc.	0	0	0	1	1	0
% Inc.	0.0	0.0	0.0	1.5	1.5	0.0
Lipoma, benign, primary						
Inc.	0	0	0	1	1	1
% Inc.	0.0	0.0	0.0	1.5	1.5	1.5
Liposarcoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Sarcoma, undifferentiated, malignant, primary						
Inc.	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0
Schwannoma, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Schwannoma, malignant, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Small Intestine, Duodenum	(49)	(69)	(60)	(65)	(65)	(65)
Adenocarcinoma, malignant, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Polyp, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Small Intestine, Ileum	(50)	(67)	(60)	(65)	(65)	(65)
Adenocarcinoma, malignant, primary						
Inc.	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0
Spinal Cord, Thoracic	(50)	(70)	(60)	(65)	(65)	(65)
Astrocytoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Spleen	(50)	(70)	(60)	(65)	(65)	(65)
Hemangiosarcoma, malignant, primary						
Inc.	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	1.5

**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

	(2)	(6)	(7)	(11)	(6)
Skin, Subcutis	(2)	(6)	(7)	(11)	(6)
Carcinoma, squamous cell, malignant, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Fibroma, benign, primary					
Inc.	0	1	3	2	0
% Inc.	0.0	1.7	5.0	3.3	0.0
Fibrosarcoma, malignant, primary					
Inc.	0	1	2	1	2
% Inc.	0.0	1.7	3.3	1.7	3.3
Skin, Subcutis (continued)	(2)	(6)	(7)	(11)	(6)
Fibrous histiocytoma, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Hemangiosarcoma, malignant, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Lipoma, benign, primary					
Inc.	0	0	0	1	1
% Inc.	0.0	0.0	0.0	1.7	1.7
Liposarcoma, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Sarcoma, undifferentiated, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Schwannoma, benign, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Schwannoma, malignant, primary					
Inc.	0	0	0	2	0
% Inc.	0.0	0.0	0.0	3.3	0.0
Small Intestine, Duodenum	(60)	(60)	(60)	(60)	(60)
Adenocarcinoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Polyp, benign, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Small Intestine, Ileum	(60)	(60)	(60)	(60)	(60)
Adenocarcinoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Spinal Cord, Thoracic	(60)	(60)	(60)	(60)	(60)
Astrocytoma, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Spleen	(60)	(60)	(60)	(60)	(60)
Hemangiosarcoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	E
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65

Protocol Tissues

Stomach, Nonglandular	(49)	(69)	(60)	(65)	(65)	(65)
Leiomyosarcoma, malignant, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Testes	(50)	(70)	(60)	(65)	(65)	(65)
Adenoma, interstitial cell, benign, primary						
Inc.	0	1	0	3	1	1
% Inc.	0.0	1.4	0.0	4.6	1.5	1.5
Thymus Gland	(44)	(65)	(56)	(65)	(64)	(63)
Thymoma, malignant, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Thyroid Gland	(50)	(69)	(59)	(65)	(65)	(64)
Adenoma, c-cell, benign, primary						
Inc.	3	6	3	8	10	5
% Inc.	6.0	8.6	5.0	12.3	15.4	7.7
Adenoma, follicular cell, benign, primary						
Inc.	0	2	7	0	0	0
% Inc.	0.0	2.9	11.7	0.0	0.0	0.0
Carcinoma, c-cell, malignant, primary						
Inc.	0	0	1	0	1	0
% Inc.	0.0	0.0	1.7	0.0	1.5	0.0
Carcinoma, follicular cell, malignant, primary						
Inc.	0	2	0	0	1	0
% Inc.	0.0	2.9	0.0	0.0	1.5	0.0
Urinary Bladder	(50)	(70)	(59)	(65)	(65)	(65)
Papilloma, transitional cell, benign, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Zymbal's Gland	(0)*	(67)	(60)	(0)	(0)	(0)*
Carcinoma, zymbals gland, malignant, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0

Inc. - Incidence

% - Percent

() - Total number examined

* - Non-Protocol Tissue

(b) (4)

**Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

Stomach, Nonglandular	(60)	(60)	(60)	(60)	(60)
Leiomyosarcoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Testes	(60)	(60)	(60)	(60)	(60)
Adenoma, interstitial cell, benign, primary					
Inc.	0	2	1	1	2
% Inc.	0.0	3.3	1.7	1.7	3.3
Thymus Gland	(54)	(58)	(56)	(56)	(57)
Thymoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Thyroid Gland	(60)	(60)	(60)	(60)	(60)
Adenoma, c-cell, benign, primary					
Inc.	1	5	6	3	9
% Inc.	1.7	8.3	10.0	5.0	15.0
Adenoma, follicular cell, benign, primary					
Inc.	2	0	1	1	2
% Inc.	3.3	0.0	1.7	1.7	3.3
Carcinoma, c-cell, malignant, primary					
Inc.	0	1	1	0	0
% Inc.	0.0	1.7	1.7	0.0	0.0
Carcinoma, follicular cell, malignant, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Urinary Bladder	(60)	(60)	(60)	(59)	(60)
Papilloma, transitional cell, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Zymbal's Gland	(53)	(0)*	(54)	(58)	(57)
Carcinoma, zymbals gland, malignant, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0

Inc. - Incidence

% - Percent

() - Total number examined

* - Non-Protocol Tissue

Study	A	B	C	D	D	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65

Non-Protocol Tissues

Adipose Tissue, Brown	(0)	(70)	(1)	(0)	(0)	(0)
Hibernoma, benign, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Hibernoma, malignant, primary						
Inc.	0	2	0	0	0	0
% Inc.	0.0	2.9	0.0	0.0	0.0	0.0
Bone	(1)	(0)	(0)	(0)	(0)	(0)
Osteosarcoma, malignant, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Bone, Rib	(0)	(0)	(0)	(0)	(0)	(1)
Osteoma, benign, primary						
Inc.	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	1.5
Cavity, Abdominal	(0)	(2)	(2)	(1)	(1)	(1)
Fibroma, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Fibrosarcoma, malignant, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Lipoma, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Neuroendocrine tumor, benign, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Sarcoma, undifferentiated, malignant, primary						
Inc.	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	1.5	0.0	0.0
Schwannoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Cavity, Thoracic	(0)	(2)	(0)	(0)	(0)	(0)
Hibernoma, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Ears	(0)	(0)	(1)	(0)	(0)	(0)
Fibroma, benign, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Cavity, Thoracic	(0)	(2)	(0)	(0)	(0)	(0)
Hibernoma, benign, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Ears	(0)	(0)	(1)	(0)	(0)	(0)
Fibroma, benign, primary						
Inc.	0	0	1	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0
Mammary Gland	(2)	(69)*	(1)	(2)	(5)	(10)
Adenoma, benign, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Fibroadenoma, benign, primary						
Inc.	0	1	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0
Mediastinum	(0)	(0)	(0)	(1)	(1)	(0)
Sarcoma, undifferentiated, malignant, primary						
Inc.	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0

Study	<u>G</u>	<u>H</u>	<u>I</u>	<u>J</u>	<u>K</u>
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Non-Protocol Tissues

Adipose Tissue, Brown	(0)	(0)	(0)	(0)	(1)
Hibernoma, benign, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Hibernoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Bone	(0)	(0)	(1)	(0)	(1)
Osteosarcoma, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Bone, Rib	(0)	(0)	(0)	(0)	(0)
Osteoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Cavity, Abdominal	(1)	(1)	(0)	(1)	(2)
Fibroma, benign, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Fibrosarcoma, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Lipoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Neuroendocrine tumor, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Sarcoma, undifferentiated, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Schwannoma, malignant, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Cavity, Thoracic	(0)	(0)	(1)	(4)	(0)
Hibernoma, benign, primary					
Inc.	0	0	1	2	0
% Inc.	0.0	0.0	1.7	3.3	0.0
Ears	(0)	(0)	(0)	(0)	(0)
Fibroma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Cavity, Thoracic	(0)	(0)	(1)	(4)	(0)
Hibernoma, benign, primary					
Inc.	0	0	1	2	0
% Inc.	0.0	0.0	1.7	3.3	0.0
Ears	(0)	(0)	(0)	(0)	(0)
Fibroma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Mammary Gland	(0)	(6)	(7)	(5)	(1)
Adenoma, benign, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0
Fibroadenoma, benign, primary					
Inc.	0	2	1	2	0
% Inc.	0.0	3.3	1.7	3.3	0.0
Mediastinum	(0)	(0)	(0)	(0)	(0)
Sarcoma, undifferentiated, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

(b) (4)
Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08

Study	A	B	C	D	D	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1
Number of Animals	50	70	60	65	65	65
Oral Mucosa	(0)	(0)	(0)	(0)	(0)	(0)
Carcinoma, squamous cell, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Primary Site Unknown	(0)	(0)	(0)	(0)	(1)	(0)
Adenocarcinoma, malignant, primary						
Inc.	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0
Tail	(2)	(0)	(0)	(1)	(0)	(0)
Keratoacanthoma, benign, primary						
Inc.	1	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0
Tooth/Teeth	(0)	(11)	(0)	(0)	(0)	(0)
Osteosarcoma, malignant, primary						
Inc.	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0

(b) (4)
Historical Control Neoplastic Data
Male Sprague Dawley Rat
2 Year Studies
12/02 to 03/08

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60
Oral Mucosa	(0)	(0)	(1)	(0)	(0)
Carcinoma, squamous cell, malignant, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0
Primary Site Unknown	(0)	(0)	(0)	(0)	(0)
Adenocarcinoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Tail	(1)	(0)	(0)	(1)	(2)
Keratoacanthoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Tooth/Teeth	(0)	(0)	(2)	(0)	(0)
Osteosarcoma, malignant, primary					
Inc.	0	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0

Inc. - Incidence
 % - Percent
 () - Total number examined
 * - Protocol Tissue

(b) (4) **Historical control Neoplastic Data in female SD Rats Between 12/26/2002 and 1/15/2007**

(b) (4) Historical Control Neoplastic Data Female Sprague Dawley Rat 2 Year Studies							
Study	A	B	C	D	D	E	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	4/25/2002	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	3/2/2004	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravaginal	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1	C-1
Number of Animals	50	70	60	65	65	50	65
Protocol Tissues							
Adrenal Glands	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Adenoma, cortical, benign, primary							
Inc.	0	5	1	4	1	2	2
% Inc.	0.0	7.1	1.7	6.2	1.5	4.0	3.1
Carcinoma, cortical, malignant, primary							
Inc.	2	1	3	2	0	0	0
% Inc.	4.0	1.4	5.0	3.1	0.0	0.0	0.0
Ganglioneuroma, benign, primary							
Inc.	0	1	0	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Pheochromocytoma, benign, primary							
Inc.	0	1	1	3	2	1	1
% Inc.	0.0	1.4	1.7	4.6	3.1	2.0	1.5
Pheochromocytoma, complex, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pheochromocytoma, malignant, primary							
Inc.	0	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Brain	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Astrocytoma, malignant, primary							
Inc.	0	1	0	0	0	1	2
% Inc.	0.0	1.4	0.0	0.0	0.0	2.0	3.1
Granular cell tumor, benign, primary							
Inc.	0	0	1	0	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0	0.0
Meningioma, benign, primary							
Inc.	0	0	0	1	0	0	0
% Inc.	0.0	0.0	0.0	1.5	0.0	0.0	0.0
Reticulosis, malignant, primary							
Inc.	0	0	1	1	0	0	0
% Inc.	0.0	0.0	1.7	1.5	0.0	0.0	0.0
Clitoral Glands	(0)*	(59)	(0)*	(47)	(47)	(1)*	(0)*
Adenocarcinoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, squamous cell, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Heart	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Schwannoma, malignant, primary							
Inc.	0	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Injection Site, Neoplasm	(0)	(0)*	(0)*	(0)	(0)	(0)*	(0)
Fibrous histiocytoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kidneys	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Adenoma, tubular cell, benign, primary							
Inc.	0	1	0	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Carcinoma, tubular cell, malignant, primary							
Inc.	0	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0	0.0
Lipoma, benign, primary							
Inc.	0	0	1	0	1	0	0
% Inc.	0.0	0.0	1.7	0.0	1.5	0.0	0.0
Nephroblastoma, benign, primary							
Inc.	0	0	0	1	0	0	0
% Inc.	0.0	0.0	0.0	1.5	0.0	0.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

	(60)	(60)	(59)	(60)	(60)
Adrenal Glands	(60)	(60)	(59)	(60)	(60)
Adenoma, cortical, benign, primary					
Inc.	2	2	2	3	4
% Inc.	3.3	3.3	3.3	5.0	6.7
Carcinoma, cortical, malignant, primary					
Inc.	1	0	1	0	0
% Inc.	1.7	0.0	1.7	0.0	0.0
Ganglioneuroma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Pheochromocytoma, benign, primary					
Inc.	1	1	0	0	1
% Inc.	1.7	1.7	0.0	0.0	1.7
Pheochromocytoma, complex, malignant, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Pheochromocytoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Brain	(60)	(60)	(60)	(60)	(60)
Astrocytoma, malignant, primary					
Inc.	1	0	0	0	2
% Inc.	1.7	0.0	0.0	0.0	3.3
Granular cell tumor, benign, primary					
Inc.	0	0	0	0	2
% Inc.	0.0	0.0	0.0	0.0	3.3
Meningioma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Reticulosis, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Clitoral Glands	(57)	(0)*	(56)	(59)	(57)
Adenocarcinoma, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Carcinoma, squamous cell, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Heart	(60)	(60)	(60)	(60)	(60)
Schwannoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Injection Site, Neoplasm	(59)	(0)*	(0)*	(0)*	(0)*
Fibrous histiocytoma, malignant, primary					
Inc.	2	0	0	0	0
% Inc.	3.3	0.0	0.0	0.0	0.0
Kidneys	(60)	(60)	(60)	(60)	(60)
Adenoma, tubular cell, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Carcinoma, tubular cell, malignant, primary					
Inc.	0	0	2	0	0
% Inc.	0.0	0.0	3.3	0.0	0.0
Lipoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Nephroblastoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

Inc. - Incidence
% - Percent
() - Total number examined

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	E	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	4/25/2002	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	3/2/2004	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravaginal	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1	C-1
Number of Animals	50	70	60	65	65	50	65
Protocol Tissues							
Large Intestine, Cecum	(50)	(70)	(60)	(64)	(65)	(50)	(65)
Fibroma, benign, primary							
Inc.	0	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0	0.0
Liver	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Adenoma, hepatocellular, benign, primary							
Inc.	0	0	3	2	2	0	0
% Inc.	0.0	0.0	5.0	3.1	3.1	0.0	0.0
Carcinoma, hepatocellular, malignant, primary							
Inc.	0	0	0	1	0	0	0
% Inc.	0.0	0.0	0.0	1.5	0.0	0.0	0.0
Cholangiocarcinoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lung	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Adenoma, bronchiolar alveolar, benign, primary							
Inc.	0	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	0.0	2.0	0.0
Mammary Gland	(50)	(70)	(59)	(65)	(63)	(50)	(65)
Adenocarcinoma, malignant, primary							
Inc.	11	14	18	27	24	9	14
% Inc.	22.0	20.0	30.0	41.5	36.9	18.0	21.5
Adenoma, benign, primary							
Inc.	4	3	3	4	1	1	1
% Inc.	8.0	4.3	5.0	6.2	1.5	2.0	1.5
Fibroadenoma, benign, primary							
Inc.	11	23	22	26	21	14	35
% Inc.	22.0	32.9	36.7	40.0	32.3	28.0	53.8
Multicentric Neoplasm	(3)	(0)	(2)	(3)	(1)	(2)	(1)
Hemangiosarcoma, malignant, multicentric							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lymphoma, malignant, multicentric							
Inc.	0	0	1	1	0	1	1
% Inc.	0.0	0.0	1.7	1.5	0.0	2.0	1.5
Mast cell tumor, malignant, multicentric							
Inc.	0	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0	0.0
Sarcoma, histiocytic, malignant, multicentric							
Inc.	3	0	1	2	0	1	0
% Inc.	6.0	0.0	1.7	3.1	0.0	2.0	0.0
Nose, Level C	(0)*	(70)	(60)	(0)	(0)	(0)*	(0)*
Adenoma, benign, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ovaries	(49)	(70)	(60)	(65)	(65)	(49)	(65)
Adenoma, tubulostromal, benign, primary							
Inc.	0	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	0.0	2.0	0.0
Granulosa cell tumor, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sex-cord/stromal tumor, benign, primary							
Inc.	0	0	1	1	1	0	0
% Inc.	0.0	0.0	1.7	1.5	1.5	0.0	0.0
Sex-cord/stromal tumor, malignant, primary							
Inc.	0	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0	0.0
Thecoma, benign, primary							
Inc.	0	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	0.0	2.0	0.0

(b) (4)
Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60
Protocol Tissues					
Large Intestine, Cecum	(60)	(60)	(60)	(60)	(60)
Fibroma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Liver	(60)	(60)	(60)	(60)	(60)
Adenoma, hepatocellular, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Carcinoma, hepatocellular, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Cholangiocarcinoma, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Lung	(60)	(60)	(60)	(60)	(60)
Adenoma, bronchiolar alveolar, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Mammary Gland	(60)	(60)	(60)	(60)	(60)
Adenocarcinoma, malignant, primary					
Inc.	15	5	10	20	12
% Inc.	25.0	8.3	16.7	33.3	20.0
Adenoma, benign, primary					
Inc.	1	0	0	1	1
% Inc.	1.7	0.0	0.0	1.7	1.7
Fibroadenoma, benign, primary					
Inc.	23	28	17	16	25
% Inc.	38.3	46.7	28.3	26.7	41.7
Multicentric Neoplasm	(2)	(0)	(5)	(2)	(4)
Hemangiosarcoma, malignant, multicentric					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Lymphoma, malignant, multicentric					
Inc.	1	0	2	1	0
% Inc.	1.7	0.0	3.3	1.7	0.0
Mast cell tumor, malignant, multicentric					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Sarcoma, histiocytic, malignant, multicentric					
Inc.	1	0	3	0	4
% Inc.	1.7	0.0	5.0	0.0	6.7
Nose, Level C	(60)	(0)*	(60)	(60)	(60)
Adenoma, benign, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Ovaries	(60)	(60)	(60)	(60)	(60)
Adenoma, tubulostromal, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Granulosa cell tumor, malignant, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Sex-cord/stromal tumor, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Sex-cord/stromal tumor, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Thecoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	E	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	4/25/2002	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	3/2/2004	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravaginal	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1	C-1
Number of Animals	50	70	60	65	65	50	65

Protocol Tissues

	(50)	(70)	(60)	(65)	(64)	(50)	(65)
Pancreas							
Adenoma, islet cell, benign, primary							
Inc.	1	0	3	1	2	1	2
% Inc.	2.0	0.0	5.0	1.5	3.1	2.0	3.1
Carcinoma, acinar cell, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, islet cell, malignant, primary							
Inc.	0	0	0	1	3	0	0
% Inc.	0.0	0.0	0.0	1.5	4.6	0.0	0.0
Parathyroid Glands	(37)	(50)	(45)	(46)	(58)	(46)	(53)
Adenoma, benign, primary							
Inc.	0	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Pituitary Gland	(50)	(70)	(59)	(65)	(65)	(50)	(65)
Adenoma, pars distalis, benign, primary							
Inc.	37	52	44	55	49	42	55
% Inc.	74.0	74.3	73.3	84.6	75.4	84.0	84.6
Carcinoma, pars distalis, malignant, primary							
Inc.	2	6	0	0	0	0	0
% Inc.	4.0	8.6	0.0	0.0	0.0	0.0	0.0
Skin	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Adenoma, basal cell, benign, primary							
Inc.	0	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0	0.0
Adenoma, sebaceous cell, benign, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, basal cell, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, squamous cell, malignant, primary							
Inc.	1	0	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Keratoacanthoma, benign, primary							
Inc.	0	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Skin, Subcutis	(3)	(3)	(4)	(3)	(3)	(2)	(1)
Fibroma, benign, primary							
Inc.	0	0	1	0	0	0	1
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0	1.5
Fibrosarcoma, malignant, primary							
Inc.	0	2	1	2	3	1	0
% Inc.	0.0	2.9	1.7	3.1	4.6	2.0	0.0
Hemangioma, benign, primary							
Inc.	1	0	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Hibernoma, benign, primary							
Inc.	0	0	1	0	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0	0.0
Lipoma, benign, primary							
Inc.	0	0	1	0	0	1	0
% Inc.	0.0	0.0	1.7	0.0	0.0	2.0	0.0
Liposarcoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Osteosarcoma, malignant, primary							
Inc.	1	0	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Sarcoma, histiocytic, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

	(60)	(60)	(60)	(60)	(60)
Pancreas	(60)	(60)	(60)	(60)	(60)
Adenoma, islet cell, benign, primary					
Inc.	2	0	1	0	0
% Inc.	3.3	0.0	1.7	0.0	0.0
Carcinoma, acinar cell, malignant, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Carcinoma, islet cell, malignant, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Parathyroid Glands	(49)	(47)	(54)	(46)	(50)
Adenoma, benign, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Pituitary Gland	(60)	(60)	(60)	(60)	(60)
Adenoma, pars distalis, benign, primary					
Inc.	45	49	48	46	50
% Inc.	75.0	81.7	80.0	76.7	83.3
Carcinoma, pars distalis, malignant, primary					
Inc.	1	0	2	1	2
% Inc.	1.7	0.0	3.3	1.7	3.3
Skin	(60)	(60)	(60)	(60)	(60)
Adenoma, basal cell, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Adenoma, sebaceous cell, benign, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Carcinoma, basal cell, malignant, primary					
Inc.	0	0	1	0	1
% Inc.	0.0	0.0	1.7	0.0	1.7
Carcinoma, squamous cell, malignant, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Keratoacanthoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Skin, Subcutis	(2)	(3)	(4)	(3)	(1)
Fibroma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Fibrosarcoma, malignant, primary					
Inc.	0	0	1	2	0
% Inc.	0.0	0.0	1.7	3.3	0.0
Hemangioma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Hibernoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Lipoma, benign, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Liposarcoma, malignant, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Osteosarcoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Sarcoma, histiocytic, malignant, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies**

Study	12/02 to 03/08						
Experimental Start Date	A	B	C	D	D	E	F
Experimental Termination Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	4/25/2002	2/11/2004
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravaginal	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1	C-1
Number of Animals	50	70	60	65	65	50	65

Protocol Tissues

	(3)	(3)	(4)	(3)	(3)	(2)	(1)
Skin, Subcutis (continued)							
Sarcoma, synovial, malignant, primary							
Inc.	0	1	0	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Schwannoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Small Intestine, Jejunum	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Leiomyoma, benign, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spinal Cord, Cervical	(50)	(70)	(60)	(65)	(65)	(0)*	(65)
Astrocytoma, malignant, primary							
Inc.	0	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Spinal Cord, Thoracic	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Astrocytoma, malignant, primary							
Inc.	1	0	0	0	0	0	0
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Stomach, Nonglandular	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Papilloma, squamous cell, benign, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thymus Gland	(45)	(66)	(56)	(62)	(64)	(44)	(62)
Thymoma, benign, primary							
Inc.	0	0	0	0	1	0	0
% Inc.	0.0	0.0	0.0	0.0	1.5	0.0	0.0
Thyroid Gland	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Adenoma, c-cell, benign, primary							
Inc.	1	8	5	5	3	5	5
% Inc.	2.0	11.4	8.3	7.7	4.6	10.0	7.7
Adenoma, follicular cell, benign, primary							
Inc.	0	1	1	0	1	1	2
% Inc.	0.0	1.4	1.7	0.0	1.5	2.0	3.1
Carcinoma, c-cell, malignant, primary							
Inc.	2	0	0	0	0	0	0
% Inc.	4.0	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, follicular cell, malignant, primary							
Inc.	0	0	1	0	0	1	0
% Inc.	0.0	0.0	1.7	0.0	0.0	2.0	0.0
Tongue	(0)*	(70)	(60)	(65)	(65)	(50)	(65)
Papilloma, squamous cell, benign, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Urinary Bladder	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Papilloma, transitional cell, benign, primary							
Inc.	0	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	0.0	2.0	0.0
Uterus with Cervix	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Adenoma, benign, primary							
Inc.	0	1	0	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Adenocarcinoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carcinoma, squamous cell, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fibroma, benign, primary							
Inc.	0	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	0.0	2.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

	(2)	(3)	(4)	(3)	(1)
Skin, Subcutis (continued)					
Sarcoma, synovial, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Schwannoma, malignant, primary					
Inc.	0	1	0	0	0
% Inc.	0.0	1.7	0.0	0.0	0.0
Small Intestine, Jejunum	(60)	(60)	(60)	(60)	(60)
Leiomyoma, benign, primary					
Inc.	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	1.7
Spinal Cord, Cervical	(60)	(60)	(60)	(60)	(60)
Astrocytoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Spinal Cord, Thoracic	(60)	(60)	(60)	(60)	(60)
Astrocytoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Stomach, Nonglandular	(60)	(60)	(60)	(60)	(60)
Papilloma, squamous cell, benign, primary					
Inc.	1	0	0	0	0
% Inc.	1.7	0.0	0.0	0.0	0.0
Thymus Gland	(58)	(56)	(58)	(58)	(59)
Thymoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Thyroid Gland	(60)	(60)	(60)	(60)	(60)
Adenoma, c-cell, benign, primary					
Inc.	4	4	6	7	6
% Inc.	6.7	6.7	10.0	11.7	10.0
Adenoma, follicular cell, benign, primary					
Inc.	0	0	0	1	1
% Inc.	0.0	0.0	0.0	1.7	1.7
Carcinoma, c-cell, malignant, primary					
Inc.	0	0	2	0	0
% Inc.	0.0	0.0	3.3	0.0	0.0
Carcinoma, follicular cell, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

Tongue	(60)	(60)	(60)	(60)	(60)
Papilloma, squamous cell, benign, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Urinary Bladder	(60)	(60)	(59)	(60)	(60)
Papilloma, transitional cell, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Uterus with Cervix	(60)	(60)	(60)	(60)	(60)
Adenoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Adenocarcinoma, malignant, primary					
Inc.	2	0	0	0	0
% Inc.	3.3	0.0	0.0	0.0	0.0
Carcinoma, squamous cell, malignant, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Fibroma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	E	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	4/25/2002	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	3/2/2004	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravaginal	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1	C-1
Number of Animals	50	70	60	65	65	50	65

Protocol Tissues

Uterus with Cervix (continued)	(50)	(70)	(60)	(65)	(65)	(50)	(65)
Granular cell tumor, benign, primary							
Inc.	0	1	6	1	0	4	0
% Inc.	0.0	1.4	10.0	1.5	0.0	8.0	0.0
Hemangiosarcoma, malignant, primary							
Inc.	0	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	0.0	2.0	0.0
Leiomyoma, benign, primary							
Inc.	1	0	0	0	0	0	1
% Inc.	2.0	0.0	0.0	0.0	0.0	0.0	1.5
Leiomyosarcoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Polyp, stromal, benign, primary							
Inc.	3	3	6	9	5	3	5
% Inc.	6.0	4.3	10.0	13.8	7.7	6.0	7.7
Sarcoma, stromal, malignant, primary							
Inc.	0	0	0	0	0	1	0
% Inc.	0.0	0.0	0.0	0.0	0.0	2.0	0.0
Schwannoma, malignant, primary							
Inc.	0	0	0	0	0	0	1
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Vagina	(50)	(70)	(60)	(65)	(65)	(50)	(64)
Granular cell tumor, benign, primary							
Inc.	0	1	1	0	0	3	0
% Inc.	0.0	1.4	1.7	0.0	0.0	6.0	0.0
Zymbal's Gland	(0)*	(69)	(55)	(0)	(0)	(0)*	(0)*
Carcinoma, zymbals gland, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Inc. - Incidence
% - Percent
() - Total number examined
* - Non-Protocol Tissue

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	G	H	I	J	K
Experimental Start Date	3/10/2004	3/30/2004	5/4/2004	12/22/2004	1/13/2005
Experimental Termination Date	3/1/2006	3/29/2006	5/3/2006	12/20/2006	1/15/2007
Route of Administration	Subcutaneous	Oral Gavage	Intranasal	Oral Gavage	Oral Gavage
Intervals Used	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-1
Number of Animals	60	60	60	60	60

Protocol Tissues

	(60)	(60)	(60)	(60)	(60)
Uterus with Cervix (continued)	(60)	(60)	(60)	(60)	(60)
Granular cell tumor, benign, primary					
Inc.	0	0	2	1	0
% Inc.	0.0	0.0	3.3	1.7	0.0
Hemangiosarcoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Leiomyoma, benign, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Leiomyosarcoma, malignant, primary					
Inc.	0	0	0	1	0
% Inc.	0.0	0.0	0.0	1.7	0.0
Polyp, stromal, benign, primary					
Inc.	2	2	2	3	2
% Inc.	3.3	3.3	3.3	5.0	3.3
Sarcoma, stromal, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Schwannoma, malignant, primary					
Inc.	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0
Vagina	(60)	(60)	(60)	(60)	(60)
Granular cell tumor, benign, primary					
Inc.	0	0	0	4	0
% Inc.	0.0	0.0	0.0	6.7	0.0
Zymbal's Gland	(55)	(1)*	(56)	(60)	(58)
Carcinoma, zymbals gland, malignant, primary					
Inc.	1	1	0	1	0
% Inc.	1.7	1.7	0.0	1.7	0.0

Inc. - Incidence

% - Percent

() - Total number examined

* - Non-Protocol Tissue

(b) (4)

**Historical Control Neoplastic Data
Female Sprague Dawley Rat
2 Year Studies
12/02 to 03/08**

Study	A	B	C	D	D	E	F
Experimental Start Date	12/28/2000	5/3/2001	4/25/2001	5/24/2001	5/24/2001	4/25/2002	2/11/2004
Experimental Termination Date	12/26/2002	3/29/2003	5/2/2003	5/30/2003	5/30/2003	3/2/2004	2/9/2006
Route of Administration	Subcutaneous	Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Intravaginal	Intravenous
Intervals Used	0 to Termination	0 to Termination	Terminal	Terminal	Terminal	Terminal	Terminal
Control Group	C-1	C-1	C-1	C-1	C-2	C-1	C-1
Number of Animals	50	70	60	65	65	50	65
Non-Protocol Tissues							
Adipose Tissue, Brown	(0)	(70)	(1)	(0)	(0)	(0)	(0)
Hibernoma, malignant, primary							
Inc.	0	0	1	0	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0	0.0
Cavity, Abdominal	(0)	(3)	(0)	(0)	(0)	(0)	(0)
Carcinoma, malignant, primary							
Inc.	0	1	0	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Leiomyoma, benign, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liposarcoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mesothelioma, malignant, primary							
Inc.	0	1	0	0	0	0	0
% Inc.	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Schwannoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cavity, Thoracic	(0)	(1)	(2)	(0)	(0)	(1)	(0)
Hibernoma, benign, primary							
Inc.	0	0	1	0	0	0	0
% Inc.	0.0	0.0	1.7	0.0	0.0	0.0	0.0
Hibernoma, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lipoma, benign, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neuroendocrine tumor, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mediastinum	(0)	(0)	(3)	(1)	(0)	(1)	(0)
Fibrosarcoma, malignant, primary							
Inc.	0	0	0	1	0	0	0
% Inc.	0.0	0.0	0.0	1.5	0.0	0.0	0.0
Zymbal's Gland	(0)	(69)*	(55)*	(0)*	(0)*	(0)	(0)
Carcinoma, zymbals gland, malignant, primary							
Inc.	0	0	0	0	0	0	0
% Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Inc. - Incidence
% - Percent
() - Total number examined
* - Protocol Tissue

The end of historical control rat neoplastic data

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/s/

FRED K ALAVI

10/25/2010

Histopath tables for the lorcaserin mouse and rat carci studies

TODD M BOURCIER

10/25/2010

Additional reference information from carci studies.

Tertiary Pharmacology/Toxicology Review

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

David Jacobson-Kram, Ph.D., DABT, Associate Director for Pharmacology and
Toxicology OND IO

NDA: 22-529

Agency receipt date: 12/22/2009

Drug: Lorcaserin HCl

Sponsor: Arena Pharmaceuticals

Indication: weight management, including weight loss and maintenance of weight loss

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments:

The pharm/tox reviewer and supervisor recommended that lorcaserin not be approved for the proposed indication. This recommendation was based on the finding of drug-related tumors in a 2-year rat bioassay.

Discussion:

The pharm/tox reviewer and supervisor have reviewed the nonclinical findings in detail in their respective reviews. The carcinogenicity evaluation of lorcaserin consisted of two-year studies in rats and mice in which lorcaserin was administered orally by gavage. The protocols for these studies were submitted to the agency prior to initiation of the studies and the executive carcinogenicity assessment committee recommended doses of 0, 25, 50 and 100 mg/kg in mice and 0, 10, 30 and 100 mg/kg in rats. The maximum dose was selected based on a maximum tolerated dose in both species. The studies were conducted with these doses.

The division reviewed these studies and the results were presented to the executive carcinogenicity assessment committee on August 10, 2010. The committee concluded that there were no drug-related tumor findings in the mouse. Several tumors were considered to be drug-related in the rat. The tumors considered drug-related in rats as shown in the committee minutes are listed below.

Males

Brain: Astrocytoma at HD. Numerical, non-statistically significant increase in astrocytoma at mid-dose also considered drug-related.

Liver: Hepatocellular adenoma and carcinoma combined, at HD.

Mammary: Adenocarcinoma and fibroadenoma combined, at MD & HD.

Skin, subcutis: Fibroma at MD & HD

Skin: Squamous Carcinoma at HD. Numerical, non-statistically significant increase in squamous carcinoma at MD also considered drug-related.

Schwannoma (all sites) at HD. Numerical, non-statistically significant increase at the MD also considered drug-related.

Thyroid: Follicular cell adenoma at HD.

Females

Mammary: Adenocarcinoma + fibroadenoma at LD, MD, HD

Questions have been raised as to the appropriateness of combining adenocarcinoma and fibroadenoma in mammary tissue. However, combining these tumor types is routine in carcinogenicity study analysis and has been historically accepted. For example, the National Toxicology Program currently combines these tumor types and has done so for many years (see McConnell et al., JNCI, 1986, 76:283-289.) As noted below, distinguishing these tumor types can be challenging and a full understanding of the relationship between fibroadenomas and adenocarcinomas has not been reached in the literature. In addition, although not reflected in the meeting minutes, fibroadenomas alone were statistically significantly elevated in female rats at all drug doses.

As described in the pharm/tox review and supervisory memo, distinguishing between adenocarcinoma and fibroadenoma apparently proved challenging in this study. For example, at week 96 of the rat carcinogenicity study, 72 of 75 female animals in the high dose group were diagnosed with mammary adenocarcinomas while 36 of 75 were diagnosed with mammary fibroadenomas. However, in the information submitted to the NDA describing results at the end of the study, 60 of 75 female animals in the high dose group were diagnosed with mammary adenocarcinomas and 45 of 75 were diagnosed with mammary fibroadenomas. In addition, it appears that more tumors were reclassified from adenocarcinoma to fibroadenoma in the mid and high dose groups (20 reclassifications) than in the low and control dose groups (2 reclassifications). This difficulty in diagnosis and other irregularities with the data were noted by the execCAC and further supported combining these tumors for analysis.

Other drugs that cause mammary tumors in rodents have been approved. However, these drugs are for different indications that may carry a different risk/benefit profile and the mode of action by which these drugs produce the mammary tumors is typically defined. An elevation in prolactin was a potentially plausible mechanism for the increase in mammary tumors seen with lorcaserin and warranted further investigation. However, the applicant was not successful in establishing this or any other mechanism for the mammary tumor formation induced by lorcaserin. Therefore, it is not yet possible to dismiss the mammary tumors as irrelevant to humans.

Of the other tumors identified as related to drug treatment, the astrocytomas in the brain are probably of most concern. The incidence of these tumors in male rats was 1, 0, 4 and 8 in control, 10, 30 and 100 mg/kg, respectively. The high dose was statistically significantly elevated. Although the mid dose was not statistically elevated, the executive carcinogenicity assessment committee felt that the numerical increase observed at the mid dose was still likely to be drug-related in part due to the relative uncommonness of this tumor. The risk that these astrocytoma findings represent to humans is difficult to quantify because a mode of action for their formation has not been elucidated and because lorcaserin partitions preferentially into brain tissue in comparison to blood. Establishing a margin between therapeutic tissue levels and levels that resulted in tumor

formation is difficult given the absence of information on drug levels in human brain and the variability of brain levels measured in rats and monkeys.

Conclusions:

The pharm/tox supervisor has noted three major deficiencies in his memo. These are:

- 1) The exposure response relationship for lorcaserin-emergent mammary adenocarcinoma is unresolved.
- 2) An unidentified mode of action and unclear safety margin exists for lorcaserin-emergent brain astrocytoma.
- 3) Mode of action for lorcaserin-emergent mammary fibroadenoma is unresolved.

Addressing these deficiencies may allow a better assessment of risk to patients. If the diagnostic uncertainty was resolved for the mammary adenocarcinomas then it may be possible to better understand the exposure response relationship for these tumors and whether an acceptable margin exists between human exposure and exposures associated with these tumors. A better understanding of the margin between human brain levels of lorcaserin and the level associated with astrocytomas or an understanding of a mode of action for the astrocytomas may permit a better understanding of whether this finding represents a risk to humans. If a mode of action for the fibroadenomas was identified and its relevance to humans understood then the human risk could be better assessed. If the diagnostic uncertainty for the mammary tumors was resolved, then even in the absence of a mode of action for fibroadenoma it may be possible to re-evaluate whether the risk for this tumor type is acceptable in balance with whatever clinical benefit is demonstrated.

Lorcaserin carries an unquantifiable level of risk for tumor formation in humans at the proposed dose based on the current information. As with any nongenotoxic carcinogen, the risk of tumor development is likely to be small with short term exposure and increase with longer durations of exposure. Short term use of lorcaserin may minimize the risk of tumor formation. However, the acceptability of short term clinical use of this drug and the ability of the clinical benefit to sufficiently balance even a relatively small tumorigenic risk from short term use would need to be considered.

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/s/

PAUL C BROWN
10/21/2010

DAVID JACOBSON KRAM
10/21/2010



Memorandum

**Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products**

NDA SECONDARY REVIEW

Date:	20 October 2010
NDA #	22-529
Sponsor:	Arena Pharmaceuticals
Drug:	Lorcaserin HCl 5HT2C agonist
Primary Reviewer:	Fred Alavi, Ph.D.
Secondary Reviewer:	Todd Bourcier, Ph.D.

Arena Pharmaceuticals is seeking marketing approval for lorcaserin, proposed trade name Lorqess, as a treatment for obesity. Lorcaserin, a new molecular entity, is a heterocyclic benzazepine intended to selectively activate the 5HT2C serotonin receptor subtype. Serotonin acts in a milieu of other episodic and chronic regulatory signals that converge on central sites of appetite control, including the hypothalamic arcuate nucleus. The anorectic properties of serotonin involve not only 5HT2C, but also receptor subtypes 5HT1B and 1D. As a ‘selective’ agonist of 5HT2C, lorcaserin would be expected to activate anorectic POMC hypothalamic nuclei which promotes satiety and reduces food intake, resulting in a loss of body weight. The 5HT2C-targeted approach of lorcaserin, while important for minimizing known adverse effects of activating other serotonin receptor subtypes, may also have contributed to the relatively modest weight loss achieved in clinical studies.

Dr. Fred Alavi, the primary pharm/tox reviewer, recommends *against approval* of NDA 22529. Dr. Alavi’s primary concern is that lorcaserin was identified as a non-genotoxic carcinogen inducing multiple tumor types in 2-year lifetime studies in rats. Among the tumor types observed, the occurrence of mammary and brain tumors are of most concern regarding human risk assessment because no safety margin was identified for the former, and the safety margin is uncertain for the latter. Importantly, the applicant has provided inadequate information regarding lorcaserin’s tumorigenic mode of action, which is critical for evaluating human risk when safety margins are absent or are uncertain. *I share Dr. Alavi’s concern and concur with his recommendation against approval of lorcaserin.*

Mammary Tumors

No safety margin was identified in female rats for mammary tumors, which emerged within 7-fold of the proposed clinical dose of 10mg bid. Mammary tumors emerged in male rats at 17-fold the clinical dose, which is notable because such tumors in males are less common. The relevance of the mammary tumors to human risk is dismissed by the applicant and their consultant, (b) (6) for a variety of reasons. Chief among them is the supposition that prolactin mediates mammary tumors in rodents administered lorcaserin, similar to the tumorigenic mode

of action identified for anti-dopaminergic drugs. The applicant also argues that in human subjects, prolactin is not significantly elevated and does not represent an increased risk for breast cancer. I agree with Dr. Alavi's argument that the data submitted by the applicant is unpersuasive that prolactin plays an intermediary role in the rodent mammary tumors that emerged with lorcaserin. Most convincingly, lorcaserin repeatedly failed to cause a robust and sustained increase in serum prolactin in rats, and the marginal increases that were observed occurred under experimental conditions that bear little resemblance to the rats used in the formal carcinogenicity study. Comparison of lorcaserin to the anti-dopaminergic agent haloperidol and the serotonergic agent dexfenfluramine in the mechanistic studies further eroded support of an intermediary role of prolactin. Given the lack of a safety margin and an unresolved tumorigenic mode of action, the relevance of these findings in rats to human risk cannot be dismissed. Additional studies that address the tumorigenic mode of action of lorcaserin based on prolactin and, more importantly, on non-prolactin hypotheses may clarify the risk of this finding to the intended obese patient population.

An issue raised at the September advisory committee meeting was that the incidence of mammary adenocarcinoma and fibroadenoma should not be combined for statistical purposes because of the distinct cellular lineage of these tumor types. Given this criticism, it is odd that the applicant provided exactly this analysis in the NDA (Section 2.6.6, page 39-40, Tables 15 & 16). Distinguishing these two tumor types by histological exam alone can be difficult, particularly when both tumor types are present in the same animal. Indeed, there is evidence of such diagnostic difficulty in the study with lorcaserin (discussed below). This alone justifies a statistical analysis of the mammary tumors separately as well as combined. The FDA statistician evaluated these two tumor types alone and in combination, and the results mirror those found by the applicant: the incidence of fibroadenoma alone was statistically significantly increased at all doses of lorcaserin in females, whether or not combined with adenocarcinoma, and the incidence of adenocarcinoma was statistically significantly increased in females only at the high dose (by both trend and pair-wise testing). The numerical increases in adenocarcinoma and fibroadenoma in males was not statistically significant unless the tumor types were combined, in which case significance was achieved at the mid- and high doses. Despite the absence of statistical significance for each tumor separately in male rats, they are nonetheless considered related to lorcaserin because the incidences exceed the concurrent and historical control values and fall along a dose-response curve. In short, including an analysis of fibroadenoma and adenocarcinoma combined does not change the statistical outcome or our interpretation of the data.

I believe the underlying argument of the above criticism is that adenocarcinoma presents the greater risk to humans than fibroadenoma does, and because lorcaserin increased adenocarcinoma only at very high drug levels, any risk to human subjects is negligible. Also, the argument has been made that fibroadenoma does not progress to adenocarcinoma in rodents (or humans). I do not agree that the increase in fibroadenoma within 7-fold of clinical exposure equates to an absence of human risk. The acceptability of an increased risk of fibroadenoma (e.g., patient distress, increased monitoring and clinical intervention) will be addressed by the clinical review team. Of more concern, I agree with Dr. Alavi that distinguishing between adenocarcinoma and fibroadenoma apparently proved challenging to the applicant. For example, twenty cases of adenocarcinoma were reclassified to fibroadenoma in the mid- and high dose groups compared to only three cases reclassified in the control and low dose groups after the rat study was nearly complete. This imbalance does not simply reflect more cases at the higher

doses to reclassify; for example, the applicant reports a ‘final’ number of 34 and 35 cases of adenocarcinoma at the low and mid-dose groups, respectively, yet 8 cases were reclassified in the mid dose compared to 1 case in the low dose group. This imbalanced pattern of tumor reclassification favoring lorcaseerin is concerning and, at least, indicates a degree of diagnostic uncertainty from the primary and peer-reviewing pathologists. Additional observations raise further concern that lorcaseerin may have reduced latency and increased the aggressiveness of adenocarcinoma in the low and mid-doses, an effect that would not be obvious from final tumor incidence data:

First, the incidence of adenocarcinoma was consistently higher in all dose groups from weeks 55 to 96 of the study, which could not be explained by the difference in the number of animals evaluated at each time point.

Second, mammary adenocarcinoma metastasized to the lung in groups administered lorcaseerin but not in control, with an incidence of 0, 2, 7, and 6 for the control, low, mid, and high doses, respectively.

Third, at all doses of lorcaseerin, palpable nodules were detected earlier and a greater number of animals died at an earlier point in the study with mammary tumors listed as the cause of death (fibroadenoma and/or adenocarcinoma), suggesting reduced latency of tumor emergence.

Fourth, a confusing yet concerning pattern is observed in the number of females found with multiple masses (as opposed to a single mass) of adenocarcinoma: 9, 21, 13, and 33 at the control, low, mid, and high doses, respectively.

Finally, we have identified gross errors in the pathology reports of 2 cases, thus far, for female rats in the high dose group. In one case, female #4202 is reported as having all tissues ‘within normal limits’ despite the description a large ($\geq 20\text{mm}$) axillary mass present about 10 weeks prior to euthanasia. In the second, female #4212 is listed as having died of a mammary tumor, yet no mammary tumor is described in the histology report for this animal. These issues are sufficiently concerning to warrant re-evaluation of all mammary and lung tissues from the study by an FDA-appointed pathologist from an agency within HHS (e.g., National Toxicology Program), as has been done previously to clarify histological diagnoses. In addition, the clinical relevance of the apparent increased aggressiveness of adenocarcinoma has not been adequately addressed; presumably, the applicant dismisses this observation based on the mechanistic studies interpreted as evidence of an intermediary role for prolactin, an interpretation of the data that we do not share.

Astrocytoma

Lorcaseerin increased brain astrocytoma in male rats at the mid and high doses using standard histological sampling and detection methods in the 2 year study. A more extensive evaluation of the brain tissue (e.g., increased sampling, gliosis marker staining, etc.) could conceivably uncover new cases of astrocytoma, a possibility that could be addressed at the same time the mammary tissues are being re-evaluated. As submitted, the tumor signal emerged at exposure 17-fold higher than clinical exposure, with a safety margin of 5-fold (i.e., highest non-tumorigenic exposure) based on plasma drug levels. Because astrocytomas are located in the CNS, which is also the site of pharmacodynamic action, comparison of drug levels in the brain across species is the more appropriate analysis for calculating a safety margin. Lorcaseerin

preferentially partitions to brain tissue in monkeys and rats, but brain levels in human subjects require assumptions because distribution of lorcaserin to the CNS was not assessed in clinical studies. If one assumes that brain partitioning in humans resembles rats, then the safety margin remains unchanged (5-fold). But if monkeys are more predictive, then the safety margin increases to ~14-fold. It is feasible that brain partitioning in human subjects exceeds both rats and monkeys, in which case the safety margin is less than 5-fold. This latter possibility may not be remote; for example, neurological adverse events of euphoria occurred in human subjects at a dose ~4-fold higher than sought for marketing, whereas adverse neurological effects were present only at substantially higher exposures in rats and monkeys. Demonstration of a sufficiently robust safety margin based on comparative drug levels in the brain would go far to mitigate human risk. Admittedly, this may be problematic to address directly because it would involve determining levels of lorcaserin in brain tissue of human subjects by imaging modalities that likely require the use of radiolabeled drug. The uncertain safety margin can alternatively be addressed by providing evidence that the tumorigenic mode of action for lorcaserin-emergent astrocytoma bears little relevance to human risk. This approach would entail identification of key events in lorcaserin's mode of action in inducing astrocytoma, and demonstration that those key events do not or are unlikely to occur in human biology.

Other tumor types

It is important to consider that lorcaserin also increased the incidence of benign subcutis fibroma, squamous carcinoma of the skin, and malignant schwannoma in male rats at the mid- and high doses. Liver and thyroid follicular cell neoplasms were also increased at the high dose. The increase in benign fibroma may have occurred at all doses if one considers the increase from 4.6% in control to 11% at the low dose as drug-related. It is notable for a non-genotoxic compound to result in this array of tumor types affecting multiple tissues. Tumors of the peripheral/central nervous system and skin/subcutis are not shared by marketed centrally acting dopaminergic or serotonergic drugs or by current obesity medications. No studies or credible explanation was provided to address the spectrum of tumors induced by lorcaserin or the mechanism by which lorcaserin increased these tumors, so risk assessment must be based on the difference in exposure between rats and the clinical dose in humans. These tumors occurred at exposure 17-fold higher than the clinical dose, with a safety margin of 5x (i.e., tumors were not observed in rats at exposure 5-fold higher than the clinical dose). Unlike astrocytoma, these tumors could be detected early should they arise with long-term exposure to lorcaserin in human subjects, but this presupposes that an increased susceptibility to tumor development represents an acceptable risk for lorcaserin. The acceptability of a 5x safety margin to these tumor types must take into consideration the benefits that lorcaserin provides to the obese patient population.

The applicant and their consultant (b) (6) provided a variety of arguments that the tumor types identified in rats are either species specific, gender specific, secondary to generalized toxicity, occur only at 'very high' drug levels, or are otherwise irrelevant to human biology. Dr. Alavi's and my rebuttal to these arguments are detailed in the FDA review and the Advisory Committee briefing document. In brief, Dr. Alavi, the FDA Executive Carcinogenicity Assessment Committee, and I found the applicant's explanations either without merit, without adequate experimental support, or without due consideration of alternative hypotheses.

Other potential safety issues pertinent to lorcaserin and to serotonergic drugs in general were encountered in the preclinical development program. These included primarily the degree of selectivity of lorcaserin for 5HT_{2C} versus 5HT_{2A} and B, which has implications for potential

neurological and cardiac toxicity. Although the pharmacology data support the selectivity of lorcaserin for 5HT_{2C} versus 2A and 2B, the actual potency of lorcaserin for these receptors is uncertain. The uncertainty arose from submission of new data in the NDA showing ~10-fold higher receptor potency than was originally reported during IND development. This change in potency changes our view of the potential for lorcaserin to activate off-target receptors at drug concentrations reached in human subjects, especially in the CNS. Reasonable efforts could be made with additional studies to clarify the potency of lorcaserin for the 5HT₂ receptor subtypes, specifically by studies that control expression of receptor density.

The neurological and cardiac assessments conducted in animals did not identify severe toxicities of relevance that would be anticipated if 5HT_{2A} and 2B were activated by lorcaserin, despite achieving drug concentrations that exceed the *in vitro* receptor potency estimates. Short-comings in some of the neurological assessments and limitations in the ability to screen for drug-induced valvulopathy in animals render these assessments less than definitive, and it is difficult to predict that lorcaserin will be devoid of such toxicities should it be approved for marketing. Nevertheless, additional preclinical studies are unlikely to provide insight relevant to neurological and cardiac risks of long-term use of lorcaserin that cannot be gained from well-designed clinical studies in the obese patient population.

In summary, there are three major deficiencies in the nonclinical data that Dr. Alavi and I consider most pertinent to our regulatory recommendation:

1) The exposure response relationship for lorcaserin-emergent mammary adenocarcinoma is unresolved.

- Reasons for the imbalanced pattern of tumor type reclassification are unclear and indicate diagnostic uncertainty.
- Lorcaserin appears to decrease latency and increase aggressiveness of adenocarcinoma at low and mid-doses (7 to 24-fold clinical dose). The clinical relevance of this observation has not been adequately addressed.
- Re-evaluation of mammary tissue by FDA-appointed pathologists (e.g., from National Toxicology Program), or at least pathologists selected in consultation with the FDA, would clarify diagnoses, verify safety margins to clinical exposure, and permit re-evaluation of clinical risk. Additional data would be required to address the clinical relevance of the apparent increase in adenocarcinoma aggressiveness with lorcaserin. Mode of action data of minimal relevance to human subjects, for example, would mitigate clinical risk regardless of safety margins.

2) An unidentified mode of action and unclear safety margin exists for lorcaserin-emergent brain astrocytoma.

- Evaluation of additional tissue samples from all experimental groups would verify the dose-response relationship for brain tumors; note, this recommendation is not an absolute requirement.
- Additional information regarding the distribution of lorcaserin to the CNS in animals and human subjects would be needed to clarify the safety margin to brain tumors in rats. A robust difference in clinical exposure and exposure resulting in astrocytoma in rats would mitigate clinical risk.

- Mode of action data would be needed to address human relevance if a safety margin is near clinical exposure or cannot be clarified. Mode of action data of minimal relevance to human subjects, for example, would mitigate clinical risk regardless of safety margins.

3) Mode of action for lorcaserin-emergent mammary fibroadenoma is unresolved.

- Lorcaserin increases fibroadenoma at the lowest dose tested in female rats (~7-fold the clinical dose). A dose of lorcaserin that does not increase fibroadenoma was not identified.
- Prolactin mode of action data is unconvincing. Lorcaserin repeatedly failed to robustly and consistently increase serum prolactin in rats.
- Additional studies are recommended to address the tumorigenic mode of action and human relevance, which would permit re-assessment of human risk.
- It is recognized that from the clinical perspective of the review team, an increased risk of fibroadenoma with lorcaserin may be tolerable and compatible with drug approval regardless of the tumorigenic mode of action.

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/s/

TODD M BOURCIER

10/20/2010

Memo recommending Complete Response

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 22-529
Supporting document/s: electronic NDA
Applicant's letter date: Dec 22, 2009
CDER stamp date: Dec 23-2009
Product: LORQESS[®] (Lorcaserin HCl)
Indication: treatment of obesity
Applicant: Arena Pharmaceuticals
Review Division: DMEP
Reviewer: Fred Alavi, Ph.D.
Supervisor/Team Leader: Todd Bourcier, Ph.D.
Division Director: Mary Parks, MD
Project Manager: Patricia Madara

Disclaimer

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: Not recommended for approval until rodent carcinogenicity findings are clarified.

1.1.2 Additional Non-Clinical Recommendations:

The mode of action for mammary neoplasm and brain astrocytoma needs to be addressed. The reviewer recommends re-evaluation of mammary and brain tissue slides by an independent panel of pathologists. Since brain tumors are small and can be easily missed, more sectioning of brain tissue is recommended. The reviewer also recommends analysis of brain lorcaserin distribution in both male and female rats due to significant gender differences in lorcaserin kinetics in rats.

1.1.3 Labeling- Not applicable at this stage

1.2 Brief Discussion of Nonclinical Findings

Lorcaserin is a new molecular entity designed to selectively bind serotonin 2C receptors (5HT_{2C}) in the brain. The sponsor is seeking approval of lorcaserin for a weight loss indication. To assess the safety of lorcaserin, standard toxicological assessments of lorcaserin were carried out. Evaluations included genotoxicity, rat and monkey toxicology and carcinogenicity studies in mice and rats. The reproductive toxicity of lorcaserin was assessed in rats and rabbits. The toxicological assessments identified two major findings of clinical concern, a) neoplastic tumors in male and female rats and b) renal tubular regeneration and degeneration in a 12-month monkey study.

The genotoxicity and carcinogenicity of lorcaserin was assessed in a standard battery of genotoxicity tests and 2-year rodent bioassays, respectively. Lorcaserin was not genotoxic nor mutagenic in the standard *in-vitro* and *in-vivo* genotoxicity assays. The mouse carcinogenicity study was initiated with lorcaserin doses of 0, 25, 50 and 100 mg/kg. Oral administration of 100 mg/kg of lorcaserin (7.5 and 15x the clinical dose of 10 mg BID based on AUC) resulted in acute increase in mortality in both male and female mice within 16 days of the study initiation leading to a reduction of the lorcaserin dose after consultation with eCAC. The cause of death at 100 mg/kg was not determined but suspected to be neuronal in origin since lorcaserin can partition into the mouse brain up to 25x the plasma levels. The sharp increase in deaths in mice was unexpected since 100 mg/kg and higher doses of lorcaserin were tolerated for as long as 13 weeks. The mouse study was continued (Day 19) with lower lorcaserin doses of 0, 5, 25 and 50 mg/kg. The top dose of 50 mg/kg (4 and 7x the MRHD) was without any further incidence of mortality. In fact, there were no statistically significant changes in

any of the parameters evaluated in the 2-year mouse study with lorcaserin doses up to 50 mg/kg. The incidence of mammary adenocarcinoma in control, 5, 25 and 50 mg/kg (2/75, 1/65, 1/65, 4/75 mice) were not significant. The NOAEL for mouse carcinogenicity was 50 mg/kg (4x the MRHD in female and 7x the MRHD in males, based on AUC).

The rat 2-year carcinogenicity study was carried out with 0, 10, 30 and 100 mg/kg of lorcaserin (C, LD, MD and HD, respectively). Lorcaserin significantly and dose-dependently increased mortality at all doses in females due to mammary tumors and in HD males due to various types of tumors. Lorcaserin dose-dependently increased the number of deaths by mammary tumor and decreased the survival time (latency) in female rats. Both fibroadenoma and adenocarcinoma were fatal in female rats.

Lorcaserin Dose, mg/kg	0	10	30	100
Number of death due to mammary tumors in female rats				
Number of animals per group	65	65	65	75
Due to fibroadenoma	2	9	14	10
Due to adenocarcinoma and or fibro	13	22	29	50
Combined	15	31	43	68

There were nearly twice as many dead female rats due to mammary tumors at LD than in control. Fatality due to tumors occurred earliest in HD females at WK 42 vs. WK 61 in HD males. Since deaths were caused by tumors and weight loss in the HD males is a function of pharmacological activity of lorcaserin, the top dose of 100 mg/kg did not exceed the maximum tolerated dose (MTD) and the rat study was accepted as valid by the reviewer and eCAC (meeting minutes in Appendix A).

Lorcaserin resulted in numerous tumors in both male and female rats. These tumors occurred much earlier in lorcaserin treated rats. The first appearance of nodules in lorcaserin treated female was 11 to 13 weeks earlier than control females while in males they occurred 10 to 23 weeks earlier than control males.

Lorcaserin Dose, mg/kg	0	10	30	100
First tumor appearance, Weeks				
Female rats	33	24	20	20
Male rats	50	40	38	27

The two prominent tumors were mammary (adenocarcinoma and fibroadenoma) and brain. In females, the incidence of mammary fibroadenoma alone or in combination with adenocarcinoma were increased at every dose level ($p < 0.0001$) with no safety margin ($< 7x$ the MRHD). The incidence of adenocarcinoma was increased only in the HD females but numerically, the number of adenocarcinoma in LD and MD females was

higher than control and historical background. In males, the combined incidence of mammary fibroadenoma and adenocarcinoma was also significantly increased in MD and HD groups with a 5 fold safety margin.

Incidence of mammary tumors in male and female SD rats in the
2-year carcinogenicity study

Lorcaserin Dose, mg/kg	0	10	30	100
Mammary Tumors in Male Rats				
AUC Exposure Multiples	-	5x	17x	55x
Adenocarcinoma @ (historical range: 0 - 2%)	0	0	2/65 (3%)	2/75 (3%)
Fibroadenoma @ (historical range: 0 - 3.3%)	0	1/65 (1.5%)	4/65 (6%)	6/75 * (8%)
Combined	0	1	6 *	8 **
Mammary Tumors in Female SD Rats				
Exposure multiples	--	7x	24x	82x
Adenocarcinoma @ (historical range: 8.3 - 37%)	28/65 (43%)	34/65 (52%)	35/65 (54%)	60/75 ** (80%)
Fibroadenoma @ (historical range: 22 - 54%)	20/65 (31%)	47/65 ** (72%)	53/65 ** (82%)	45/75 ** (60%)
Combined	40	56 **	61 **	70 **

* p value <0.05, ** p value <0.01

Although the incidences of mammary tumors in the interim TK female rats were not included in the analysis, the incidence of adenocarcinoma was common finding in lorcaserin treated female rats.

Lorcaserin Dose, mg/kg	0	10	30	100
Mammary tumors in TK female SD Rats (n = 5-14/group)				
Adenocarcinoma	0/5	7/14	6/14	7/10
Fibroadenoma	3/5	5/14	8/14	5/10

Both fibroadenoma and adenocarcinoma were fatal in female rats. Division and eCAC recommended combining tumors originating from the same tissue. The sponsor also had analyzed mammary tumors individually and in combination. Combining benign (fibroadenoma) with malignant (adenocarcinoma) for statistical analysis was justified and logical for several reasons: **a)** a number of adenocarcinoma cases in female rats were reclassified to fibroadenoma, suggesting that distinguishing adenocarcinoma from fibroadenoma was difficult for the reviewing pathologist, **b)** both mammary fibroadenoma and adenocarcinoma originate from the same tissue with epithelial lineage, **c)** mammary tumor development in rodents is generally recognized to progress from hyperplasia to benign to malignant, **d)** combining mammary tumors in rats is an accepted practice used by other sponsors and the sponsor of this application.

As shown in the table below, the number of adenocarcinoma in the lorcaserin treated groups started decreasing after WK 96 even though more animals remained to be analyzed, suggesting the distinction between adenocarcinoma and fibroadenoma is not clear cut.

Changes in diagnosis of adenocarcinoma and fibroadenoma over time
(from Wk 55 until the final NDA submission)

Mammary Adenocarcinoma Incidence over time in Female Rats (main study)				
Data Update (Week)	Control	10 mg/kg	30 mg/kg	100 mg/kg
Week 55 update	0/1	2 / 4	5 / 7	13 / 15
Week 68 update	2 / 5	6 / 6	16 / 18	45 / 46
Week 88 update	16 / 28	27 / 38	36 / 45	72 / 74
Week 96 update	20 / 39	34 / 50	43 / 57	72 / 75
Week 104 update	30 / 65	35 / 65	35 / 65	63 / 75
Final update	29 / 65	35 / 65	36 / 65	62 / 75
Final NDA	28 / 65	34 / 65	35 / 65	60 / 75

Mammary Fibroadenoma Incidence over time in Female Rats (main study)				
Data Update (Week)	Control	10 mg/kg	30 mg/kg	100 mg/kg
Week 55 update	0 / 1	1 / 4	3 / 7	2 / 15
Week 68 update	1 / 10	1 / 11	5 / 18	20 / 46
Week 88 update	4 / 28	16 / 38	24 / 45	35 / 74
Week 96 update	10 / 39	27 / 50	36 / 57	36 / 75
Week 104 update	20 / 65	47 / 65	60 / 65	53 / 75
Final update	20 / 65	48 / 65	56 / 65	51 / 75
Final NDA	20 / 65	47 / 65	53 / 65	45 / 75

The lorcaserin-related increase in mammary tumors were hypothesized by the sponsor to be mediated indirectly by action of lorcaserin on prolactin since prolactin is a known intermediary hormone in rodent mammary tumorigenesis for several drugs including antipsychotic anti-dopaminergic drugs, such as haloperidol. This mode of action sounded reasonable at the time; however, the mechanistic studies provided by the sponsor thus far have failed to persuasively demonstrate a link between lorcaserin-emergent mammary tumors and prolactin, as it has been demonstrated for haloperidol. Lorcaserin had no effect on serum prolactin in female rats and reduced prolactin in males by 50% in the rat carcinogenicity study.

Serum Prolactin at week 55 and 56 in TK rats in the carcinogenicity study		
Lorcaserin, mg/kg	Serum prolactin at WK 55 in male rats	Serum prolactin at WK 56 in female rats, ng/ml
0	57.8 ± 32 *	115 ± 80
10	28.2 ± 12	130 ± 56
30	29.9 ± 11	106 ± 68
100	23.6 ± 16	117 ± 63

* p vales < 0.05

In multiple supportive GLP studies, haloperidol robustly increased serum prolactin under all circumstances (intact, ovariectomized) while lorcaserin did not. When rats were ovariectomized and replenished with estradiol and progesterone, a minimal increase in prolactin was seen with lorcaserin. Since the conditions of the study were rather contrived and nothing like those present in the rat carcinogenicity study, the scientific value of the study is questionable.

Dexfenfluramine, a nonselective serotonin agonist, also mildly increased prolactin levels supposedly by increasing brain serotonin, which lorcaserin does not. Dexfenfluramine does not cause mammary tumors in rodents despite the mild increase in prolactin, suggesting that a small increase in prolactin is unlikely to lead to mammary tumors.

Serum Prolactin Analysis				Study: (b) (4) 370002/TX08007		
Group:	Sexually Intact Female			Ovariectomized Females		
	Vehicle	Lorc	D-Fen	Vehicle	Lorc	D-Fen
Prolactin, ng/ml						
Day 9	15.0	6.2	42.10	10.7	3.1	21.50
Day 20	11.7	9.1	98.1 *	4.6	4.7	12.6 *

With no role definitively attributable to prolactin, one has to conclude that lorcaserin increased mammary tumors in rats by a direct or indirect mechanism independent of prolactin.

The second prominent tumor identified was the increased incidence of brain astrocytoma in HD male rats ($p < 0.0001$). Numerically, the number of astrocytoma in MD males was greater than control and the historical background, and the Division and eCAC consider this numerical increase related to drug treatment.

Incidence of adenocarcinoma and schwannoma (all sites) in male rats

Lorcaserin dose, mg/kg	0 n=65	10 n=65	30 n=65	100 n=75
AUC Exposure Multiples	-	5x	17x	55x
Nervous System Tumors in Male Rats				
Astrocytoma @ (historical range 0 to 5%)	1 (1.5%)	0	4 (6%)	8 ** (10.7%)
Malignant Schwannoma @ (historical range, 0-3.3%)	0	0	2 (3%)	9** (12%)

The sponsor has argued that astrocytoma in rats derive from a microglial lineage compared to an astrocytic lineage as occurs in humans, and therefore astrocytoma in rats does not have a human counterpart. The issue of cell lineage of rat astrocytoma has been known for more than 20 years and the issue is unsettled as they are still officially classified as astrocytoma in rats. The sponsor has also suggested that the absence of a significant increase in astrocytoma in female rats indicates that astrocytoma is gender specific. Although this is a plausible explanation, the fact that female rats were dying 7 to 17 weeks earlier than males and had significantly shorter

duration of exposure than males also bears consideration. Also, brain exposure in female rats may have differed from males, because there appears to be a significant gender difference in plasma drug exposure in rats. It should be noted that there was a total of 20 cases of astrocytoma in the rat study of which only one was found in the control group, suggesting that astrocytoma was indeed consistently more common in rats administered lorcaserin.

Incidence of astrocytoma in the main and the interim TK animals (WK 52)
in the 2-year rat carcinogenicity study

Lorcaserin dose, mg/kg		0 n=65	10 n=65	30 n=65	100 n=75
Main study, astrocytoma	M	1	0	4	8
	F	0	2	0	1
TK study, astrocytoma	M	0	0	0	1
	F	0	0	1	2
Total astrocytoma (20)		1	2	5	12

Risk assessment for astrocytoma is complicated by the fact that human brain lorcaserin exposure is unknown. Lorcaserin is a CNS drug and highly partitions to brain relative to plasma (35x the plasma in rats and 10x the plasma in monkeys), so a safety margin based on plasma levels is less acceptable than a safety margin based on brain levels of drug. If the brain exposure data in monkeys extends to humans (10x the plasma), then a sufficient safety margin exists for astrocytoma (14x the MRHD). But if one assumes a human brain partition similar to rats, the safety margin is reduced to only 5x the clinical dose, which raises our level of concern.

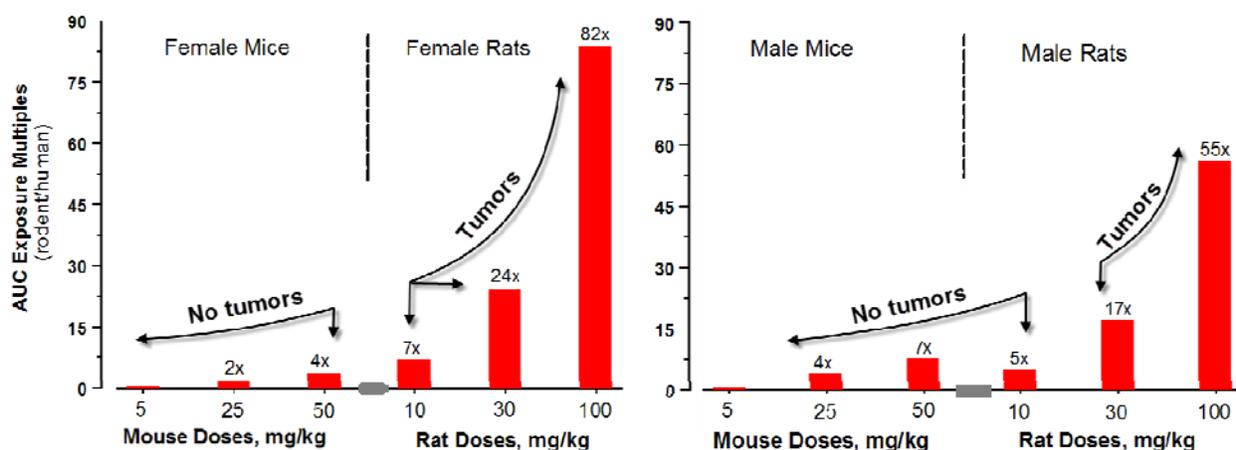
Exposure multiple based on estimated brain concentrations of lorcaserin in humans		
Brain : Plasma Ratio	10 mg/kg (No astrocytoma)	30 mg/kg (astrocytoma)
Assuming 10x →	14 x	50x
Assuming 25x →	5 x	17x

Other significant tumors associated with lorcaserin in male rats were skin (squamous cell carcinoma and subcutis fibroma) and malignant schwannoma, with drug-related increases occurring at 17-fold the clinical dose. The clinical relevance of these tumor types is uncertain. Also, hepatocellular adenoma and carcinoma occurred at 100 mg/kg, or 55x the clinical dose, which presents a minimal potential risk to human subjects.

Overall, lorcaserin was considered to be a non-genotoxic carcinogen in rats. Lorcaserin dose-dependently increased mammary tumors at all doses in female rats (<7x the MRHD) and at ≥ 30 mg/kg (≥ 17 x the MRHD) in male rats. The incidence of astrocytoma was significantly increased in HD males (100 mg/kg) and was numerically

higher than the control and historical background in MD males (30 mg/kg). With no brain exposure data, the safety margin for astrocytoma is difficult to predict.

Lorcaserin exposure in mice and rats relative to the clinical dose of 10 mg BID, based on AUC (1.02 $\mu\text{g}\cdot\text{h}/\text{ml}$)



The absence of significant increases in mammary tumors in mice at the maximum tolerated dose of 50 mg/kg is not considered evidence of a species specificity because the highest drug exposure in mice was equal to or less than the lowest drug exposure in rats. Furthermore, since lorcaserin partitions to the brain in mice (25x the plasma) less than that in rats (35x the plasma), the brain exposure to lorcaserin did not cover the same range seen in rats, suggesting that the absence of tumors in mice were primarily due to lower exposure to lorcaserin.

Renal Tubular findings in monkey

In the 12-month monkey study (2, 10, 50 and 125 mg/kg; 1, 6, 37 and 61x the clinical dose of 10 mg BID on AUC), lorcaserin resulted in minimal to moderate renal tubular regeneration and degeneration at ≥ 10 mg/kg in monkeys. The severity and incidence of tubular regeneration and degeneration increased with dose and persisted at the end of the 4-week recovery in some animals at 50 and 125 mg/kg. Lower doses were not included in the recovery phase. Examination of renal slides from the rodent studies were not consistent. Some early studies (i.e. 3-month rat) had identified a renal signal but there were no such findings in the 6-month or the 2-year rat carcinogenicity study. Although rats are prone to glomerulosclerosis, cynomolgus monkeys are not, thus making the renal signal in monkeys an important adverse effect. The reason for concern stems from the high concentration and function of 5HT_{2A} receptors in the kidney. Activation of 5HT_{2A} has been shown to result in tubular hypertrophy in rodents. The relevance of renal degeneration at this point is not certain since there has been no notable renal signal in clinical studies. Whether there are renal tubular changes in humans is currently unknown.

Nonclinical safety issues relevant to clinical use

The most prominent non-clinical finding of potential relevance to chronic use of lorcaserin in human subjects is the increased incidence of mammary and brain tumors in the 2-year rat carcinogenicity study. Prolactin as the intermediately hormone for mammary tumors is a plausible explanation but studies provided by the sponsor to date have failed to show a clear relationship between lorcaserin and prolactin. If the effect of lorcaserin on mammary tissue is by direct activation of off target receptors (5HT_{2A} or 5HT_{2B}), resembling that of 5HT_{2B} activation of heart valves, then the long-term risk to humans is substantial.

The high incidence of astrocytoma in male rats at ≥ 30 mg/kg is a concern due to the absence of a reliable estimate of safety margins. Although the cell lineage and site concordance of rat astrocytoma to humans remains unresolved, the fact that lorcaserin is a CNS active drug with significant partitioning to brain tissue makes it a long-term clinical risk unless a reliable safety margin can be established or a mode of action that is irrelevant to human biology is demonstrated.

2 Drug Information

2.1 Drug: Lorcqess ®

2.1.1 CAS Registry Number: 856681-05-5

2.1.2 Generic Name: Lorcaserin hydrochloride

2.1.3 Code Name: APD356 hemihydrate, AR226173 hydrochloride hemihydrate

2.1.4 Chemical Name:

(*R*)-8-Chloro-1-methyl-2,3,4,5 tetrahydro-1*H*-3-benzazepine hydrochloride hemihydrate

2.1.5 Molecular Formula/Molecular Weight: C₁₁H₁₅Cl₂N.5H₂O, MW [REDACTED] g/mol

2.1.6 Structure:



2.1.7 Pharmacologic class: Serotonin receptor 2 C (5HT_{2C}) agonist

2.2 Relevant IND/s, NDA/s:

IND 69888 (Arena pharmaceuticals),
Sibutramine (IND 27,624, NDA 20-632, Abbott/Knoll)
IND [REDACTED] (b) (4)
Dexfenfluramine (NDA 20344)

2.3 Clinical Formulation: 10 mg lorcaserin hydrochloride tablets

2.3.1 Drug Formulation

Active ingredient: 10.4 mg of APD356 Hemihydrate (10.4 mg tablets)

Inactive ingredients: Silicified microcrystalline cellulose, hydroxypropyl cellulose, croscarmellose Na, magnesium stearate and (b) (4)

Component	Grade	Function	mg/tablet	%w/w
Core				
Lorcaserin HCl Hemihydrate	Arena	Drug substance	10.4 ^a	10.4
Silicified microcrystalline cellulose ^b	(b) (4)			(b) (4)
Hydroxypropyl cellulose	NF			
Croscarmellose sodium	NF			
Magnesium stearate	NF			
(b) (4)	(b) (4)			
(b) (4)	USP			

^a Equivalent to 10 mg lorcaserin HCl.

2.3.2 Comments on Novel Excipients: All the excipient are GRAS

2.3.3 Comments on Impurities/Degradants of Concern: There are several very minor impurities in the product of which only the (b) (4) exceeded \geq (b) (4). The potential genotoxicity of the impurities were examined using MultiCASE software that evaluates for structural activity relationship of the impurities to the available database. Three of the 12 minor impurities (b) (4) were found to have the potential for genotoxicity. Since the exposures to these impurities were less than the daily allowance of (b) (4) under the genotoxic impurities guidance (b) (4) no specific safety analysis is required and thus deemed to be safe. Residual solvents (b) (4) in the drug substance were less than the accepted ICH limits.

2.4 Proposed Clinical Population and Dosing Regimen: (b) (4)

2.5 Regulatory Background. Lorcaserin IND was submitted to FDA on May 25, 2004 with some clinical experience in trials carried out in the UK. The rat and mouse carcinogenicity study protocols were submitted on Jan 25 of 2005 and May 23, 2006,

respectively. Upon initiation of mouse carcinogenicity study, the unexpected rise in mortality within 16 days administration of 100 mg/kg of lorcaserin, the sponsor requested dose adjustment to 50 mg/kg. Mid-way (63 weeks) through the rat carcinogenicity study the sponsor submitted a 15-day safety report on May 31, 2007 (#0047), showing a high incidence of mammary tumors in females and brain tumors in male and female rats. At the time of the submission, the sponsor was 8 months to the 2-year clinical study #3182. The Division recommended changes to the consent form to reflect the preliminary data describing higher than normal incidence of mammary tumors and brain tumors in the ongoing study. The Division requested bimonthly updates of mammary and brain tumor incidence as histopathology evaluation of dead rats became available (page 148). In the 3rd bimonthly update on March 10, 08 (WK 96) with all the HD females necropsied, there was an apparent dose-dependent increase in incidence of malignant mammary tumors (adenocarcinoma) in female rats at all doses. The division met with the sponsor to discuss the mode of action for mammary tumors and the possibility of a clinical hold. The Division allowed the ongoing phase 3 studies to continue since the data from other groups in the rat study were still missing, prolactin was a reasonable explanation of mode of action, and there were no mammary tumors in mice. The Division requested a draft report of the rat and mouse carcinogenicity studies as soon as possible and requested changes to the clinical protocol to include analysis of human serum prolactin. The bimonthly updates continued until the rat study was completed and draft report of the rat study was submitted (Feb 3, 2009).

3 Studies Submitted:

- Acute toxicology studies in rats and monkeys
- PK, TK studies including brain distribution in mice, rats and monkeys
- Standard battery of *in-vitro* and *in-vivo* genotoxicity tests
- Mechanistic studies exploring role of prolactin
- 3- and 6-month SD rat toxicology studies
- 3-month dose ranging study in CD-1 mice
- 3- and 12-month cynomolgus monkeys studies
- 2-year rat and mouse carcinogenicity studies
- Rat fertility and reproductive studies
- Rat and rabbit embryofetal developmental studies
- Rat pre- and post-developmental studies

3.1 Studies Reviewed: All the above

3.2 Studies Not Reviewed: None

3.3 Previous Reviews Referenced: Toxicology studies up to 3-months in mice, rats, and monkey studies, genotoxicity studies as well as some of the reproductive toxicology studies were review under lorcaserin IND 69,888.

4 Pharmacology

4.1 Primary Pharmacology

The FDA briefing document for the September advisory committee meeting reviews additional information regarding serotonin receptor selectivity and the nonclinical neurological and cardiac assessment of lorcaserin.

Lorcaserin is a chiral compound (r-racemate, purity >98%) isolated from S-racemate. *In-vivo* and *in-vitro* studies have not found any chiral inversion of lorcaserin. Rats pretreated with 5HT_{2C} antagonist (SB242084) had reduced response to lorcaserin suggesting that the appetite suppressant effect of lorcaserin is mediated via 5HT_{2C} receptor (K_i 23 nM). Lorcaserin (R-configuration) and its S-enantiomer binding have been tested for affinity to 76 other receptor types, ion channels and transporters (appendix B). The Neither enantiomers displayed significant inhibition of non-serotonergic receptors at tested concentrations of 1 μM. Lorcaserin has approximately 14 fold and 100 fold selectivity over 5HT_{2A} and 5HT_{2B} receptors, respectively. Lorcaserin was selective to 5HT_{2C} in rats but in monkeys, lorcaserin affinity to 5HT_{2C} and 5HT_{2A} and 5HT_{2B} were similar. Since 5HT_{2C} are primarily located in the CNS, the potential non-CNS effect is likely to be a consequence of central effects of lorcaserin in rats.

Lorcaserin-mediated increase in inositol phosphate (IP) accumulation in HEK293 cells expressing 5-HT₂ receptors.¹

Compound	5-HT _{2A} EC ₅₀ (nM) [± SEM]	5-HT _{2A} Cmpd Max/5-HT Max [± SEM]	5-HT _{2B} EC ₅₀ (nM) [± SEM]	5-HT _{2B} Cmpd Max/ 5-HT Max [± SEM]	5-HT _{2C} EC ₅₀ (nM) [± SEM]	5-HT _{2C} Cmpd Max/ 5-HT Max [± SEM]
5-HT	122 [±4]	1.00	35 [±3]	1.00	22 [±1]	1.00
Lorcaserin	123 [±15]	0.84 [±0.12]	1,000 [±80]	1.0 [±0.004]	9 [±1]	1.0 [±0.004]

¹Values represent the mean ± SEM of EC₅₀ determinations. 5-HT₂ Compound Max/5-HT Max refers to ratio of the maximal stimulation of inositol phosphate accumulation (percent control) observed with the highest concentration of test compound (10 μM) divided by the maximal stimulation of inositol phosphate accumulation observed in the presence of the highest concentration of 5-HT (10 μM) obtained in the same experiment.

Summary of lorcaserin binding affinities (K_i) for human and rat 5-HT_{2A} and 5-HT_{2C} receptors

h5-HT _{2A} K _i (nM) [±SD]	r5-HT _{2A} K _i (nM) [±SD]	h5-HT _{2C} K _i (nM) [±SD]	r5-HT _{2C} K _i (nM) [±SD]
149 [±34]	150 [±35]	23 [±4]	15 [±3]

In addition to CNS, 5HT_{2A} is also expressed in platelets, fibroblast and cardiovascular cells as well as the peripheral neuronal cells. Lysergic acid (LSD) exhibits agonist

activity at both receptors. These data were interpreted as indicating that the 5HT_{2A} receptor might be the initiating site of action for hallucinogens.

Binding of Lorcaserin, Lorcaserin Enantiomer and Metabolites to Recombinant Rat and Cynomolgus Monkey 5-HT₂ Receptors

Receptor / Radioligand	Lorcaserin ^a	Lorcaserin Enantiomer (AR226175) ^b	Metabolite M1 (AR244208) ^c	Metabolite M2 (AR235734) ^d	Metabolite M5 (AR306388) ^e
	K _i (n) ^f [95% confidence interval], (nM)				
Rat 5-HT_{2A} [¹²⁵ I]DOI	81 (13) [59 – 110]	114 (20) [94 – 138]	> 10,000 (3)	457 (3) [440 -467]	> 10,000 (3)
Rat 5-HT_{2B} [¹²⁵ I]DOI	114 (6) [95 – 137]	227 (6) [188 – 274]	> 10,000 (4)	407 (4) [288 - 566]	> 10,000 (4)
Rat 5-HT_{2C} [¹²⁵ I]DOI	16 (8) [11 – 23]	20 (10) [14 – 28]	> 10,000 (3)	110 (3) [58 - 208]	> 10,000 (3)
Monkey 5-HT_{2A} [¹²⁵ I]DOI	157 (7) [109 – 226]	319 (9) [222 – 459]	> 10,000 (4)	302 (4) [261 - 349]	> 10,000 (4)
Monkey 5-HT_{2B} [¹²⁵ I]DOI	127 (7) [99 – 162]	260 (7) [190 – 356]	> 10,000 (4)	417 (4) [274 - 633]	> 10,000 (4)
Monkey 5-HT_{2C} [¹²⁵ I]DOI	122 (6) [107 – 139]	242 (6) [214 – 275]	> 10,000 (3)	851 (3) [730 - 990]	> 10,000 (3)

The binding studies also included evaluation of the prominent metabolite, M1 and minor metabolites M2 and M5. M1 and M5 were inactive but M2 did show some activity at 5HT_{2C} and 5HT_{2A} (EC₅₀ = 30 to 35 nM) with minimal activity at 5HT_{2B} (17 fold more selective). However, M2 had also appeared to have some inhibitory properties toward rat α₂-adrenergic (65% inhibition) and human β₁-adenergeic receptors (56% inhibition) (appendix B for receptor binding table).

Potential off target activity was measured at well-known receptors, ion channels and enzymatic systemic. Lorcaserin showed no notable binding to non-serotonin receptors. The EC₅₀ for dopamine and serotonin transporter was minimal at 23 and 49 μM, respectively.

Effect of lorcaserin on monamine transporter binding, uptake, and release¹

Transporter	IC ₅₀ Binding (μM)	IC ₅₀ Uptake (μM)	EC ₅₀ Release (μM)
Norepinephrine	14	2.5	>100
Dopamine	33	12	23
Serotonin	1	1.4	49

¹ NE uptake and release was measured using rat hypothalamic synaptosomes, DA uptake and release were measured using rat striatal synaptosomes, and 5-HT release was measured using rat brain synaptosomes. For each determination, eight concentrations of lorcaserin were tested in duplicate.

Drug activity related to proposed indication: Lorcaserin has been shown to reduce body weight in animal models as well as in humans. . Chronic administration of 4.5, 9, 18 and 36 mg/kg of lorcaserin to male and female Levin dietary induced obese (DIO) for 4 weeks reduced body weight and food intake.

Effects of APD356 on Appetite, Weight, Body Composition and Plasma Markers for Dyslipidemia in Rats

Mean ± SEM	Treatment Group						Recovery Group	
	Vehicle	Sibutramine (6 mg/kg q.d.g)	APD356 (mg/kg b.i.d.)				Vehicle	APD356
			4.5	9	18	36		
Female								
Food intake (g)								
Average daily (wk 1)	14.7 ± 0.7	6.5 ± 0.7	10.6 ± 0.6	8.3 ± 0.5	7.4 ± 0.4	5.8 ± 0.6	15.8 ± 0.7	17.4 ± 0.8
Average daily (wk 2)	15.6 ± 0.6	13.2 ± 0.8	14.1 ± 0.8	12.2 ± 0.3	11.9 ± 0.3	11.3 ± 1.1	16.9 ± 0.9	19.9 ± 1.2
Average daily (wk 3)	15.5 ± 0.6	15.3 ± 0.7	14.9 ± 0.8	12.9 ± 0.3	12.5 ± 0.3	12.1 ± 0.8	16.9 ± 0.9	18.6 ± 0.8
Average daily (wk 4)	16.1 ± 0.6	16.4 ± 0.7	15.3 ± 0.7	13.8 ± 0.6	13.5 ± 0.3	11.8 ± 0.7	16.9 ± 1.2	18.9 ± 1.1
total food intake	432.7 ± 15.4	359.4 ± 15.8**	384.6 ± 19.2	330 ± 7.3***	317.3 ± 7.4***	275.8 ± 18.6***	465.1 ± 24.1	523.9 ± 22.8
Body weight (g)								
Baseline	267 ± 9.1	275 ± 7.0	266 ± 10.2	267 ± 8.5	268 ± 10.8	259 ± 10.4	303 ± 21.2	279 ± 18.8
end of wk 1	274 ± 10.3	244 ± 5.5	262 ± 9.7	256 ± 7.9	256 ± 10.3	247 ± 10.2	307 ± 21.1	293 ± 20.0
end of wk 2	282 ± 10.8	240 ± 2.8	270 ± 10.8	263 ± 8.9	259 ± 9.8	248 ± 7.0	315 ± 22.8	314 ± 23.6
end of wk 3	291 ± 10.5	243 ± 2.2	277 ± 12.2	268 ± 7.3	263 ± 10.0	249 ± 7.2	322 ± 23.0	327 ± 22.6
end of wk 4	300 ± 12.1	252 ± 2.7	286 ± 12.7	277 ± 7.7	271 ± 10.4	254 ± 6.5	331 ± 25.7	340 ± 26.0
Change from baseline (%)	11.9 ± 1.4	-8.2 ± 2.1***	7.1 ± 2.0	3.8 ± 0.7**	1.6 ± 0.9***	-1.7 ± 2.1***	10.3 ± 2.8	22.1 ± 2.7**

* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 ANOVA w/ Dunnett's Multiple Comparison test

Effects of APD356 on Appetite, Weight, Body Composition and Plasma Markers for Dyslipidemia in Male and Female Levin DIO Rats (Cont.)

Mean ± SEM	Treatment Group						Recovery Group	
	Vehicle	Sibutramine (6 mg/kg q.d.)	APD356 (mg/kg b.i.d.)				Vehicle	APD356
			4.5	9	18	36		
Females								
Average Fat Mass								
Average Fat Mass	87.8 ± 15	51.1 ± 4.6*	70.1 ± 11.8	67.5 ± 3.3	55 ± 4.7	44.8 ± 9.1		
Average Lean Mass								
Average Lean Mass	182.6 ± 4.6	176.1 ± 4.5	190.8 ± 5.4	184.1 ± 5.1	185 ± 3.7	157.1 ± 25.9		
Serum Lipids								
Triglycerides								
Triglycerides	69 ± 17.9	56 ± 9.3	51.6 ± 10.3	71.9 ± 12.3	63.7 ± 7.8	74.8 ± 7.8		
Total Cholesterol								
Total Cholesterol	61.3 ± 4.0	62.6 ± 5.1	64 ± 4.7	65.3 ± 1.8	64.3 ± 2.7	65.4 ± 4.4		
HDL Cholesterol								
HDL Cholesterol	54.0 ± 2.0	55.3 ± 2.8	56.9 ± 3.3	57.2 ± 1.2	58.9 ± 2.0	56.9 ± 4.5		
Male								
Food intake (g)								
Average daily (wk 1)								
Average daily (wk 1)	21.1 ± 0.5	11.7 ± 0.7	15.7 ± 0.6	13.4 ± 0.6	11.3 ± 0.4		20.0 ± 0.3	24.5 ± 0.7
Average daily (wk 2)								
Average daily (wk 2)	21.9 ± 0.5	18.1 ± 0.4	18.9 ± 0.8	16.7 ± 0.7	16.8 ± 0.5		20.7 ± 0.4	24.6 ± 0.6
Average daily (wk 3)								
Average daily (wk 3)	21.1 ± 0.5	18.4 ± 0.7	19.9 ± 0.5	17.6 ± 0.6	19.0 ± 0.5		20.6 ± 0.4	23.7 ± 0.7
Average daily (wk 4)								
Average daily (wk 4)	21.1 ± 0.5	18.2 ± 0.5	19.0 ± 0.3	18.5 ± 0.5	18.7 ± 0.4		20.5 ± 0.6	22.4 ± 0.5
Total food intake	596.5 ± 12.8	465 ± 12.9***	514 ± 14.6***	462.6 ± 13.7***	460.4 ± 10.4***		573 ± 9.3	666 ± 16.3***

* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 ANOVA w/ Dunnett's Multiple Comparison test

Effects of APD356 on Appetite, Weight, Body Composition and Plasma Markers for Dyslipidemia in Male and Female Levin DIO Rats

Mean ± SEM	Treatment Group						Recovery Group	
	Vehicle	Sibutramine (6 mg/kg <i>q.d.</i>)	APD356 (mg/kg b.i.d.)				Vehicle	APD356
4.5			9	18	36			
Male								
Body weight (g)								
Baseline	462 ± 9.8	460 ± 9.7	467 ± 11.9	460 ± 14.3	457 ± 9.8	537 ± 18.9	498.0 ± 19.7	
end of wk 1	481 ± 10.2	442 ± 10.0	471 ± 11.8	454 ± 14.0	444 ± 9.6	549 ± 19.1	530 ± 18.2	
end of wk 2	505 ± 11.2	458 ± 10.7	488 ± 12.5	465 ± 14.7	459 ± 9.8	564 ± 19.3	558 ± 19.3	
end of wk 3	525 ± 11.9	471 ± 11.7	508 ± 12.6	480 ± 14.5	478 ± 10.0	579 ± 20.3	580 ± 20.3	
end of wk 4	543 ± 12.6	479 ± 12	524 ± 13.2	495 ± 14.1	495 ± 10.5	591 ± 20.5	597 ± 20.1	
Change from baseline (%)	17.7 ± 0.8	4.0 ± 0.8 ^{***}	12.4 ± 0.7 ^{***}	7.7 ± 0.9 ^{***}	8.4 ± 1.0 ^{**}	10.4 ± 0.7	20.9 ± 1.3 ^{***}	
Average Fat Mass	197.0 ± 15.4	111.3 ± 4.7 ^{***}	170.6 ± 13.5	155.0 ± 10.4 [*]	139.6 ± 10 ^{**}			
Average Lean Mass	317.9 ± 13.6	339.9 ± 11.4	317.4 ± 6.6	303.3 ± 9.6	319.7 ± 5.0			
Serum Lipids								
Triglycerides	164 ± 22.8	70.9 ± 7.1 ^{***}	89.6 ± 8.3 ^{**}	77.9 ± 8.5 ^{***}	129.5 ± 19.6			
Total Cholesterol	71.3 ± 2.7	62.5 ± 3.0	74.1 ± 3.8	66.9 ± 1.0	54.8 ± 2.5 ^{***}			
HDL Cholesterol	55.3 ± 1.5	55.3 ± 2.0	62.5 ± 2.1 [*]	57.3 ± 1.0	48.6 ± 1.6 [*]			

* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 ANOVA w/ Dunnett's Multiple Comparison test

Phase IIb clinical study showed a modest decrease in BW with 10 mg QD (-1.8 kg), 15 mg QD (-2.6 kg) and 10 mg BID (-3.6 kg) lorcaserin in obese human subjects. Based on this study the sponsor chose 10 mg BID as the clinical dose.

4.2 Secondary Pharmacology

Published data suggest that drug-induced decreases in BW can reduce obesity associated diabetes, hyperlipidemia, insulin resistance, hypertension. The benefits of weight loss stemming from the reduction in risk factors (blood lipids, cardiovascular etc.) have been demonstrated in obese animal studies. The sponsor has proposed to evaluate the potential cardiovascular benefits, effect on mood and quality of life of lorcaserin treatment (10 mg BID) in phase III clinical trials.

4.3 Safety Pharmacology

Safety pharmacology studies have been reviewed in earlier reviews and only a brief summary is provided for reference. Lorcaserin induced a dose-dependent reduction in locomotor activity and increased periods of inactivity in animals. In the 28-day rat toxicology study there were no clinical sign of CNS toxicity at doses up to 50 mg/kg (>24 X clinical dose, based on AUC). Some rats at 50 mg/kg were hypersensitive. An incidence of tremor was noted in 1 male rat at 50 mg/kg. As a CNS active drug, lorcaserin has the potential to be addictive and cause psychological disturbances in humans; therefore the neurobehavioral effect of lorcaserin in humans was also closely monitored.

In the cardiovascular safety study, single oral doses of lorcaserin up to 100 mg/kg had no effect on cardiovascular parameters (MAP, HR, ECG, and QT) in monkeys (telemetry up to 20 hr post dose). When the action potential effect was examined in isolated Purkinje fiber assay, lorcaserin significantly prolonged action potential duration at 90% (ADP₉₀) in isolated canine Purkinje fiber assay at 30 μ M (6.96 μ g/ml vs. clinical of 85 ng/ml) but had no effect on ADP₆₀ (3, 10 and 30 μ M). In hERG channel study, lorcaserin significantly inhibited hERG (I_{Kr}) current at all concentrations in a dose-dependent manner with IC₅₀ =14 μ M (3.25 μ g/ml) but the potential clinical significance is minimal since the concentrations at which these findings were observed are several multiples higher than the anticipated plasma concentrations in humans (C_{max} of 85 ng/ml at 20 mg/kg). Therefore, lorcaserin is unlikely to prolong action potential in humans. A definitive human QT study in human subjects was conducted and reviewed by FDA.

In a recent review article McCann et al (278(8): 666-672, JAMA, 1977) discussed long-term use of fenfluramine on brain serotonin neurons, body weight, and pulmonary function in animals and humans. They reported that fenfluramine caused dose-related, long-lasting reductions in serotonin axonal markers in all the animal species tested at doses similar to doses used in humans. The primary human adverse findings were related to development of primary pulmonary hypertension. In animal studies where high doses of fenfluramine and dexfenfluramine were administered for longer than 2 weeks, degeneration of 5-HT nerve terminals throughout the forebrain of animals were reported. The degeneration of 5HT nerve terminals was characterized by depletion of tissue 5-HT, decreased 5-HT biosynthesis, and loss of 5-HT transporters. These effects are believed to be caused by accumulation of drug molecules into 5-HT nerve terminals and subsequent cytotoxic effects of these drugs or their metabolites. Although there is no data to suggest that lorcaserin may share the same features with fenfluramine, the potential for neurotoxicity exist since rat data found significant accumulation of lorcaserin (13 to 30 fold) in rat brain relative to plasma. It should be noted that no such findings were reported in rats and it is not known if such damage occurs in humans or if there are clinical consequences. Other potential CNS effects of chronic use of 5HT drugs is a condition called serotonin syndrome, a potentially dangerous and fatal condition characterized by a hyperserotonergic state. Serotonergic syndrome which may occur when a combination of two or more serotonergic drugs are taken is relatively rare but could occur if lorcaserin is used in combination with another 5HT drug such as SSRIs. The sudden death in some of the high dose animal toxicology studies reported with lorcaserin could have potentially been caused by a hyperserotonergic state.

Abuse liability: As a CNS drug, serotonin receptor agonists including lorcaserin have the potential to activate 5HT_{2A} if drug concentrations are sufficiently high. Activation of 5HT_{2A} is known to result in psychological disturbances such as hallucinations. The potential CNS effect of lorcaserin was examined in a series of studies in animals. In these studies, low doses of lorcaserin did not appear to result in adverse CNS effects, however since there was no reliable positive control in these studies and doses of lorcaserin were limited to 5 mg/kg, the absence of CNS behavioral effects is not reassuring. The crude assessment of behavioral effects of lorcaserin in the toxicology studies found no notable adverse effect.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Lorcaserin (APD356), prominently an R-enantiomer (>98% purity) is a highly bioavailable compound in animals (F: 93%, 51% and 37% in rats, monkeys and dogs respectively). Food had no effect on lorcaserin bioavailability in rats or humans. Studies have found minimal chiral inversion. Exposure to lorcaserin increased in a dose-proportional manner in oral pharmacokinetic studies. As a 5HT_{2C} receptor agonist, the appetite suppressive effect of lorcaserin is exerted at the brain level and thus drug entry and accumulation in the brain are fundamental for its pharmacological effect. Studies in rats have shown lorcaserin concentrations in the brain to reach 13 to 30 times the plasma concentration. Whether brain exposure in humans is as high as that seen in rats is unknown. Since a nominal dose of 10 mg BID (AUC of 1.02 µg.h/ml) was able to reduce BW in humans, one may conclude that there is significant brain exposure to lorcaserin in humans. Lorcaserin is significantly metabolized to prominent metabolites (7-hydroxy and N-hydroxy metabolites). Since the metabolites identified in human liver microsomes were also found in animals (rats, rabbits and cynomolgus monkey), the toxicology profile of the metabolites has been assessed and no additional toxicology studies of metabolites are needed. Lorcaserin reversibly increased hepatic microsomal enzymes (CYP1A, CYP2B, CYP1A1 and CYP2B1/2) in rats. It had no effect on CYP3A4 or CYP2C9. Lorcaserin minimally inhibited human CYP2D6 (2.9 µM), CYP2C19 (53 µM), CYP2C9 (61 µM), CYP1A2 (200 µM) and CYP3A4 (200 µM). Lorcaserin moderately bound plasma proteins in humans (~74%), monkeys (~73%), rabbits (~62%), rats (~70%) and mice (~67%). Lorcaserin is rapidly metabolized to 2 major metabolites, namely M1(lorcaserin sulfamate, APD244208) and M5 (N-carbamoyl glucuronide). The remaining metabolites constituted a fraction of total in plasma. Several metabolites (M2, M3, M4 and M6) that were identified *in-vitro* were also found in some instances in urine and feces of the tested species. Neither M1 nor M5 showed any pharmacological activity, however, minor M2 showed some agonistic potency to human 5HT_{2C} and 5HT_{2A} with 17 fold selectively to 5HT_{2B}.

Absorption

Bioavailability of lorcaserin in male SD rats (2 to 30 mg/kg), male cynomolgus monkeys (1.5 to 100 mg/kg) and dogs (6 mg/kg) were 93%, 51% and 37%, respectively. Food had no effect on lorcaserin bioavailability in rats; however, a delay in absorption was noted in fed animals. Similarly, clinical PK study found no food effect on PK parameters as predicted by rat study. In the cyclosporine-sensitive drug transporter model, Lorcaserin did not show signs of active efflux. Hepatic extraction of lorcaserin was relatively low as demonstrated after jugular and portal vein administration of 5 mg/kg to male rats (~32%). Analysis of brain levels of lorcaserin in rats found 13 to 35 fold higher brain levels than plasma suggesting an active transport system may be involved. At significantly higher brain exposure levels, one would expect significant PD effect in rats, yet only the highest doses in toxicology studies minimally affected appetite and BW.

Distribution

Brain and plasma concentration profiles for lorcaserin after oral administration to male SD rats (single 10 mg/kg) were comparable with respect to $t^{1/2}$ (plasma 4.9 hr, brain 4.7 hr). The t_{max} for brain was 1 hr versus 0.25 hr to t_{max} for plasma concentrations in this study. At 24 hr post-dose, the ratio of lorcaserin concentrations in brain over plasma were 11.5 to 30 fold (μg lorcaserin per g brain/ μg lorcaserin per ml plasma) suggesting a role for active transport of lorcaserin across the BBB and minimal active efflux from the brain. Whether high brain to plasma exposure also occurs in humans is unknown at this point.

Tissue distribution

Plasma ^{14}C levels approached background by 120 hr after single oral dose of lorcaserin. In most tissues the t_{max} for ^{14}C -APD356 derived radioactivity was within the 1 to 2 hr post-dose. As expected, ^{14}C -APD356-derived radioactivity was highest in tissues associated with drug pathway (gastrointestinal, stomach, small intestine and bladder). The lungs exhibited relatively high concentrations of ^{14}C -APD356-derived radioactivity (t_{max} between 1 to 2 hrs). Earlier study had found brain exposure to be approximately 11 to 30 times greater than plasma. The ratio of ^{14}C -lorcaserin in plasma to whole blood was between 1.5 and 1.6 through 24 hr post-dose, suggesting differential distribution to the plasma (75% to 80% of the total). In the in vitro human whole blood distribution study (200 ng/ml of lorcaserin), approximately 95% of lorcaserin was found in the plasma similar to the radioactivity study results in rats.

Brain tissue Distribution

In series of drug distribution studies, the brain and cerebrospinal fluid (CSF) levels of lorcaserin and its prominent metabolites (M1 and M5) were evaluated in rats, mice and monkeys

In one of the earliest brain distribution studies (PDR-03-0097) in 2004, a single 10 mg/kg dose of lorcaserin was given to male SD rats (PO) with plasma and brain analysis determined over a 40 hr time period (part 1). In part 2, single lorcaserin doses of 2, 10, 50 and 100 mg/kg were given to male SD rats (PO) with brain and plasma lorcaserin analysis carried out at 1 and 24 hr post dose. This initial analysis did not include M1 metabolite exposure. Based on the single dose test, the AUC for lorcaserin in the brain was about 13x greater than plasma AUC. When plasma concentrations were compared at different dose levels, the brain to plasma lorcaserin ratio varied from 16 to 34 fold suggesting that lorcaserin brain levels may vary with dose.

Pharmacokinetic Parameters for APD356 after a Single Oral Dose in Male Rats (10 mg/kg).¹

Parameter	Plasma	Brain	Brain/Plasma Ratio
λ_z (hr ⁻¹)	0.142	0.148	
$t_{1/2}$ (hr)	4.9	4.7	
AUC _{last} (hr•µg/mL or g)	3.202	44.010	
AUC _{INF} (hr•µg/mL or g)	3.322	44.132	13.3
%AUC _{extrap}	3.6	0.3	
t_{max} (hr)	0.25	1	
C _{max} (µg/mL or µg/g)	0.760	6.110	
AUMC _{last} (hr•hr•µg/mL or g)	17.401	267.221	
MRT _{last} (hr)	5.4	6.1	

¹Values were determined from the averaged plasma concentration versus time profiles from Table 3.

Average Plasma and Brain Concentrations, and Brain/Plasma Ratios for APD356 One Hour After a Single Oral Dose in Male Rats at 2, 10, 50 or 100 mg/kg.¹

Time = 1 hr

Dose (mg/kg)	Plasma (ng/mL)		Brain (ng/g)		Brain/Plasma Ratio	
	Mean	SD	Mean	SD	Mean	SD
2	75	14	2480	261	34.0	9.3
10	299	75	9740	2475	32.5	0.8
50	1650	52	26070	2495	15.8	2.0
100	1400	451	29740	8034	21.5	1.0

Average Plasma and Brain Concentrations, and Brain/Plasma Ratios for APD356 Twenty-Four Hours After a Single Oral Dose in Male Rats at 2, 10, 50 or 100 mg/kg.¹

Time = 24 hr

Dose (mg/kg)	Plasma (µg/mL)		Brain (µg/g)		Brain/Plasma Ratio	
	Mean	SD	Mean	SD	Mean	SD
2	BLQ ²	-	26	24	NA ³	-
10	28	12	765	308	27.4	2.9
50	301	71	9830	588	33.9	7.9
100	471	228	12970	4681	28.6	3.5

¹Values are from Table 4.

²BLQ: Below limit of quantitation of 1 ng/mL.

³NA: not available (no plasma concentrations at 24 hr post-dose).

In a rat study (PDR-08-218), 10 mg/kg of lorcaserin HCl hemihydrate was administered by gavage to male Sprague Dawley CD rats for 14 days. The plasma, CSF and brain tissue levels of parent and APD244208 metabolite (M1) were measured on Day 1 and Day14 similar to mouse study. As expected lorcaserin was rapidly absorbed from the GI into the systemic circulation to CSF and finally to the brain. Lorcaserin AUC and C_{max} in the brain were 24x and 21x greater than plasma levels in male rats. Repeated administration of lorcaserin for 14 days increased plasma AUC by 75% but brain levels were increased by 20%. Contrary to parent drug, the prominent metabolite of lorcaserin was not reaching the brain in significant amounts. Although brain levels were nearly 24x

greater than plasma, the CSF levels were fraction of plasma levels suggesting that lorcaserin in the CNS removed and stored/trapped in the brain tissue in rats similar to mice. As noted in mice, the M1 exposure in the brain was a fraction (~110 fold lower) of plasma levels suggesting that any CNS effect is unlikely to be due to M1 metabolite in rats. Overall, the rat study found up to 24x fold higher brain lorcaserin levels than plasma. Exposure to metabolite in the brain and to parent in the CSF was negligible.

APD356 Pharmacokinetic Parameters after Oral Administration of APD356 to Male Sprague-Dawley Rats at 10 mg/kg/day for 14 Days

Parameter	APD356					
	Day 1			Day 14		
	Plasma	CSF	Brain	Plasma	CSF	Brain
$t_{1/2}$ (hr)	3.83	7.44	3.43	2.89	3.41	2.58
t_{max} (hr)	0.500	0.500	0.500	0.500	4.00	1.00
C_{max} ($\mu\text{g/mL}$ or g) ^a	0.309 (0.079)	0.0634 (0.0148)	7.09 (1.56)	0.373 (0.199)	0.0639 (0.0496)	7.78 (1.23)
AUC_{last} (hr· $\mu\text{g/mL}$ or g)	1.43	0.434	50.1	2.51	0.564	60.3
Accumulation Index (AUC_{last}) Day 14/Day 1	-	-	-	1.76	1.30	1.20
Tissue/Plasma Ratio (AUC_{last})	-	0.303	35.0	-	0.225	24.0

^a C_{max} values are Mean (SD), n = 5-6 / time point

AR244208 Pharmacokinetic Parameters after Oral Administration of APD356 to Male Sprague-Dawley Rats at 10 mg/kg/day for 14 Days

Parameter	AR244208 after APD356					
	Day 1			Day 14		
	Plasma	CSF	Brain	Plasma	CSF	Brain
$t_{1/2}$ (hr)	4.85	12.2	8.42	3.66	7.75	4.03
t_{max} (hr)	8.00	2.00	8.00	8.00	1.00	8.00
C_{max} ($\mu\text{g/mL}$ or g) ^a	14.8 (4.5)	0.0123 (0.0255)	0.122 (0.027)	15.9 (4.3)	0.00424 (0.00273)	0.141 (0.0559)
AUC_{last} (hr· $\mu\text{g/mL}$ or g)	214	0.0607	2.02	214	0.0466	1.88
Accumulation Index (AUC_{last}) Day 14/Day 1	-	-	-	1.00	0.768	0.931
Tissue/Plasma Ratio (AUC_{last})	-	0.000284	0.00944	-	0.000218	0.00879

^a C_{max} values are Mean (SD), n = 5-6 / time point

In a third rat study (PDR-08-014), a higher dose of lorcaserin HCl hemihydrate (30 mg/kg) was administered by gavage to male Sprague Dawley CD rats for 14 days. The plasma, CSF and brain tissue levels of parent and APD244208 metabolite (M1) were measured on Day 1 and Day14 as noted before. As expected lorcaserin was rapidly absorbed from the GI into the systemic circulation to CSF and finally trapped in the brain.

Lorcaserin AUC and C_{max} in the brain were 35x and 17.3x greater than plasma levels in male rats at the higher dose of 30 mg/kg. The increase in lorcaserin from 10 to 30 mg/kg appeared to increase brain exposure in rats (24 fold vs. 35 fold) suggesting that higher doses may lead to significantly greater brain levels. This may explain why there were notable CNS findings in rat carci study but not in mice.

Repeated administration of lorcaserin increased brain AUC (64%) slightly more than plasma (43%). Although increase in plasma AUC at 30 mg/kg was dose-proportional to 10 mg/kg, the brain AUC at 30 mg/kg was greater (37%) than dose-proportional, suggesting that lorcaserin might accumulate to a greater degree at higher doses. Although CSF levels increase in proportion to plasma levels of lorcaserin, lorcaserin levels in CSF were about 3 and 116x lower than plasma and brain levels, respectively. It appears that as lorcaserin enters the CSF, it quickly partitions to brain tissue. Unlike

the parent drug, the exposure to M1 metabolite in CSF and brain were 25x and 110x lower than plasma, respectively. It appears that 34 fold higher M1 metabolite levels in plasma than lorcaserin did not result in higher CSF and brain levels suggesting that lorcaserin is transported to the brain by some unknown mechanism.

Overall, the rat study at 30 mg/kg found that lorcaserin partitions at a 35 to 1 ratio in brain versus plasma. Higher dose of lorcaserin results in greater than dose-proportional increase in brain exposure. The M1 metabolite stayed was largely excluded from the CNS, staying in the systemic circulation.

APD356 Pharmacokinetic Parameters after Oral Administration of APD356 to Male Sprague-Dawley Rats at 30 mg/kg/day for 14 Days

Parameter	APD356								
	Day 1 ^a			Day 14 ^b			Accumulation Index Day 14/Day 1		
	Plasma	CSF	Brain	Plasma	CSF	Brain	Plasma	CSF	Brain
t _{1/2} (hr)	10.4	19.3	19.6	4.23	4.26	19.1	-	-	-
C _{max} (µg/mL or g)	0.525 (0.164)	0.104 (0.022)	8.92 (2.10)	1.59 (0.77)	0.289 (0.055)	27.6 (4.9)	2.84 (0.71)	2.91 (0.85)	3.03 (0.75)
AUC _{last} (hr·µg/mL or g)	4.95	1.59	151	7.07	2.13	247	1.43	1.35	1.63

^a Day 1 Plasma, CSF and brain concentrations corresponding to the plasma C_{max} at t_{max} of 0.5 hr (Mean (SD), n = 5-6 / time point)

^b Day 14 Plasma, CSF and brain concentrations corresponding to the plasma C_{max} at t_{max} of 1.0 hr (Mean (SD), n = 5-6 / time point)

AR244208 Pharmacokinetic Parameters after Oral Administration of APD356 to Male Sprague-Dawley Rats at 30 mg/kg/day for 14 Days

Parameter	AR244208 after APD356								
	Day 1 ^a			Day 14 ^b			Accumulation Index Day 14/Day 1		
	Plasma	CSF	Brain	Plasma	CSF	Brain	Plasma	CSF	Brain
t _{1/2} (hr)	20.0	12.7	16.4	7.89	5.81	7.73	-	-	-
C _{max} (µg/mL or g)	16.0 (3.5)	0.0127 (0.0085)	0.153 (0.037)	15.5 (3.0)	0.0068 (0.0029)	0.176 (0.038)	1.03 (0.40)	0.751 (0.545)	1.24 (0.49)
AUC _{last} (hr·µg/mL or g)	320	0.191	3.14	241	0.110	2.76	0.753	0.576	0.876

^a Day 1 Plasma, CSF and brain concentrations corresponding to the plasma C_{max} at t_{max} of 0.5 hr (Mean (SD), n = 5-6 / time point)

^b Day 14 Plasma, CSF and brain concentrations corresponding to the plasma C_{max} at t_{max} of 1.0 hr (Mean (SD), n = 5-6 / time point)

APD356 Tissue and Plasma Ratios

Parameter	APD356 Tissue/Plasma Ratio					
	Day 1 ^a		Day 14 ^b		Day 14/Day1	
	Brain	CSF	Brain	CSF	Brain	CSF
C _{max} (µg/mL or g)	17.6 (3.8)	0.208 (0.053)	20.2 (8.0)	0.213 (0.089)	1.17 (0.512)	1.13 (0.559)
AUC _{last} (hr·µg/mL or g)	30.5	0.321	35.0	0.301	1.15	0.938

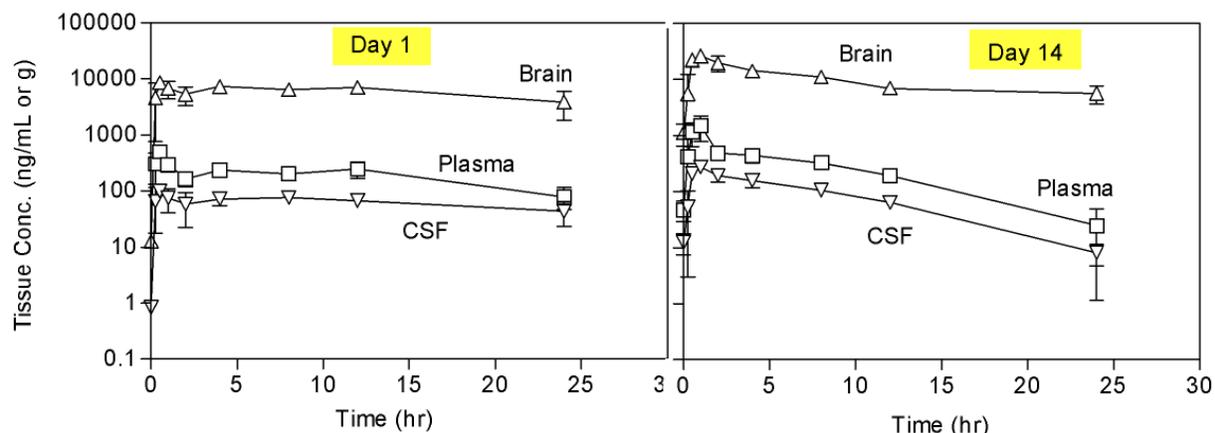
AR244208 Tissue and Plasma Ratios

Parameter	AR244208 Tissue/Plasma Ratio					
	Day 1 ^a		Day 14 ^b		Day 14/Day1	
	Brain	CSF	Brain	CSF	Brain	CSF
C _{max} (µg/mL or g)	0.0098 (0.0021)	0.0008 (0.0005)	0.0113 (0.0004)	0.0004 (0.0002)	1.21 (0.32)	0.661 (0.238)
AUC _{last} (hr·µg/mL or g)	0.0098	0.0006	0.0115	0.0005	1.16	0.765

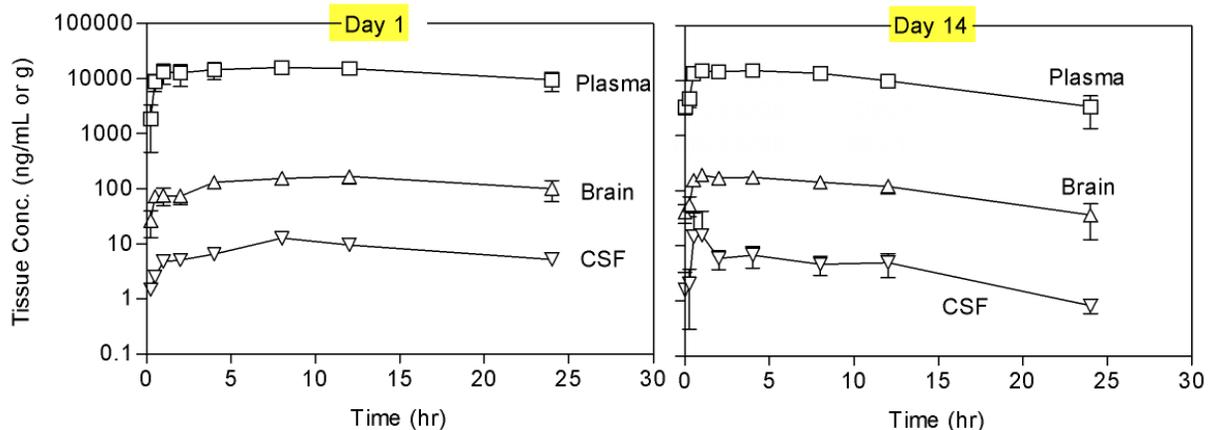
^a Day 1 Plasma, CSF and brain concentrations corresponding to the plasma C_{max} at t_{max} of 8.0 hr (Mean (SD), n = 5-6 / time point)

^b Day 14 Plasma, CSF and brain concentrations corresponding to the plasma C_{max} at t_{max} of 4.0 hr (Mean (SD), n = 5-6 / time point)

APD356 Concentration versus Time Curves after Oral Administration of APD356 at 30 mg/kg/Day to Male Sprague- Dawley Rats for 14 Days



AR244208 Concentration versus Time Curves after Oral Administration of APD356 at 30 mg/kg/Day to Male Sprague- Dawley Rats for 14 Days



In a mouse study (PDR-08-219), lorcaserin HCl hemihydrate was administered orally to ad lib fed male CD-1 mice (35.7 ± 2.9 g, ^{(b) (4)}) at 50 mg/kg for 14 days and plasma levels as well as brain and cerebrospinal fluid (CSF) levels of parent and APD244208 metabolite (M1, HSO₃-APD356) were evaluated on Day 1 and Day 14. The brain tissue AUC and C_{max} in male mice were 26x and 22x greater than plasma levels in male mice. Although there was no notable change in AUC exposure with repeated dosing of lorcaserin, the C_{max} on Day 14 was increased by 38%. Interestingly the CSF levels for both lorcaserin and APD244208 metabolite were minimal relative to plasma and brain levels providing evidence that as soon as either parent or metabolite enters the CSF, it is shifted to the brain tissue. Relative to lorcaserin, there was no or very little M1 in the brain. In fact the brain levels of M1 metabolite were 126 to 190 fold lower than plasma suggesting minimal CNS role for M1 metabolite. The unexpected accumulation of lorcaserin in the brain suggests that even lower doses of lorcaserin could lead to significant and excessive brain exposure levels in mice.

Overall, the 14-day mouse study found significant lorcaserin accumulation with no notable change in M1 metabolite suggesting that any CNS effect of lorcaserin is likely to be sole responsibility of lorcaserin in mice.

APD356 Pharmacokinetic Parameters after Oral Administration of APD356 to Male CD-1 Mice at 50 mg/kg/day for 14 Days

Parameter	APD356					
	Day 1			Day 14		
	Plasma	CSF	Brain	Plasma	CSF	Brain
$t_{1/2}$ (hr)	2.87	5.44	2.65	2.29	3.11	2.17
t_{max} (hr)	0.500	1.00	1.00	0.500	0.500	0.500
C_{max} ($\mu\text{g/mL}$ or g) ^a	1.46 (0.42)	0.394 (0.103)	32.1 (9.9)	2.04 (0.86)	0.351 (0.0883)	44.4 (15.0)
AUC_{last} (hr· $\mu\text{g/mL}$ or g)	6.06	1.29	150	5.05	1.14	133
Accumulation Index (AUC_{last}) Day 14/Day 1	-	-	-	0.833	0.884	0.887
Tissue/Plasma Ratio (AUC_{last})	-	0.213	24.8	-	0.226	26.3

^a C_{max} values are Mean (SD), n = 3-6 / time point

AR244208 Pharmacokinetic Parameters after Oral Administration of APD356 to Male CD-1 Mice at 50 mg/kg/day for 14 Days.

Parameter	AR244208 after APD356					
	Day 1			Day 14		
	Plasma	CSF	Brain	Plasma	CSF	Brain
$t_{1/2}$ (hr)	2.87	5.43	3.93	2.86	2.28	3.28
t_{max} (hr)	2.00	2.00	1.00	1.00	8.00	1.00
C_{max} ($\mu\text{g/mL}$ or g) ^a	37.0 (12.2)	0.678 (0.389)	0.274 (0.0452)	53.8 (11.9)	0.312 (0.224)	0.332 (0.0436)
AUC_{last} (hr· $\mu\text{g/mL}$ or g)	406	3.63	3.22	462	3.16	2.43
Accumulation Index (AUC_{last}) Day 14/Day 1	-	-	-	1.14	0.871	0.755
Tissue/Plasma Ratio (AUC_{last})	-	0.00894	0.00793	-	0.00684	0.00526

^a C_{max} values are Mean (SD), n = 3-6 / time point

In a preliminary monkey study (ARN-20080418), a single dose of 10 mg/kg of lorcaserin HCl hemihydrate was administered orally to 2 naïve male Cynomolgus monkeys ^{(b) (4)} and plasma levels as well as brain and cerebrospinal fluid (CSF) levels of parent and APD244208 metabolite (M1, HSO₃-APD356) were determined. The brain concentrations were about 7 to 9x the plasma concentrations at 4.5 and 24 hrs post dose. Although plasma M1 metabolite (APD244208) exposure was about 16 x greater than lorcaserin, the brain and CSF concentrations of M1 metabolite was fraction of the plasma M1 concentrations.

Plasma pharmacokinetic parameters of APD356 and HSO₃-APD356 generated following a single oral dose of 10 mg/kg (free base) in the male Cynomolgus Monkeys (Samples were collected from C2 and C3 for up to 4 and 24 hrs, respectively.)

Parameter	APD356	
	C2 (051809)	C3 (051913)
Dose (mg/kg)	10	10
C_{max} ($\mu\text{g/mL}$)	0.16	1.00
T_{max} (h)	4.0	4.0
AUC (inf) ($\mu\text{g}\cdot\text{h/mL}$)	NA*	9.97
AUC(0-t) ($\mu\text{g}\cdot\text{h/mL}$)	0.49	9.74
$T_{1/2}$ (h)	NA*	4.2

Parameter	HSO ₃ -APD356	
	C2 (051809)	C3 (051913)
Dose (mg/kg)	10	10
C_{max} ($\mu\text{g/mL}$)	0.51	17.60
T_{max} (h)	4.0	4.0
AUC (inf) ($\mu\text{g}\cdot\text{h/mL}$)	NA*	262.59
AUC(0-t) ($\mu\text{g}\cdot\text{h/mL}$)	14.68	240.19
$T_{1/2}$ (h)	NA*	6.52

The plasma-brain partition ratios of APD356 and HSO₃-APD356 at 4 and 24 hours following a single oral dose of 10 mg/kg APD356 in the male Cynomolgus Monkeys

	Time point (hr)	Brain Concentration (ng/g)	Plasma Concentration (ng/mL)	*Brain/Plasma Ratio
APD356	4.5	1852	245	7.56
	24.5	452	50.6	8.94
HSO ₃ -APD356	4.5	35.9	5260	0.007
	24.5	16.1	2300	0.007

* Brain/Plasma Ratio=Brain concentration (ng/g) / Plasma concentration (ng/mL)

The plasma-CSF partition ratios of APD356 and HSO₃-APD356 at 4 and 24 hours following a single oral dose of 10 mg/kg APD356 in the male Cynomolgus Monkeys

	Time point (hr)	CSF Concentration (ng/mL)	Plasma Concentration (ng/mL)	*CSF/Plasma Ratio
APD356	4.5	17.9	245	0.0731
	24.5	5.46	50.6	0.108
HSO ₃ -APD356	4.5	LLOQ	5260	-
	24.5	1.39	2300	0.0006

*CSF/Plasma Ratio=CSF concentration (ng/mL) / Plasma concentration (ng/mL)

The preliminary study in monkey found significant accumulation (~8x) of lorcaserin but not M1 metabolite after single 10 mg/kg dose of lorcaserin.

In a monkey study (ARN-20080419), 10 mg/kg of lorcaserin HCl hemihydrate was administered orally to naïve male Cynomolgus monkeys (b) (4) for 7 days and plasma levels as well as brain and cerebrospinal fluid (CSF) levels of parent and APD244208 metabolite (M1, HSO₃-APD356) were evaluated on Day 1 and Day 7. The brain AUC was 10x greater than the plasma AUC in male monkeys. The brain to plasma ratio on Day 7 was about 21% greater than the ratio on Day 1 suggesting slight accumulation in the brain with repeated oral administration of lorcaserin.

APD356 Pharmacokinetic Parameters in Male Cynomolgus Monkeys after Oral Administration of APD356 at 10 mg/kg/day for Seven Days

Parameter	APD356					
	Day 1			Day 7		
	Plasma	CSF	Brain	Plasma	CSF	Brain
t _{1/2} (hr)	4.27	5.23	4.87	4.48	4.99	4.69
t _{max} (hr)	4.00	8.00	8.00	2.00	2.00	2.00
C _{max} (µg/mL or g)	0.549	0.0434	3.87	1.44	0.134	11.0
AUC _{last} (hr·µg/mL or g)	5.84	0.614	48.9	7.22	0.812	73.2
Accumulation Index (AUC _{last} Day 7/Day 1)	-	-	-	1.24	1.32	1.50
Tissue/Plasma Ratio (AUC _{last})	-	0.105	8.37	-	0.112	10.1

Although the mean ratio of brain to plasma was about 10 fold in monkeys, the ratio ranged from 3 fold to 23 fold suggesting that not all animals accumulate lorcaserin in the brain tissue to the same extent. Animals capable of accumulating more drug may show significant CNS effect. With this large variance in brain exposure, the CNS safety can

not be reliably extended from one animal to another.

APD356 Individual Brain, CSF and Plasma Concentrations at Various Terminal Time Points in Male Cynomolgus Monkeys from Group A and Group B after oral dosing of 10 mg/kg APD356

Group	Monkey#	Terminal Time (hr)	Brain ^a (ng/g)	CSF ^a (ng/mL)	Plasma ^a (ng/mL)	Brain/Plasma Ratio ^b	CSF/Plasma Ratio ^c
A Day 1	M1	1	600	7.75	211	2.84	0.0367
	M2	2	1800	22.4	250	7.20	0.0896
	M3	4	619	7.39	131	4.73	0.0564
	M4	4	3200	59.8	1010	3.17	0.0592
	M5	4	3480	44.1	506	6.88	0.0872
	M6	8	3870	43.4	507	7.63	0.0856
	M7	12	2540	31.8	172	14.8	0.185
	M8	24	395	5.38	37.4	10.6	0.144
	M9	24	546	7.10	49.8	11.0	0.143
	M10	24	301	4.15	13.1	23.0	0.317
B Day 7	M11	0	172	2.89	19.3	8.91	0.150
	M12	1	1340	18.0	293	4.57	0.0614
	M13	2	11000	134	1440	7.64	0.0931
	M14	4	10000	98.8	1130	8.85	0.0874
	M15	4	7920	71.9	780	10.2	0.0922
	M16	4	7550	76.7	624	12.1	0.123
	M17	8	3100	34.5	178	17.4	0.194
	M18	12	1860	21.5	159	11.7	0.135
	M19	24	615	3.97	49.5	12.4	0.0802
	M20	24	347	5.01	32.8	10.6	0.153
	M21	24	320	8.09	39.0	8.21	0.207

^a Study animals were anesthetized with pentobarbital prior to tissue collection.

^b Brain/Plasma Ratio = Brain concentration (ng/g) / Plasma concentration (ng/mL)

^c CSF/Plasma Ratio = CSF concentration (ng/mL) / Plasma concentration (ng/mL)

Although lorcaserin accumulated in the monkey brain, accumulation multiples was not. Although the plasma M1 exposure was high relative to parent drug in all species, very little of it reached the CSF. There was very little accumulation of M1 metabolite in monkeys or any other species suggesting that a) M1 metabolite was unlikely to play a CNS role, b) there is no active transport for M1, c) M1 profile in rodents and monkeys is likely to extent to humans.

HSO₃-APD356 Pharmacokinetic Parameters in Male Cynomolgus Monkeys after Oral Administration of APD356 at 10 mg/kg/Day for Seven Days

Parameter	HSO ₃ -APD356 after APD356					
	Day 1			Day 7		
	Plasma	CSF	Brain	Plasma	CSF	Brain
t _{1/2} (hr)	4.45	3.41	4.21	5.41	12.8	5.61
t _{max} (hr)	8.00	12.00	8.00	2.00	2.00	2.00
C _{max} (µg/mL or g)	17.9	0.0116	0.173	35.1	0.0198	0.285
AUC _{last} (hr·µg/mL or g)	162	0.106	1.58	263	0.126	2.27
Accumulation Index (AUC _{last}) Day 7/Day 1	-	-	-	1.62	1.19	1.44
Tissue/Plasma Ratio (AUC _{last})	-	0.000654	0.00975	-	0.000479	0.00863

The HPLC assay used by the laboratory in (b) (4) had a LLOQ that ranged from 1 to 5 ng/ml for lorcaserin.

The LLOQ and Quantification Ranges of the Analytes in Different Matrices

Matrix	APD356 (ng/mL)		HSO ₅ -APD356 (ng/mL)	
	LLOQ	Range	LLOQ	Range
Plasma	1	1-1000	20	20-20,000
CSF	1	1-200	0.5	0.5-100
Brain homogenate	5	5-5000	1	1-200

Overall, the 7-day monkey study found a 10-fold increase in brain lorcaserin exposure relative to plasma. The brain accumulation in monkeys was not as high as those in rats (35x) and mice (25x), however wide range of variation in the brain lorcaserin accumulation among monkeys (3 to 23x) suggests that some animals may accumulate significantly more lorcaserin in the brain than other thus leading to wide range of pharmacological and toxicological response. Exposure to M1 metabolite in monkeys was nearly 90 fold less than lorcaserin. Similar to rodents, high plasma M1 exposure did not lead to high brain exposure in monkeys.

Protein binding

Lorcaserin protein binding ranged from 66 to 76% across species. Percent binding of lorcaserin to human plasma proteins was 72.9% in females and 75.1% in males. Lorcaserin binding to human plasma proteins (mixed gender) did not change with lorcaserin starting concentrations of 0.1 to 10 µM. Plasma protein binding in mice was approximately 66%.

Species	% Bound Male	% Bound Female
Human (Caucasian)	75.1 ± 4.55	72.9 ± 5.54
Monkey (Cynomolgus)	70.1 ± 3.58	76.0 ± 4.33
Rat (SD)	72.3 ± 4.53	67.1 ± 4.72
Mouse (CD-1)	65.3 ± 0.67	67.6 ± 2.76
Rabbit (NZW)	63.3 ± 0.67	60.6 ± 0.54
Dog (beagle)	66.4 ± 4.81	67.7 ± 3.54

Metabolism

Enantiomeric conversion

Potential enantiomeric conversion of lorcaserin was determined in plasma samples obtained from male SD rats (50 to 500 mg/kg, PO) and male cynomolgus monkeys (100 and 300 mg/kg, PO). Plasma samples collected from 0.5 to 24 hr post-dose were analyzed by a chiral HPLC method for lorcaserin and its (S)-enantiomer (AM-0012). No change in the amount of the optical isomer was observed in any of the plasma samples. Potential enantiomeric conversion of lorcaserin was also measured *in vitro* in microsomes prepared from SD rat, cynomolgus monkey and human liver. No change in the amount of the optical isomer was observed after *in vitro* incubation with rat, monkey, or human liver microsomes. Metabolism of lorcaserin in these incubations resulted in 20% to 50% loss of lorcaserin.

Species comparison of microsomal metabolism

¹⁴C-APD356 was incubated with human hepatic microsomes and microsomes from a variety of other species, including those used in toxicology studies (i.e., male and female SD rat, male and female cynomolgus monkey, and female New Zealand White rabbit). Incubations were conducted at 37°C, and included an NADPH generating system, starting with 30 µM APD356. Turnover of ¹⁴C-APD356 in human liver microsomes was relatively low, and 5 hr incubation was required to obtain significant amounts of metabolites. Human liver microsomes produced 7 metabolites that were identified by distinct HPLC retention times. All metabolites were produced by hepatic microsomes from other species, including male and female rat, male and female monkey and female rabbit, thus metabolite safety were adequately assessed.

Identification of Metabolites

Lorcaserin (APD356) is rapidly and extensively metabolized in nearly all the species. The prominent circulating metabolite of lorcaserin is **M1** also known as lorcaserin sulfamate (APD244208), see table blow. The second most prominent metabolite is **M5**, an N-carbamoyl glucuronide conjugate of lorcaserin. All the metabolites identified in human liver microsomes have been also identified in mouse liver microsomes. After oral administration of lorcaserin to humans only minor levels of 7-hydroxy metabolite was observed. Lorcaserin is directly conjugated to lorcaserin sulfamate (APD244208) *in vivo*.

The remaining minor metabolites that constituted fraction of total drug exposure were **M3** (*N*-hydroxy-APD356), **M4** (1-hydroxy-APD356), **M8** (4-keto-*N*-hydroxy glucuronide of APD356), **M9** (*N*-hydroxy-*O*-glucuronide of lorcaserin), **M10** (sulfate of 7-hydroxy APD356), **M11** (*O*-glucuronide of 7-hydroxy-APD356), **M12** (*O*-glucuronide of 5-hydroxy APD356), **M13** (*N*-glucuronide of APD356), and **M14** (*N*-oxide-*N*-glucuronide of APD356), **M15** (glucuronide of an oxidative metabolite of APD356) and **M16** (glucuronide of 9-hydroxy APD356).

Minor M2, M3, M4 and M6 metabolites were identified *in-vitro* but not in plasma, however, there was some exposure to M3 and M4 in urine and feces.

Metabolic profile of lorcaserin in plasma, urine and feces in different species

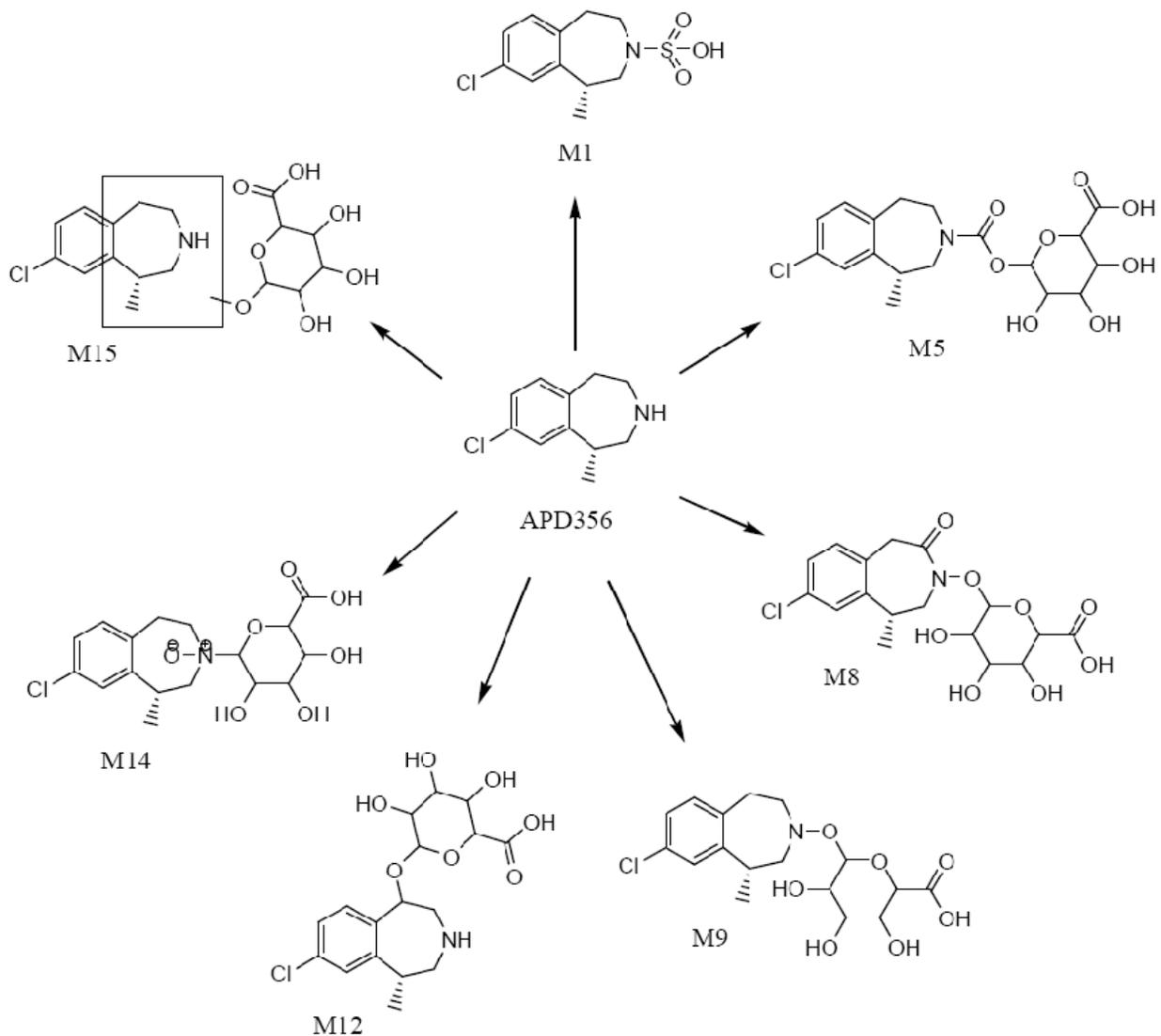
Parent & metabolites	Mice	Rats	Monkeys	Humans
	Plasma			
Lorcaserin	1.4%	2%-5%(M-F)	5%	12%
M 1	98.6%	94%-86% (M-F)	84.8%	37.8%
M5	@	@	2.8%	7.4%
M8		1.7-2.7%(M-F)		5.7%
M9			2.5%	
M10			1%	3.1%
M13			1.8%	4%
M14			1.1%	1.8%
M16			0.8%	
U (1, 2, 3)	2.2%,2.9%,1.8%	0.4% (U2)		
U (4, 8, 9)		0.8%, 2%,1.2%		
Urine				
Lorcaserin		5%	0.9%	
M1	58%	35.5%,41.3% (M-F)	20.7%	3.2%
M3		5.4%		
M4		1.9%		
M5	0.8%		19.3%	33.4%
M8	2.6%	2%	1.8%	12.2%
M9	2.5%	1.5%	12.5%	7.1%
M10			4.7%	6.8%
M11		2.5%	1.8%	3%
M12	6.2%	1.7%	2.6%	1.3%
M13		2.2%	0.6%	2%
M14	1.3%	1.4%	3.9%	9.4%
M15	0.5%	0.9%	0.7%	2.8%
M16			0.5%	2.2%
U (1, 2, 3)	0.7%(U2)	0.85%(U2)	0.3%,0.5% 0.3%	2.8%, 3%, 2.2%
U (4, 5, 6)	1.3%,0.8%,0.7%	1.6%,0.7%,(U4,5)		
U (7, 8, 9)		~1%, 3.1%,2.1%		
Feces *				
M1	12%	6.2%(M)		
M3		0.1% (F)		
M4		0.9-0.1%(M-F)		
M5		1.6-0.2%(M-F)		
M8		2.3-0.3%(M-F)		
M9	0.5%	5-0.8%(M-F)		
M11		0.1% (M)		
M12	0.5%	3.1-0.2%(M-F)		
M14		1.3%(M)		
U (2, 5,)		1.1%, 2.7%		
U (8, 9,10)	1%	0.1,0.1%,1.3%		
U 11,12,13		6.5%,2.2%1.5%		
U14		1.2%		

U = Unidentified metabolites, each as percent of dose (in rats they were combined)

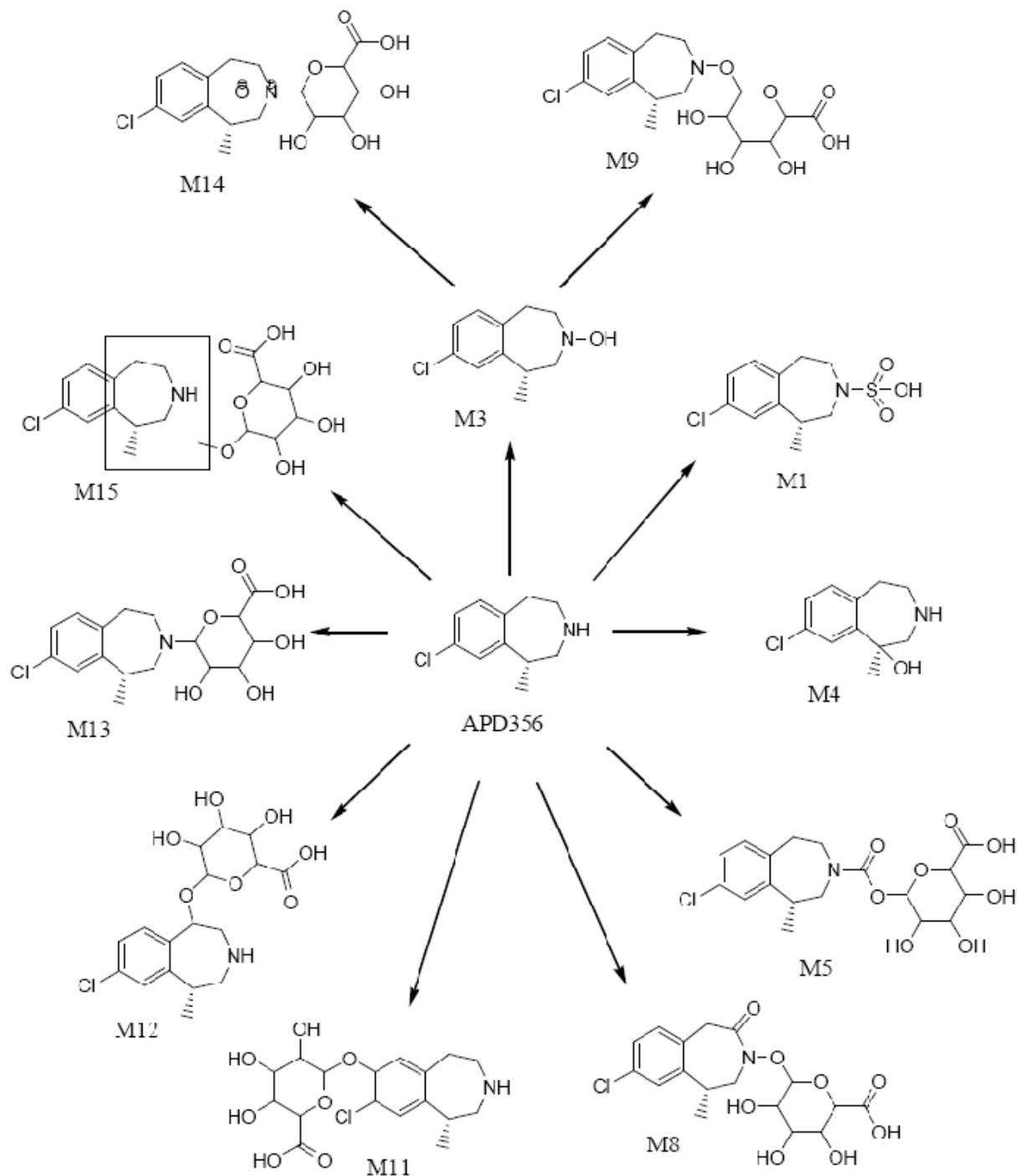
@ = Metabolite M5 was identified in the TK animals in the mouse and rat carci studies.

* = Fecal radioactivity in monkeys and humans was very low

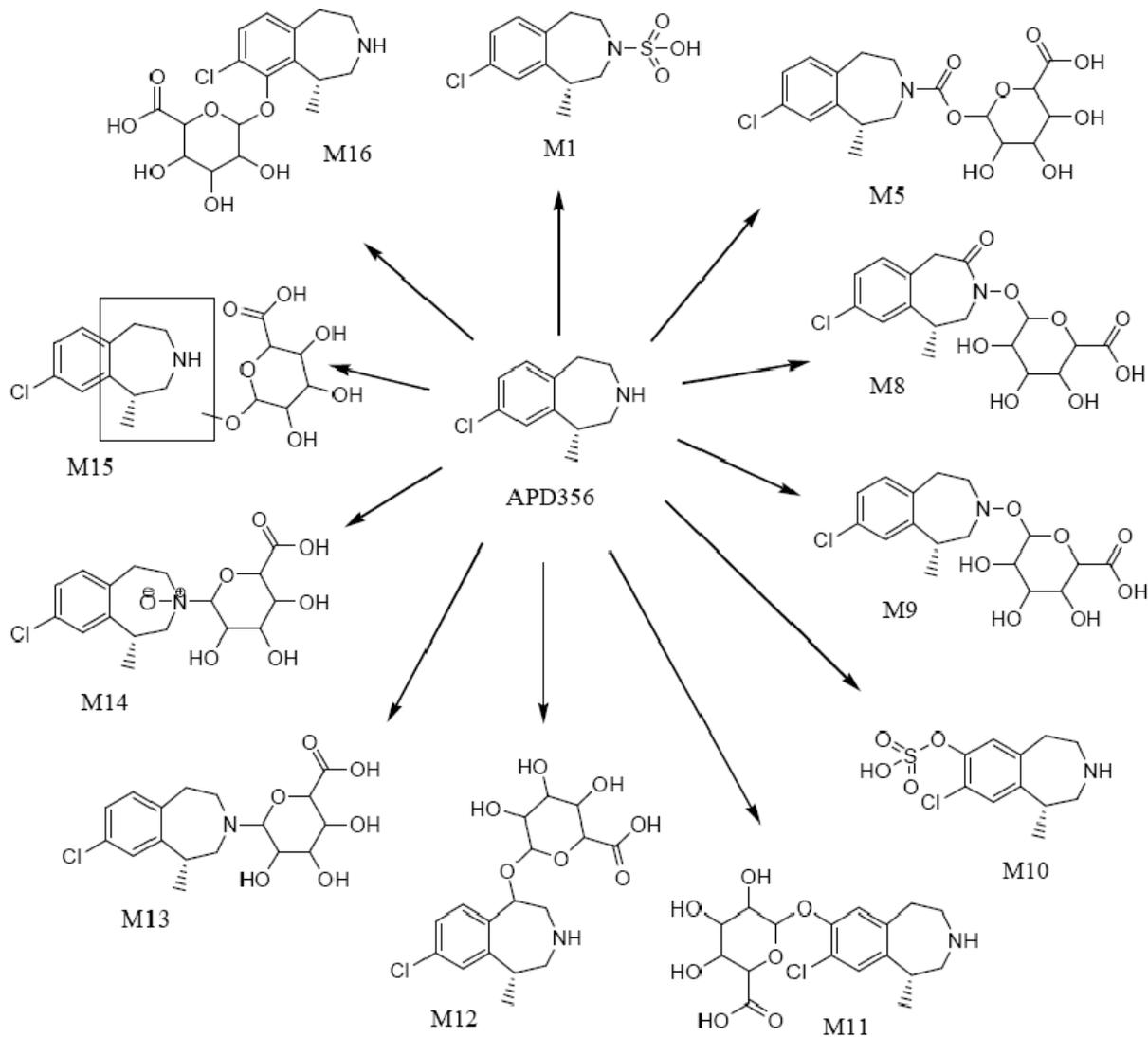
Circulating and Excreta Metabolites Identified in APD356-Dosed Mice



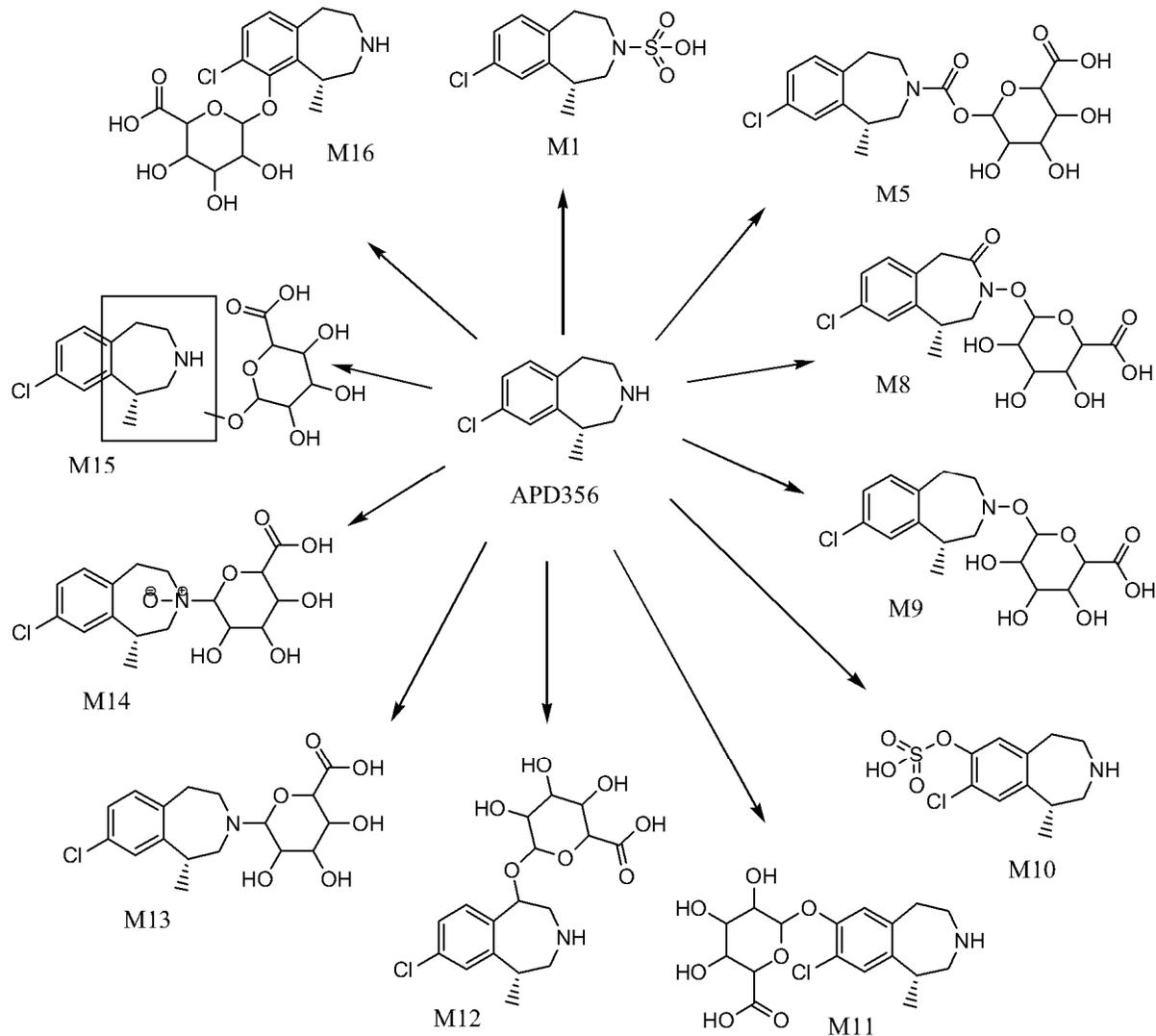
Metabolites Identified in APD356-Dosed Rats



Circulating and Urinary Metabolites Identified in APD356-Dosed Monkeys



Circulating and Urinary Metabolites Identified in APD356-Dosed Human Subjects



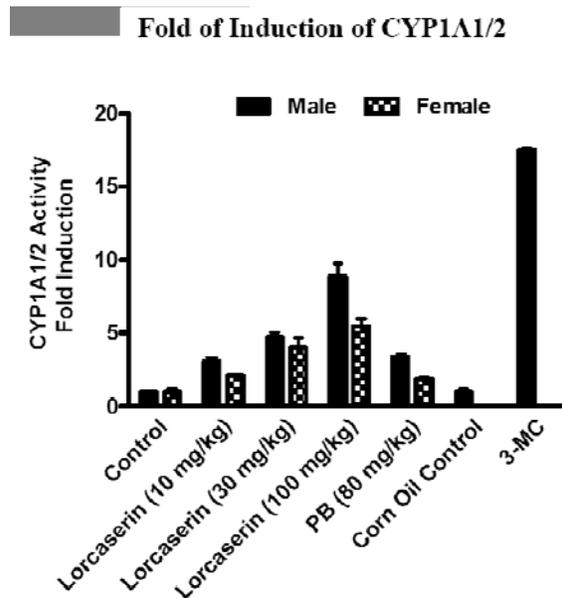
Induction of hepatic enzymes

The ability of lorcaserin (APD356) to induce liver enzymes was evaluated in a 28-day rat study at 10 and 50 mg/kg of lorcaserin. Lorcaserin induced liver microsomal enzymes in both male and female rats, marked by increase in total CYP450 content. Lorcaserin increased CYP1A, CYP2B and CYP1A1 and CYP2B1/2 in rats. The levels of these enzymes had returned to normal level at the end of the recovery phase.

Liver enzyme induction studies were also carried out using human hepatocytes incubated for 3 days with 0.2, 2 and 20 μ M of lorcaserin. In contrast to the rat study, lorcaserin concentrations up to 20 μ M had no effect on CYP1A2, CYP2C9 and CYP3A4 levels. The highest concentration of lorcaserin slightly increased only the levels of **CYP2D6** (1.85 fold) and **CYP2C19** (1.3 fold). Since the increase in CYP2B9 and CYP2C19 were less than 2 fold and occurred at 20 μ M, the potential for induction of these enzymes under normal clinical conditions is minimal.

The induction of CYP2B1/2 was evaluated in male and female SD rats due to liver and thyroid pathology findings in the toxicology studies in rats. The induction of CYP 2B1/2 and UDP-glucuronosyl transferase (UGT1A) by phenobarbital (PB) has been associated with hepatic and thyroid pathology in rats. Male and female SD rats were treated with 10, 30, and 100 mg/kg for 7 days. A separate group of rats were treated with 80 mg/kg of PB, positive control. Lorcaserin dose-dependently induced CYP2B1/2 and CYP2B1/2 mRNA expression in both male and female rats. Lorcaserin dose of 100 mg/kg appear to induce both CYP2B1/2 and UGT1A enzyme the same extent as the positive control. Lorcaserin also treatment increased hepatic mRNA expression of rat UGT1A1 and UGT1A6 suggesting that lorcaserin induction of these enzymes might be similar to the positive control.

Lorcaserin also dose-dependently induced **CYP1A1 and CYP3A1**. However, the CYP1A1 induction was much less than 3-methylcholanthrene (3-MC), a well known CYP3A1/2 inducer. In contrast to rat liver induction assay, lorcaserin at concentrations up to 20 μ M had no effect on human CYP1A1 or CYP3A1 suggesting that the liver and thyroid pathology (tumors) in rat carcinogenicity study are less likely to occur in humans at therapeutic dose of 10 mg/kg (C_{max}).



Inhibition of human liver enzymes

The ability of lorcaserin to inhibit human hepatocyte CYP 450 isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) was evaluated in human liver microsomal preparation using CYP-specific substrates.

Lorcaserin inhibited the metabolism of dextromethorphan (CYP2D6) in a concentration-dependent manner with an IC_{50} value of $3.99 \pm 0.41 \mu\text{M}$ (781 ng/mL), whereas it did not inhibit phenacetin (CYP1A2), tolbutamide (CYP2C9), (S)-mephenytoin (CYP2C19), or midazolam (CYP3A4) metabolism up to the concentration of 100 μM . Additional studies were carried out since only CYP2D6 was inhibited by lorcaserin. Lorcaserin inhibited dextromethorphan metabolism in a competitive manner with a K_i value of $2.03 \pm 0.18 \mu\text{M}$ (397 ng/mL).

In summary these studies found lorcaserin to be a capable competitive inhibitor of human CYP2D6 but not CYP1A2 (200 μM), CYP2C9 (61 μM), CYP2C19 (53 μM), or CYP3A4 (200 μM) human microsomal enzymes. The inhibition of CYP2D6 by lorcaserin suggests that lorcaserin may increase the exposure of drugs metabolized by this CYP2D6.

Excretion

Lorcaserin (APD356) is metabolized and excreted primarily by the kidneys (70 to 82%) and to some extent by feces (14 to 25%) in SD rats. When rats were dosed with ^{14}C Lorcaserin approximately 96% of the radioactivity was recovered by 120 hr in both male and female rats. Percent excretion of radioactivity was greater than 70% of dose after 24 hr, greater than 85% of dose after 48 hr, and greater than 96% of dose after 120 hr (the last time-point). Recovery of lorcaserin itself in urine was 1.7% of total oral dose in male rats and 4.0% of total oral dose in female rats (to 120 hr post-dose). Biliary excretion of lorcaserin after single oral dose of 10 or 100 mg/kg represented less than 1% of the total lorcaserin dose at both 10 and 100 mg/kg.

5.2 Toxicokinetic table

Safety margin

Species	Daily Dose, (mg/kg)	lorcaserin AUC ₀₋₂₄ (µg.h/ml)	NOAEL, (mg/kg) M/F	Safety margins based on AUC (Animal/Human)	
				male	Female
13-Week mouse Study	25	M:3.4 F:1.0		3	1
	50	M:7.6 F: 2.3	50/50	7	2
	250	M:34.8 F:9.2		34	9
	350	M:25 F:27		25	26
13-Week rat study	1	M:0.143 F:0.33	5/1	<1	<1
	5	M:0.75 F:1.71		<1	2
	50	M:16.6 F:32.5		16	32
	100	M:33.6 F:55.8		33	55
6-Month rat study	1	M:0.20 F:0.31		<1	<1
	5	M:1.19 F:2.87	5/5	1	3
	50	M:22.0 F:34.4		22	34
12-Month cynomolgus monkey study	2	M: 1.0 F: 0.6	2 / 2	1	0.6
	10	M: 7.9 F: 4.5		8	4
	50	M:43.6 F:31.4		43	31
	125	M: 50.9 F: 51		50	50
104-Week Mouse Carci Study	5	M:0.55 F:0.32		<1	<1
	25	M:3.9 F: 1.6		4	1
	50	M:7.5 F:3.7	50/50	7	4
104-Week Rat Study	10	M:4.78 F:6.7	5 / <7	5	7
	30	M:16.9 F:24.1		16.6	24
	100 ^b	M:55.9 F:83.8		55	82
Fertility and early embryonic development in rats	5	M:2.68 F: 4 ^a		3	4
	15	M:9.91 F:12 ^a	15/50	10	12
	50	M: 29.3 F:48.7 ^a		29	48
Oral Embryo-fetal development in rats	2	F:1.34			1
	10	F:7.99	10		8
	50	F:48.7			48
Oral Embryo-fetal development in rabbits	20	F: 0.155			<1
	60	F: 0.443	60		<1
	200	F:19.3			19
Pre- and postnatal development in rats	5	F:4 ^a	<5		4
	15	F:12 ^a			12
	50	F:48.7 ^a			48
Clinical Dose: lorcaserin, 10 mg BID		1.02			

^a The AUC value is derived from other existing similar studies.

^b The lorcaserin AUC in females in the 2-year rat study was about 50% higher than the AUC in female rats in the 13-week toxicology and 28-day prolactin mechanistic study (AUC 53 µg.hr/ml).

Toxicokinetic parameters collected from 3-month rat, monkey and mouse toxicology studies. The AUC for 100 mg/kg/d lorcaserin (APD356) was **50 to 66%** higher (M:55.9, F:83.8 $\mu\text{g}\cdot\text{h}/\text{ml}$) at 52 weeks in the carcinogenicity study than the AUC at the same dose level at 13 weeks in SD rats. The safety margin at 100 mg/kg in the 13-week study was 33x (M) and 55x(F) the clinical dose of 10 mg BID based on AUC vs. 55x(M) and 82x(F) in the rat carcinogenicity study at week 52.

Mean Toxicokinetic Parameters of APD356 Anhydrous in the 13 Week Oral Toxicity and Toxicokinetic Study with APD356 in Rats (Week 13)

Gender	Dose (mg/kg/day)	Total (bound and free)		Free ^a	
		C _{max} ($\mu\text{g}/\text{mL}$)	AUC _{0-24h} ($\mu\text{g}\cdot\text{h}/\text{mL}$)	C _{max} ($\mu\text{g}/\text{mL}$)	AUC _{0-24h} ($\mu\text{g}\cdot\text{h}/\text{mL}$)
Male	1	0.055	0.143	0.006	0.016
	5	0.195	0.747	0.021	0.082
	50	1.45	16.6	0.160	1.83
	100	3.09	33.6	0.340	3.70
Female	1	0.081	0.335	0.009	0.037
	5	0.363	1.71	0.040	0.188
	50	2.67	32.5	0.294	3.58
	100	4.48	55.8	0.493	6.14

^a Free fraction calculated using the following plasma protein binding percentage: Rat 89% [24]

**APD356 Toxicokinetic Summary
after Oral Administration of APD356 to Male and
Female Monkeys on Day 1, 28, and 90.**

Day	Dose (mg/kg/day)	C _{max} ($\mu\text{g}/\text{mL}$)	t _{max} (hr)	AUC _{last} ($\text{hr}\cdot\mu\text{g}/\text{mL}$)	
Male	1	2	2.0	0.910	
		10	3.0	5.251	
		75	8.7	53.588	
		125	12.3	66.894	
	28	2	0.089	3.8	1.211
		10	0.754	2.0	7.132
		75	4.162	4.8	54.966
		125	4.712	4.0	70.556
	90	2	0.097	2.8	1.148
		10	0.587	2.3	6.047
		75	4.646	3.6	61.484
		125	5.240	3.8	77.278
Female	1	2	2.0	0.409	
		10	4.5	5.145	
		75	13.0	32.287	
		125	12.7	60.943	
	28	2	0.061	3.3	0.684
		10	0.376	4.5	4.949
		75	3.303	4.0	46.610
		125	5.647	5.3	82.583
	90	2	0.097	1.9	0.495
		10	0.369	1.8	3.978
		75	3.280	3.7	42.069
		125	6.295	5.0	88.779

**AR244208 Toxicokinetic Summary
after Oral Administration of APD356 to Male
and Female Monkeys on Day 1, 28, and 90.**

Day	Dose (mg/kg/day)	C _{max} ($\mu\text{g}/\text{mL}$)	t _{max} (hr)	AUC _{last} ($\text{hr}\cdot\mu\text{g}/\text{mL}$)
Male	1	2	3.5	30.115
		10	4.5	190.079
		75	13.3	1001.307
		125	21.3	1219.650
	28	2	5.5	34.676
		10	3.3	241.469
		75	6.7	1097.068
		125	3.3	1614.467
	90	2	4.5	37.856
		10	3.0	224.826
		75	5.6	1196.400
		125	4.3	1594.492
Female	1	2	4.0	27.839
		10	5.0	181.156
		75	13.0	776.037
		125	12.7	994.025
	28	2	3.0	31.884
		10	5.5	181.848
		75	5.0	1043.438
		125	6.3	1367.967
	90	2	2.8	32.806
		10	3.0	154.791
		75	4.0	959.762
		125	4.1	945.322

APD356 Toxicokinetic Summary in Male CD-1 Mice as a Function of Oral Dose of ADP356 on Days 1, 42, and 91.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	T _{max} (hr)	AUC _{last} (hr·µg/mL)
Male 1	25	0.993	0.5	2.787
	50	1.817	1.0	7.773
	250	3.417	0.3	23.646
	350	5.440	1.0	39.765
42	25	1.380	0.5	3.253
	50	1.353	0.5	7.622
	250	4.200	0.5	34.806
	350	5.227	1.0	24.954
91	25	1.380	0.5	6.661
	50	0.806	0.3	3.910
	250 ¹	4.300	0.5	ND
	350 ²	ND ³	ND	ND

APD356 Toxicokinetic Summary in Female CD-1 Mice as a Function of Oral Dose of APD356 on Days 1, 42, and 91.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	T _{max} (hr)	AUC _{last} (hr·µg/mL)
Female 1	25	1.496	0.3	2.116
	50	1.583	0.3	3.578
	250	5.953	0.3	18.635
	350	2.873	1.0	24.337
42	25	0.634	0.5	1.034
	50	0.876	0.5	2.280
	250	2.373	0.5	9.263
	350	3.097	1.0	27.433
91	25	0.920	0.3	2.372
	50	0.610	0.3	1.110
	250 ¹	2.207	0.3	ND
	350 ²	ND ³	ND	ND

¹ Significant mortality at 250 mg/kg/day prevented a complete concentration vs. time profile.

² Significant mortality at 350 mg/kg/day prevented the collection of plasma samples for toxicokinetic analysis.

³ ND = not determined due to insufficient data from dose group.

AR244208 Toxicokinetic Summary in Male CD-1 Mice as a Function of Oral Dose of APD356 on Day 1, 42, and 91.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	T _{max} (hr)	AUC _{last} (hr·µg/mL)
Male 1	25	24.633	0.5	181.190
	50	48.567	1.0	355.410
	250	43.600	4.0	673.147
	350	50.600	2.0	876.438
42	25	29.200	0.5	283.279
	50	36.133	4.0	372.094
	250	46.667	2.0	843.510
	350	55.833	4.0	401.750
91	25	28.400	0.5	288.120
	50	21.867	0.3	213.204
	250 ¹	41.067	0.5	ND
	350 ²	ND ³	ND	ND

AR244208 Toxicokinetic Summary in Female CD-1 Mice as a Function of Oral Dose of APD356 on Day 1, 42, and 91.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	T _{max} (hr)	AUC _{last} (hr·µg/mL)
Female 1	25	46.700	0.3	233.018
	50	63.000	0.5	617.507
	250	89.033	0.5	1040.477
	350	82.933	2.0	1555.467
42	25	35.767	1.0	247.841
	50	57.333	0.5	425.471
	250	79.633	8.0	1159.035
	350	77.300	8.0	1266.653
91	25	60.500	0.3	402.943
	50	48.300	0.5	241.251
	250 ¹	77.100	2.0	ND
	350 ²	ND ³	ND	ND

¹ Significant mortality at 250 mg/kg/day prevented a complete concentration vs. time profile.

² Significant mortality at 350 mg/kg/day prevented the collection of plasma samples for toxicokinetic analysis.

³ ND = not determined due to insufficient data for dose group.

Induction of liver CYP2B1/2 and UDP-glucuronosyltransferase (UGT) in SD rates treated with lorcaserin doses of 10, 30 and 100 mg/kg of lorcaserin for 7 days. Phenobarbital (PB) was used as positive control.

Average Value^a of 7-Pentoxoresorufin O-Dealkylation by CYP2B1/2 in Male and Female Rat Liver Microsomes Treated with Lorcaserin and Phenobarbital (PB)

Treatments	Dose mg/kg/day	Male Rats		Female Rats	
		7-Pentoxoresorufin O-Dealkylation		7-Pentoxoresorufin O-Dealkylation	
		(pmol/mg protein/min)	Fold of Induction	(pmol/mg protein/min)	Fold of Induction
Control	0	26.5 ± 0.13	1.00 ± 0.01	23.1 ± 0.19	1.00 ± 0.01
Lorcaserin	10	33.5 ± 1.63	1.26 ± 0.06	30.4 ± 0.75	1.32 ± 0.03
Lorcaserin	30	55.4 ± 4.79	2.09 ± 0.18	57.8 ± 22.5	2.50 ± 0.97
Lorcaserin	100	241 ± 58.7 ^b	9.07 ± 0.75 ^b	121 ± 45.1 ^b	5.23 ± 1.95 ^b
PB	80	296 ± 19.8 ^b	11.2 ± 0.75 ^b	169 ± 18.7 ^b	7.33 ± 0.81 ^b

^a Mean ± standard deviation of three rats/dose/gender unless specified

^b Statistically significant compared to control according to Dunnett's test ($p < 0.01$).

Average Value^a of p-Nitrophenol-glucuronide Formation by UGT1A in Male and Female Rat Liver Microsomes Treated with Lorcaserin, Phenobarbital (PB), and 3-Methylcholanthrene (3-MC)

Treatments	Dose mg/kg/day	Male Rats		Female Rats	
		p-Nitrophenol-glucuronide		p-Nitrophenol-glucuronide	
		(nmol/mg protein/min)	Fold of Induction	(nmol/mg protein/min)	Fold of Induction
Control	0	12.0 ± 4.51	1.00 ± 0.38	7.41 ± 4.11	1.00 ± 0.55
Lorcaserin	10	16.4 ± 4.14	1.37 ± 0.35	11.0 ± 1.05	1.49 ± 0.14
Lorcaserin	30	21.0 ± 0.92 ^b	1.75 ± 0.08 ^b	15.3 ± 6.77	2.06 ± 0.91
Lorcaserin	100	34.4 ± 3.37 ^c	2.87 ± 0.28 ^c	23.3 ± 6.43 ^b	3.14 ± 0.87 ^b
PB	80	28.5 ± 1.99 ^e	2.39 ± 0.17 ^e	31.1 ± 5.0 ^e	4.20 ± 0.67 ^e
Corn Oil Control ^f	0	16.7 ± 2.80	1.00 ± 0.17	ND ^e	ND
3-MC ^c	25	50.8 ± 3.04 ^d	3.05 ± 0.18 ^d	ND	ND

^a Mean ± standard deviation of three rats/dose/gender unless specified.

^b Statistically significant compared to control according to Dunnett's test ($p < 0.05$).

^c Statistically significant compared to control according to Dunnett's test ($p < 0.01$).

^d Statistically significant compared to control according to Student's t-test ($p < 0.01$).

^e Not determined.

^f Mean ± standard deviation of two rats/dose, with assays done in triplicate.

Average Value^a of 4-Methylumbelliferone-glucuronide Formation by UGT1A in Male and Female Rat Liver Microsomes Treated with Lorcaserin, Phenobarbital (PB), and 3-Methylcholanthrene (3-MC)

Treatments	Dose mg/kg/day	Male Rats		Female Rats	
		4-Methylumbelliferone Glucuronide		4-Methylumbelliferone Glucuronide	
		nmol/mg protein/min	Fold of Induction	nmol/mg protein/min	Fold of Induction
Control	0	8.77 ± 2.88	1.00 ± 0.33	12.9 ± 3.35	1.00 ± 0.26
Lorcaserin	10	11.8 ± 0.47	1.35 ± 0.05	14.8 ± 0.99	1.15 ± 0.08
Lorcaserin	30	13.4 ± 0.52 ^b	1.52 ± 0.06 ^b	15.9 ± 3.22	1.24 ± 0.25
Lorcaserin	100	18.3 ± 0.66 ^b	2.09 ± 0.07 ^b	16.2 ± 1.95	1.26 ± 0.15
PB	80	17.1 ± 0.38 ^b	1.95 ± 0.04 ^b	20.8 ± 0.92 ^b	1.62 ± 0.07 ^b
Corn Oil Control ^f	0	14.2 ± 1.56	1.00 ± 0.11	ND ^e	ND
3-MC ^c	25	29.5 ± 0.38 ^d	2.08 ± 0.03 ^d	ND	ND

- ^a Mean ± standard deviation of three rats/dose/gender unless specified.
^b Statistically significant compared to control according to Dunnett's test ($p < 0.01$).
^c Not determined.
^d Statistically significant compared to control according to Student's t-test ($p < 0.01$).
^e Mean ± standard deviation of two rats/dose, with assays done in triplicate.

Average Value^a of Thyroxine (T4)-glucuronide Formation by UGT1 (UGT1A6) in Male and Female Rats Liver Microsomes Treated with Lorcaserin, Phenobarbital (PB), and 3-Methylcholanthrene (3-MC)

Treatments	Dose mg/kg/day	Male Rats		Female Rats	
		T4-Glucuronide		T4-Glucuronide	
		Peak Area Ratio	Fold of Induction	Peak Area Ratio	Fold of Induction
Control	0	1.76 ± 0.48	1.00 ± 0.27	1.68 ± 0.38	1.00 ± 0.23
Lorcaserin	10	1.86 ± 0.38	1.05 ± 0.22	2.51 ± 0.59	1.50 ± 0.35
Lorcaserin	30	2.49 ± 0.48	1.41 ± 0.27	1.56 ± 0.57	0.93 ± 0.34
Lorcaserin	100	3.59 ± 0.19 ^b	2.03 ± 0.11 ^b	2.01 ± 0.33	1.20 ± 0.20
PB	80	3.98 ± 0.61 ^b	2.26 ± 0.34 ^b	2.03 ± 0.55	1.21 ± 0.33
Corn Oil Control ^f	0	2.53 ± 0.54	1.00 ± 0.21	ND ^d	ND
3-MC ^c	25	8.11 ± 0.18 ^e	3.21 ± 0.07 ^c	ND	ND

- ^a Mean ± standard deviation of three rats/dose/gender unless specified.
^b Statistically significant compared to control according to Dunnett's test ($p < 0.01$).
^c Statistically significant compared to control according to Student's t-test ($p < 0.01$).
^d Not determined.
^e Mean ± standard deviation of two rats/dose, with assays done in triplicate.

Average Value^a of Triiodothyronine (T3)-glucuronide Formation by UGT in Male and Female Rat Liver Microsomes Treated with Lorcaserin, Phenobarbital (PB), and 3-Methylcholanthrene (3-MC)

Treatments	Dose mg/kg/day	Male Rats		Female Rats	
		T3-Glucuronide		T3-Glucuronide	
		Peak Area Ratio	Fold of Induction	Peak Area Ratio	Fold of Induction
Water Control	0	8.52 ± 1.16	1.00 ± 0.14	10.9 ± 6.36	1.00 ± 0.58
Lorcaserin	10	5.92 ± 3.16	0.70 ± 0.37	13.8 ± 4.08	1.27 ± 0.37
Lorcaserin	30	6.72 ± 1.43	0.79 ± 0.17	5.18 ± 1.45	0.47 ± 0.13
Lorcaserin	100	4.40 ± 1.69	0.52 ± 0.20	9.29 ± 0.63	0.85 ± 0.06
PB	80	12.6 ± 5.36	1.48 ± 0.63	9.91 ± 7.45	0.91 ± 0.68
Corn Oil Control ^f	0	9.06 ± 1.01	1.00 ± 0.11	ND ^b	ND
3-MC ^c	25	10.9 ± 0.54	1.20 ± 0.06	ND	ND

- ^a Mean ± standard deviation of three rats/dose/gender unless specified.
^b Not determined.
^c Mean ± standard deviation of two rats/dose, with assays done in triplicate.

Induction of liver CYP1A1/2 and CYP3A1 in SD rats treated with lorcaserin doses of 10, 30 and 100 mg/kg of lorcaserin for 7 days. 3-Methylcholanthrene (3-MC) was used as positive control.

Average Value^a of 7-Ethoxyresorufin O-Dealkylation by CYP1A1/2 in Male and Female Rat Liver Microsomes Treated with Lorcaserin, Phenobarbital (PB), and 3-Methylcholanthrene (3-MC)

Treatments	Dose	Male Rats		Female Rats	
		7-Ethoxyresorufin O-Dealkylation		7-Ethoxyresorufin O-Dealkylation	
	mg/kg/day	(pmol/mg protein/min)	Fold of Induction	(pmol/mg protein/min)	Fold of Induction
Control	0	44.1 ± 6.23	1.00 ± 0.14	73.7 ± 21.1	1.00 ± 0.29
Lorcaserin	10	136 ± 18.9 ^b	3.07 ± 0.43 ^b	156 ± 12.2	2.11 ± 0.17
Lorcaserin	30	209 ± 21.4 ^c	4.73 ± 0.49 ^c	295 ± 85.0 ^c	4.01 ± 1.15 ^c
Lorcaserin	100	391 ± 69.1 ^c	8.85 ± 1.57 ^c	405 ± 59.7 ^c	5.49 ± 0.81 ^c
PB	80	149 ± 15.7 ^b	3.38 ± 0.35 ^b	138 ± 11.2	1.87 ± 0.15
Corn Oil Control ^f	0	33.9 ± 6.98	1.00 ± 0.21	ND ^e	ND
3-MC ^e	25	594 ± 8.28 ^d	17.5 ± 0.24 ^d	ND	ND

^a Mean ± standard deviation of three rats/dose/gender unless specified.

^b Statistically significant compared to control according to Dunnett's test ($p < 0.05$).

^c Statistically significant compared to control according to Dunnett's test ($p < 0.01$).

^d Statistically significant compared to control according to Student's t-test ($p < 0.01$).

^e Not determined.

^f Mean ± standard deviation of two rats/dose, with assays done in triplicate.

Average Value^a of Testosterone 6 β -Hydroxylation by CYP3A1/2 in Male and Female Rat Liver Microsomes Treated with Lorcaserin, Phenobarbital (PB), and 3-Methylcholanthrene (3-MC)

Treatments	Dose	Male Rats		Female Rats	
		Testosterone 6 β -Hydroxylation		Testosterone 6 β -Hydroxylation	
	mg/kg/day	(pmol/mg protein/min)	Fold of Induction	(pmol/mg protein/min)	Fold of Induction
Control	0	381 ± 44.4	1.00 ± 0.12	117 ± 25.1	1.00 ± 0.21
Lorcaserin	10	604 ± 48.5	1.59 ± 0.13	128 ± 20.3	1.09 ± 0.17
Lorcaserin	30	527 ± 24.2	1.38 ± 0.06	179 ± 59.7	1.53 ± 0.51
Lorcaserin	100	824 ± 282 ^b	2.16 ± 0.74 ^b	212 ± 36.7 ^b	1.81 ± 0.31 ^b
PB	80	1103 ± 206 ^c	2.89 ± 0.54 ^c	386 ± 42.1 ^c	3.30 ± 0.36 ^b
Corn Oil Control ^e	0	398 ± 168	1.00 ± 0.42	ND ^d	ND
3-MC ^e	25	542 ± 11.4	1.36 ± 0.03	ND	ND

^a Mean ± standard deviation of three rats/dose/gender unless specified.

^b Statistically significant compared to control according to Dunnett's test ($p < 0.05$).

^c Statistically significant compared to control according to Dunnett's test ($p < 0.01$).

^d Not determined.

^e Mean ± standard deviation of two rats/dose, with assays done in triplicate.

6 General Toxicology

6.1 Single-Dose Toxicity

The single dose studies (reviewed under IND 69,888), 500 mg/kg of lorcaserin was considered MTD in rats due to death at 1000 mg/kg. Salivation, penile erection, reduced activity, tremor in addition to decrease in BW and food intake were hallmark of single dose lorcaserin in rats. Monkeys tolerated single doses up to 300 mg/kg of APD356. Similar to rats, penile erection was noted at doses ≥ 10 mg/kg. Penile erection, a pharmacological side effect of 5HT was seen in the 10 day monkey study at all dose levels (10, 100, 150 mg/kg). In addition to the decrease in activity (≥ 100 mg/kg), an increase in AST (1.8X) at doses greater than 10 mg/kg and ALT (1.4X) at 300 mg/kg were noted. The NOAEL and MTD were 10 and 100 mg/kg in the 10 day monkey study, respectively.

6.2 Repeat-Dose Toxicity

Multiple dose studies up to 3-months have been reviewed under IND 69,888. In acute 10 day studies in rats, administration of lorcaserin doses up to 500 mg/kg resulted in salivation, increased sensitivity to touch (≥ 50 mg/kg), clonic convulsion (500 mg/kg), decrease in BW and food intake. The increase in liver weight (≥ 50 mg/kg) was associated with hepatocellular vacuolation, likely due to metabolic response. Although kidney weights were increased at all doses (20 to 23%) in rats, there was no evidence of macroscopic damage. There was no evidence of any heart or brain lesions in rats at doses up to 500 mg/kg. When the same study was repeated at lower doses with a 10 day recovery, an increase in liver weight with trace hepatocellular vacuolation was noted again (≥ 30 mg/kg). During recovery, trace to mild increase in pigmented macrophage (≥ 30 mg/kg) was still present at 100 mg/kg.

Study title: 6-Month Oral Toxicity Study in Rats with a 4-Week Recovery Period

Study no.: TX03015 (b) (4) 900-046)
 Study report location: 032, Vol # 5 and page # 1-1493
 Conducting laboratory and location: (b) (4)
 Date of study initiation: April, 4, 2005
 GLP compliance: Yes
 QA statement: yes (x) no (),
 Drug, lot #, and % purity: AR 222154-A-04-001, 100%

Key Study Findings

- There were deaths in both control (2 M) and HD group (5 M, 1F). The cause of death was known only in 2 males. The remaining was considered drug-related by the reviewer. There were also deaths in HD TK study (3 M and 1 F)
- Clinical signs included salivation during WK1 and red/yellow/brown material around nose

- Significant decrease in BW gain during WK1 at 50 mg/kg (M -29% and F -49%) was only slightly lower than control (M -15% and F -7%) at the end of the study
- Slight decrease in food intake during WK1 at 50 mg/kg
- There were no ophthalmic findings
- Significant decrease in erythroid parameters were noted at 50 mg/kg with associated increase in reticulocytes
- Small changes in clinical chemistry parameters were not considered clinically meaningful
- No notable change in urinalysis except for an increase in urine volume at ≥ 5 mg/kg
- Gross findings included enlarged liver in 2 HD males and moderate renal pelvic dilatation in 1 male at MD dose (5 mg/kg)
- There was significant increase in relative kidney, liver heart and spleen weight at HD
- Minimal centrilobular hepatocellular hypertrophy in 2 MD males and minimal to moderate hepatocellular hypertrophy in 18/20 males and 16/20 females at 50 mg/kg
- Dose-related increase in centrilobular hepatocellular vacuolation at all doses in males but not females even though drug exposure was higher in female rats. No evidence of hepatic vacuolation during recovery
- Minimal to mild incidences of focal liver necrosis in 1 male and 1 female at 1 and 5 mg/kg and 1 female and 2 males at 50 mg/kg. Only 1 HD female at recovery had minimal focal liver necrosis.
- Minimal to mild increase in extramedullary hematopoiesis at 50 mg/kg
- There were no drug related valvular or cardiac or renal histopath findings in rats
- Minimal hyperplasia of mammary ducts in 2 HD female rats. There were no such findings in the recovery animals.
- The AUC exposure on Day 183 was greater than Day 1 by 1.1 to 2.6 fold in both male and female rats although exposure in females tend to be 50% greater than males. The greater exposure to parent drug in females was attributed to lower Cyp3A4 in female rats
- The most prominent metabolite, M1 (lorcaserin sulfamate, APD244208) and APD306388 (M2) were identified in both male and female rats.
- The exposure to M1 was 20 to 160 fold greater than the parent drug while the exposure to M2 was 20% less than parent drug
- The NOAEL was 5 mg/kg was approximately 1.6 to 3 fold greater than clinical exposure at 10 mg BID (AUC 1.02 $\mu\text{g}\cdot\text{h}/\text{ml}$)

Methods

Rats were treated daily with lorcaserin hemihydrate (correction factor, 1.037) for 6-months of with a 4-week recovery phase (control and HD). The lorcaserin was prepared in deionized water and administered by oral gavage. Rats in the toxicokinetic satellite groups (12/sex/group) were similarly treated for PK analysis of parent and metabolites (M1 and M5). Animals had ad lib access to food and water. The stability studies showed lorcaserin solutions were stable for 3 months at room temperature or stressed temp (40C). Solution concentrations were within 91.1 to 104.5% of the target solution concentrations.

Doses: 0, 1, 5 and 50 mg/kg
Frequency of dosing: daily

Route of administration: Oral gavage
Dose volume: 5 ml/kg/day
Formulation/Vehicle: water
Species/Strain: CD® [CrI:CD ® (SD)] rats, (b) (4)
Number/Sex/Group: 25/sex/C and HD, 20/sex/LD and MD
Age: 6 weeks old
Weight: 180-210 g males and 153-176 g females
Satellite groups: 12/sex/group
Unique study design: Liver samples were also collected at the end of the study for enzymatic analysis. In addition, **methylene blue** was used to stain blood smears for Heinz bodies.

Deviation from study protocol:

Observations and Results

Mortality

Main Study

- 2 control males (Found dead on D142 and D163)
- 5 males and 1 female at 50 mg/kg (Found dead: 1 M on D42, 1 F D49, 1 M on D103, 1 M on D172, 1 M on D126; Moribund euthanized 1 M on D107)
- The cause of death was not determined except in 2 HD males (1 male was euthanized due to shoulder jury abrasion/laceration/skin inflammation/necrosis and 1 male died due to lymphoma). Although the cause of death in other HD animals was not determined, the reviewer considered them drug-related as they may have died to seizure or other CNS toxicity.

TK study

- 3 males (D95, 118 and 119) and 1 female (D 119) at 50 mg/kg were found dead.
- No deaths at lower doses (1 and 5 mg/kg)

Clinical Signs

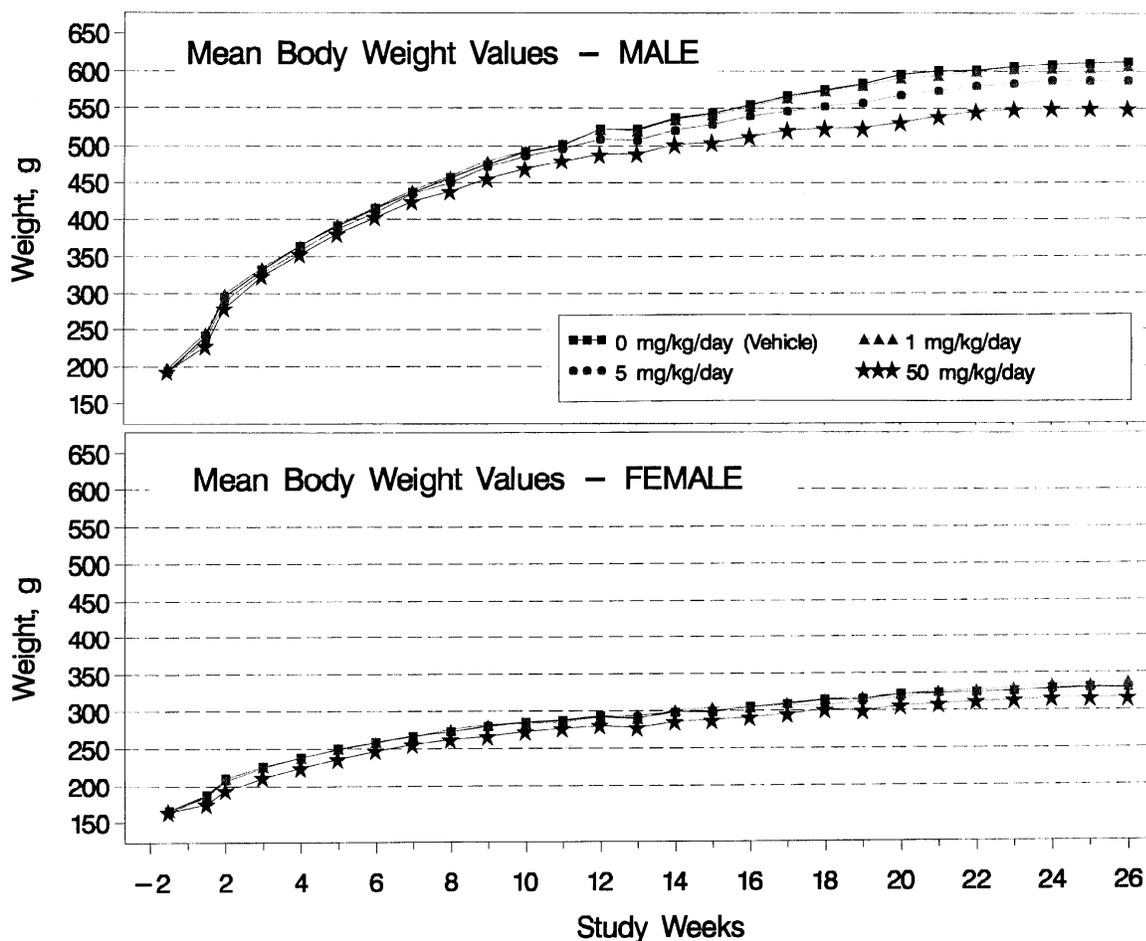
- Salivation (prominent during WK1) and red/yellow/black/brown material around the nose/mouth/eyes in both sexes at 50 mg/kg and as a minor incidence in males at 5 mg/kg
- No notable clinical signs were noted in rats during recovery period

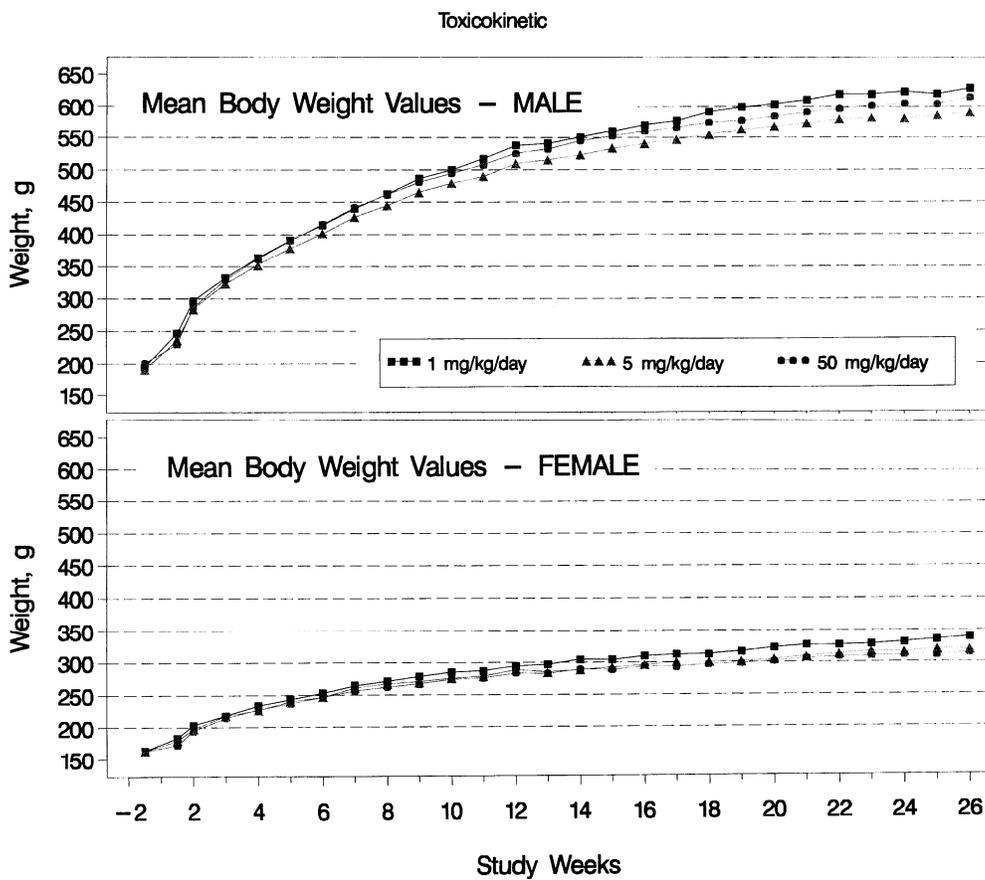
Body Weights

- Significant decrease in body weight gain in both males (-29%) and females (-49%) during the first week at 50 mg/kg and slight non-significant decrease in BW gain during week 1 at 5 mg/kg.
- The final BW (M: -10%) and BW gain (M: -15%, F: -7%) at 50 mg/kg reflected the initial decrease in BW gain during week 1.

Mean Body Weight Gain (g) With Percent Decrease From Control (Week 1 [Day -1 to 7], Weeks -1 to 13, and Weeks -1 to 26)				
Dose Group (mg/kg/day)	Male Mean Body Weight (g) Gain		Female Mean Body Weight (g) Gain	
	Week 1, Day -1 to 7 (%)		Week 1, Days -1 to 7 (%)	
0 (Vehicle)	+47.5 (NA)		+21.1 (NA)	
1	+48.5 (NA)		+20.0 (5)	
5	+43.8 (8)		+20.8 (1)	
50	+33.9 (29)		+10.7 (49)	
NA- Not applicable				
Dose Group (mg/kg/day)	Male Mean Body Weight (g) Gain		Female Mean Body Weight (g) Gain	
	Week -1 to 13 (%)	Week -1 to 26 (%)	Week -1 to 13 (%)	Week -1 to 26 (%)
0 (Vehicle)	+328.6 (NA)	+420.2 (NA)	+124.6 (NA)	+164.1 (NA)
1	+322.6 (2)	+409.8 (2)	+124.7 (NA)	+172.9 (NA)
5	+315.3 (4)	+396.5 (6)	+130.3 (NA)	+165.8 (NA)
50	+295.9 (10)	+357.0 (15)	+113.7 (9)	+152.5 (7)
NA - Not applicable				

The changes in body weight in rats in the main and TK studies





Feed Consumption

- Slight decrease in food intake at 50 mg/kg correlating to slight decrease in initial BW gain. The decreases were primarily seen in the first few days of the treatment. It appears that only the HD was capable of reducing food intake and correlated decrease in BW in rats.

Mean Food Consumption for Days 1 to 14 (g/animal/day)							
Dose Level (mg/kg/day)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6*	Days 7-14
0 (male)	21.6	21.0	21.8	24.4	24.8	23.8	26.05
0 (female)	16.2	15.6	15.4	18.1	18.2	17.6	18.97
1 (male)	22.3	21.4	22.4	24.8	25.2	25.0	26.51
1 (female)	15.7	15.7	15.5	17.9	17.9	17.6	18.99
5 (male)	20.1	20.8	20.9	23.8	23.7	24.0	26.78
5 (female)	14.9	15.0	15.9	19.4	17.6	19.7	19.31
50 (male)	14.7	17.2	18.9	22.0	21.4	23.7	24.68
50 (female)	9.9	13.1	13.6	15.9	15.5	17.1	17.68

*After this point, food consumption was measured weekly for the remainder of the study.

Ophthalmoscopy: Nothing abnormal was found at the end of the study in the treated rats

ECG: Not applicable

Hematology

- Significant decrease in RBC, Hgb and Hct in male and females at 50 mg/kg but not at lower doses
- Significant increase in reticulocytes in response to decrease in RBCs
- Recovery hematology parameters were returned to normal
- Heinz bodies were seen at termination and during recovery in the treated groups

Changes in hematology parameters in HD rats relative to controls at 13 and 26 weeks

Percent change in hematology parameters	50 mg/kg			
	WK 13		Wk 26	
	M	F	M	F
Erythrocytes, Hemoglobin & hematocrit	-10 to -14%	-7 to -11%	-6 to -10%	-7 to -10%
Neutrophils	64%	NS	NS	NS
Reticulocytes	82%	50%	80%	60%
Heinz bodies			present	present

NS= not significant

Clinical Chemistry

- Statistically significant minimal changes in clinical chemistry parameters were not considered meaningful (ALP, AST and ALT, creatinine, total bilirubin, triglycerides, urea nitrogen, plasma Na, Cl, total protein and albumin).
- There was a 1.23 fold increase in total cholesterol and 1.3 fold (30%) increase in HDL cholesterol in females at 5 and 50 mg/kg at Wk13.
- There were no meaningful difference at the end of the recovery period

Urinalysis

- Urine volume was increased at 5 and 50 mg/kg at week 13 and at week 26 except for female at 5 mg/kg
- The recovery parameters were normal in all treated groups except for lower urine volume in males.

Gross Pathology

- Moderate renal pelvic dilatation in 1 male at 5 mg/kg
- Enlarged Liver in 2 dead HD males
- No notable drug related findings except for the 2 HD males (1 Male euthanized due to visible signs of skin lesions and another died due to lymphoma)

Organ Weights

- There was no significant change in absolute organ weights except for increase in HD female liver weight (13.5%)
- HD rats had increased liver (M, F), kidney (M), heart (M, F), lung (M) and spleen weight (M,F) relative to body weight (see table below)
- Significant increase in relative heart/brain weight in HD males

- There was no change in recovery animal absolute or relative organ weights
- There were no changes in heart weight in the 13-WK rat study, however, the relative liver, spleen and kidney relative to BW were increased at 50 mg/kg

Changes in organ wt relative to BW	50 mg/kg	
	M	F
Live wt /BW	28%	19.2%
Kidney wt / BW	20 %	NC
heart wt/BW	21.8 %	10.8%
Lung wt /BW	12.8%	NC
Spleen wt /BW	31.5%	31.7%
Testes wt /BW	13.4%	NC

NC= no notable change

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings:

- Minimal centrilobular hepatocellular hypertrophy in 2 males at 5 mg/kg,
- Minimal to moderate centrilobular hepatocellular hyper trophy 18 males and 16 females at 50 mg/kg
- Dose-related increase in centrilobular hepatocellular vacuolation in males at all dose. However a few incidences of minimal hepatic vacuolation were also seen in control males. The vacuolation in control males were found in midzonal and periportal regions of the liver
- Minimal to mild incidences of focal liver necrosis in 1 male and 1 female at 1 and 5 mg/kg and 1 female and 2 males at 50 mg/kg. Only 1 HD female at recovery had minimal focal liver necrosis.
- Minimal to mild increase in incidence of extramedullary hematopoiesis and pigmented macrophages at 50 mg/kg (correlated with increase in spleen weight)
- There was no notable treatment related cardiac pathology in rats.
- Increase in incidence or severity of generalized lymphoid depletion at 50 mg/kg
- No hepatocellular or hepatic vacuolation was observed during the recovery, however residual splenic findings remained at the end of the recovery in the HD animals
- **Minimal hyperplasia of mammary ducts in 2 HD female rats. There were no such findings in the recovery animals.**
- Malignant lymphoma in spleen, thymus, sternal and femur bone marrow in 1 HD male that died before scheduled necropsy
- The sponsor considered the 2 cases of minimal hyperplasia of the mammary ductules in females a background lesion and not related to drug treatment.
- The NOAEL was 5 mg/kg in the 6-month rat study, based on the histological changes in the liver at 50mg/kg.

Histopathology of notable organs in the 26-week rat study with 1, 5 and 50 mg/kg of lorcaserin with 4 week recovery

Histopathology of most altered Tissues/organs			0	1 mg/kg	5 mg/kg	50 mg/kg
Liver	centrilobular hepatocyte hypertrophy	M			2mi,	1mi, 5ml, 12md
		F				8mi, 10ml, 1md
	vacuolation, centrilobular	M	3mi	7mi	14mi	7mi, 9 ml, 3md
		F				
	necrosis, focal	M		1mi	1ml	2mi
		F		1mi	1mi	1mi
Spleen	extramedullary hematopoiesis	M			1mi	8mi, 2ml
		F				9mi
Mammary gland	Minimal hyperplasia of mammary ductules	F				2 mi
Histopathology of Tissues in after a 4-week Recovery in Rats						
			Control	1 mg/kg	5 mg/kg	50 mg/kg
Liver	vacuolation, centrilobular	F	1mi			
		M				
	necrosis, focal	F				1mi

M=male, F= female, mi=minimal, ml= mild, md=moderate

Special Evaluation:

Heart tissue was examined for potential evidence of valvulopathy. There was no evidence of valvulopathy in rats treated with lorcaserin doses up to 50 mg/kg which was respectively in males and females 24x and 40x the clinical dose of 10 mg BID based on AUC.

Comprehensive list of histopathology report of the 6-month rat study

(b) (4) Study Number 900-046
 APD356: 6-Month Oral Toxicity Study in Rats With A 4-Week Recovery Period

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day (Vehicle)		1 mg/kg/day		5 mg/kg/day		50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		2	18	0	20	0	20	5	16
bone marrow, femur		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
depletion, adipose	- mild	0	0	0	0	0	0	0	1
hyperplasia, granulocytic	- mild	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		2	18	0	0	0	0	3	15
bone marrow, sternum		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
depletion, adipose	- minimal	0	0	0	0	0	0	0	1
hyperplasia, granulocytic	- mild	0	0	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		2	18	0	0	0	0	3	15
bone, femur		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		2	18	0	0	0	0	4	16
bone, sternum		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		2	18	0	0	0	0	4	16
brain		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
inflammation, meningeal	- minimal	1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		1	18	0	0	0	0	4	16
cavity, abdominal		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
hemorrhage	- mild	0	0	0	0	0	0	0	1
lipoma, benign, primary		0	0	0	0	0	0	0	1
heart		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
cardiomyopathy		0	11	0	0	0	0	3	7
	- minimal	0	10	0	0	0	0	3	7
	- mild	0	1	0	0	0	0	0	0
within normal limits		2	7	0	0	0	0	2	9
kidneys		(2)	(18)	(0)	(0)	(0)	(1)	(5)	(16)
calculus/calculi	- minimal	0	0	0	0	0	0	0	1
cyst	- minimal	0	1	0	0	0	0	0	0
hydronephrosis, unilateral		0	1	0	0	0	1	0	0
	- minimal	0	1	0	0	0	0	0	0
	- mild	0	0	0	0	0	1	0	0
hyperplasia, transitional cell	- minimal	0	0	0	0	0	0	0	1
infiltration, lymphocytic	- minimal	0	9	0	0	0	0	0	6
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
nephropathy, chronic progressive		2	10	0	0	0	1	2	3
	- minimal	2	8	0	0	0	1	2	3
	- mild	0	2	0	0	0	0	0	0
within normal limits		0	2	0	0	0	0	2	7

DOS - Died or euthanized on study
 SNC - Scheduled necropsy
 () - Number observed

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day (Vehicle)		1 mg/kg/day		5 mg/kg/day		50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		2	18	0	20	0	20	5	16
liver		(2)	(18)	(0)	(20)	(0)	(20)	(5)	(16)
hyperplasia, bile duct	- minimal	0	0	0	0	0	1	0	0
hypertrophy, hepatocyte, centrilobular		0	0	0	0	0	2	2	16
	- minimal	0	0	0	0	0	2	0	1
	- mild	0	0	0	0	0	0	1	4
	- moderate	0	0	0	0	0	0	1	11
infiltration, mononuclear cell	- minimal	1	18	0	20	0	20	4	16
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
necrosis, focal		0	0	0	1	0	1	0	2
	- minimal	0	0	0	1	0	0	0	2
	- mild	0	0	0	0	0	1	0	0
vacuolation, centrilobular		0	3	0	7	0	14	3	16
	- minimal	0	3	0	7	0	14	0	7
	- mild	0	0	0	0	0	0	3	6
	- moderate	0	0	0	0	0	0	0	3
vacuolation, midzonal	- minimal	0	0	0	3	0	2	0	0
within normal limits		1	0	0	0	0	0	0	0
lung		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
congestion	- moderate	0	0	0	0	0	0	1	0
edema	- mild	1	0	0	0	0	0	0	0
hemorrhage		1	1	0	0	0	0	0	1
	- minimal	0	1	0	0	0	0	0	1
	- mild	1	0	0	0	0	0	0	0
histiocytosis, alveolar		0	2	0	0	0	0	0	3
	- minimal	0	2	0	0	0	0	0	2
	- moderate	0	0	0	0	0	0	0	1
inflammation, acute	- minimal	0	0	0	0	0	0	0	1
inflammation, chronic	- minimal	0	1	0	0	0	0	0	0
inflammation, subacute		0	4	0	0	0	0	0	1
	- minimal	0	3	0	0	0	0	0	1
	- mild	0	1	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		1	12	0	0	0	0	3	12
lymph node, mandibular		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
hyperplasia, lymphocyte/plasmacyte		0	9	0	0	0	0	2	14
	- minimal	0	7	0	0	0	0	1	10
	- mild	0	0	0	0	0	0	0	4
	- moderate	0	2	0	0	0	0	1	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		2	9	0	0	0	0	2	2
lymph node, mesenteric		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
erythrocytosis/erythrophagocytosis, sinus	- minimal	0	1	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		2	17	0	0	0	0	4	16
multicentric neoplasm		(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
pancreas		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
atrophy, acinar	- minimal	0	3	0	0	0	0	0	1
inflammation, chronic	- mild	0	1	0	0	0	0	0	0
inflammation, subacute	- minimal	1	5	0	0	0	0	0	6

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day (Vehicle)		1 mg/kg/day		5 mg/kg/day		50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		2	18	0	20	0	20	5	16
pituitary gland		(2)	(17)	(0)	(0)	(0)	(0)	(5)	(16)
cyst		1	0	0	0	0	0	1	2
- minimal		1	0	0	0	0	0	1	1
- mild		0	0	0	0	0	0	0	1
hyperplasia, pars distalis	- minimal	0	1	0	0	0	0	0	0
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
within normal limits		1	16	0	0	0	0	3	14
prostate gland		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
inflammation, acute	- minimal	0	0	0	0	0	0	0	1
inflammation, subacute		1	2	0	0	0	0	0	1
- minimal		0	1	0	0	0	0	0	1
- mild		1	1	0	0	0	0	0	0
mineralization		1	16	0	0	0	0	3	10
- minimal		1	12	0	0	0	0	3	8
- mild		0	4	0	0	0	0	0	2
within normal limits		1	2	0	0	0	0	2	6
spleen		(2)	(18)	(0)	(20)	(0)	(20)	(5)	(16)
depletion, lymphoid		0	2	0	0	0	0	0	2
- minimal		0	2	0	0	0	0	0	0
- mild		0	0	0	0	0	0	0	2
hematopoiesis, extramedullary, increased		0	0	0	0	0	1	1	9
- minimal		0	0	0	0	0	1	0	8
- mild		0	0	0	0	0	0	1	1
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0
spleen		(2)	(18)	(0)	(20)	(0)	(20)	(5)	(16)
macrophages, pigmented		2	18	0	20	0	20	4	16
- minimal		0	0	0	0	0	1	0	0
- mild		2	10	0	16	0	13	1	0
- moderate		0	8	0	4	0	6	1	0
- severe		0	0	0	0	0	0	2	16
thymus gland		(2)	(18)	(0)	(0)	(0)	(0)	(5)	(16)
atrophy		0	16	0	0	0	0	2	16
- minimal		0	14	0	0	0	0	2	12
- mild		0	2	0	0	0	0	0	4
cyst	- minimal	0	0	0	0	0	0	0	1
lymphoma, malignant, multicentric		0	0	0	0	0	0	1	0

DOS - Died or euthanized on study
 SNC - Scheduled necropsy
 () - Number observed

APD356: 6-Month Oral Toxicity Study in Rats With A 4-Week Recovery Period

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day (Vehicle)		1 mg/kg/day		5 mg/kg/day		50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		0	20	0	20	0	20	1	20
adrenal glands		(0)	(20)	(0)	(0)	(0)	(0)	(1)	(20)
cystic degeneration, focal cortical		0	2	0	0	0	0	0	2
	- minimal	0	2	0	0	0	0	0	0
	- mild	0	0	0	0	0	0	0	2
fatty change, focal cortical		0	0	0	0	0	0	0	1
	- minimal	0	0	0	0	0	0	0	1
hypertrophy, focal cortical		0	0	0	0	0	0	0	1
	- mild	0	0	0	0	0	0	0	1
infiltration, mononuclear cell		0	1	0	0	0	0	0	1
within normal limits		0	17	0	0	0	0	1	16
heart		(0)	(20)	(0)	(0)	(0)	(0)	(1)	(20)
cardiomyopathy		0	7	0	0	0	0	0	4
	- minimal	0	7	0	0	0	0	0	4
infiltration, mononuclear cell		0	0	0	0	0	0	0	1
	- minimal	0	0	0	0	0	0	0	1
within normal limits		0	13	0	0	0	0	1	15
kidneys		(0)	(20)	(0)	(0)	(0)	(0)	(1)	(20)
dilatation, tubular		0	1	0	0	0	0	0	0
	- minimal	0	1	0	0	0	0	0	0
infiltration, lymphocytic		0	4	0	0	0	0	0	0
	- minimal	0	4	0	0	0	0	0	0
mineralization, pelvic		0	2	0	0	0	0	0	6
	- minimal	0	2	0	0	0	0	0	5
	- mild	0	0	0	0	0	0	0	1
mineralization, tubular		0	9	0	0	0	0	0	8
	- minimal	0	9	0	0	0	0	0	8
nephropathy, chronic progressive		0	8	0	0	0	0	0	3
	- minimal	0	8	0	0	0	0	0	3
pyelitis		0	2	0	0	0	0	0	4
	- minimal	0	2	0	0	0	0	0	4
	- mild	0	1	0	0	0	0	0	1
	- moderate	0	1	0	0	0	0	0	3
within normal limits		0	3	0	0	0	0	1	4
liver		(0)	(20)	(0)	(20)	(0)	(20)	(1)	(20)
focus of cellular alteration, eosinophilic		0	0	0	0	0	0	0	1
	- minimal	0	0	0	0	0	0	0	1
hyperplasia, bile duct		0	1	0	0	0	0	0	1
	- minimal	0	1	0	0	0	0	0	1
hypertrophy, hepatocyte, centrilobular		0	0	0	0	0	0	0	19
	- minimal	0	0	0	0	0	0	0	8
	- mild	0	0	0	0	0	0	0	10
	- moderate	0	0	0	0	0	0	0	1
infiltration, mononuclear cell		0	20	0	18	0	18	1	20
	- minimal	0	20	0	18	0	18	1	20
necrosis, focal		0	0	0	1	0	1	0	1
	- minimal	0	0	0	1	0	1	0	1
vacuolation, midzonal		0	6	0	9	0	9	0	10
	- minimal	0	6	0	9	0	9	0	10
vacuolation, periportal		0	0	0	2	0	0	0	0
	- minimal	0	0	0	2	0	0	0	0
within normal limits		0	0	0	2	0	1	0	0

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day (Vehicle)		1 mg/kg/day		5 mg/kg/day		50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
lymph node, mandibular		(0)	(20)	(0)	(1)	(0)	(0)	(1)	(20)
hyperplasia, lymphocyte/plasmacyte		0	10	0	1	0	0	0	14
	- minimal	0	9	0	1	0	0	0	8
	- mild	0	0	0	0	0	0	0	5
	- moderate	0	1	0	0	0	0	0	1
lymph node, mesenteric		(0)	(20)	(0)	(0)	(0)	(0)	(1)	(20)
within normal limits		0	20	0	0	0	0	1	20
mammary gland		(0)	(20)	(0)	(1)	(0)	(0)	(1)	(20)
adenoma, benign, primary		0	0	0	0	0	0	0	1
galactocele		0	2	0	1	0	0	0	3
	- minimal	0	2	0	1	0	0	0	1
	- mild	0	0	0	0	0	0	0	2
hyperplasia, lobular		0	0	0	0	0	0	0	2
	- minimal	0	0	0	0	0	0	0	2
inflammation, subacute		0	0	0	0	0	0	0	1
	- minimal	0	0	0	0	0	0	0	1
nerve, sciatic		(0)	(20)	(0)	(0)	(0)	(0)	(1)	(20)
degeneration, axonal/myelin		0	1	0	0	0	0	1	0
	- minimal	0	1	0	0	0	0	1	0
within normal limits		0	19	0	0	0	0	0	20
pituitary gland		(0)	(20)	(0)	(0)	(0)	(0)	(1)	(20)
angiectasis		0	0	0	0	0	0	0	1
	- minimal	0	0	0	0	0	0	0	1
cyst		0	0	0	0	0	0	0	1
	- mild	0	0	0	0	0	0	0	1
spleen		(0)	(20)	(0)	(20)	(0)	(20)	(1)	(20)
depletion, lymphoid		0	1	0	0	0	0	0	5
	- minimal	0	1	0	0	0	0	0	5
hematopoiesis, extramedullary, increased		0	0	0	0	0	0	0	9
	- minimal	0	0	0	0	0	0	0	9
urinary bladder		(0)	(20)	(0)	(0)	(0)	(0)	(1)	(20)
hyperplasia, simple transitional cell		0	0	0	0	0	0	0	1
	- mild	0	0	0	0	0	0	0	1

List of prominent histopathology findings at the end of the 4-week recovery in rats treated with lorcaserin for 6 months:

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	Recovery	
		0 mg/kg/day (Vehicle)	50 mg/kg/day
Number of Animals Examined		5	4
heart		(5)	(4)
cardiomyopathy	- minimal	3	3
within normal limits		2	1
kidneys		(5)	(4)
calculus/calculi	- mild	1	0
hydronephrosis, unilateral	- minimal	1	0
nephropathy, chronic progressive	- minimal	3	2
within normal limits		1	2
liver		(5)	(4)
infiltration, mononuclear cell	- minimal	5	4
vacuolation, centrilobular	- minimal	2	2
vacuolation, midzonal	- minimal	2	4
lung		(5)	(4)
histiocytosis, alveolar	- minimal	0	1
inflammation, subacute		5	3
	- minimal	4	3
	- mild	1	0
within normal limits		0	1
spleen		(5)	(4)
macrophages, pigmented		5	4
	- mild	4	1
	- moderate	1	3
thymus gland		(5)	(4)
atrophy		5	4
	- minimal	4	1
	- mild	1	3

() - Number observed

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	Recovery	
		0 mg/kg/day (Vehicle)	50 mg/kg/day
heart		(5)	(4)
adhesion/inflammation/fibrosis, pleural	- minimal	0	1
cardiomyopathy	- minimal	0	1
within normal limits		5	2
kidneys		(5)	(4)
calculus/calculi	- minimal	0	1
hyperplasia, transitional cell	- minimal	0	1
mineralization, pelvic	- minimal	0	2
mineralization, tubular	- minimal	2	1
nephropathy, chronic progressive	- minimal	2	1
pyelitis		1	1
	- minimal	0	1
	- mild	1	0
liver		(5)	(4)
infiltration, mononuclear cell	- minimal	5	4
necrosis, focal	- minimal	0	1
vacuolation, centrilobular	- minimal	1	0
vacuolation, midzonal	- minimal	3	1
lymph node, mandibular		(4)	(4)
hyperplasia, lymphocyte/plasmacyte		4	3
	- minimal	4	1
	- mild	0	2

Toxicokinetics

- Lorcaserin (APD356) AUC exposure increased in a dose-proportional manner in both male and female rats. AUC on day 28 was nearly twice the AUC on Day 1.
- The exposure on Day 182 (end of the study) was greater than Day 1 (1.1 to 2.6 fold)
- Lorcaserin was rapidly absorbed with T_{max} of 1.25 hrs (0.5 and 4 hrs)
- Lorcaserin exposure was nearly 50% greater in females than males at the end of the study.
- Female rats have lower CYP3A4 metabolizing capacity but since the M1 metabolite exposure was also higher in females suggest that either female rats received larger lorcaserin dose or drug was more bioavailable in them.
- Two major metabolites APD244208 (M1) and APD306388 (M5) were identified in both male and female rats. The exposure to M1 was 20 to 160 fold higher than the parent exposure while M5 exposure was 20% of the parent drug exposure.
- AR244208 was formed in male and female rats after oral administration of lorcaserin.
- A244208 exposure ranged from 20- to 160-fold higher than the concomitant lorcaserin exposure across gender, dose and day of dosing.
- AR306388 was formed in male and female rats after oral administration of lorcaserin.
- AR306388 exposure was 20% or less than the concomitant lorcaserin exposure across gender, dose and day of dosing.

Toxicokinetic Parameters of APD356 as a Function of Oral Dose on Day 1, 28, and 182.

Day	Dose (mg/kg/day)	C_{max} ($\mu\text{g/mL}$)	t_{max} (hr)	AUC_{last} ($\text{hr}\cdot\mu\text{g/mL}$)	$AUC_{(0-\text{inf})}$ ($\text{hr}\cdot\mu\text{g/mL}$)
Male					
1	1	0.041	1.0	0.164	0.229
	5	0.164	1.0	0.809	1.102
	50	0.722	1.0	7.765	8.852
28	1	0.033	1.0	0.154	0.219
	5	0.161	2.0	0.818	1.025
	50	1.730	1.0	17.626	17.800
182	1	0.044	1.0	0.205	0.274
	5	0.249	0.5	1.196	1.534
	50	2.375	1.0	22.058	22.677
Female					
1	1	0.050	2.0	0.254	0.364
	5	0.223	1.0	1.156	2.061
	50	0.833	4.0	14.099	17.888
28	1	0.050	1.0	0.230	0.327
	5	0.417	1.0	1.664	2.254
	50	2.417	1.0	29.923	32.678
182	1	0.070	1.0	0.317	0.389
	5	0.404	1.0	2.873	2.911
	50	3.333	1.0	34.438	37.722

AR244208 Toxicokinetic Summary After Oral Administration of APD356 to Male and Female Rats on Days 1, 28 and 182.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)	AUC _(0-inf) (hr·µg/mL)
Male					
1	1	1.267	4.0	18.161	23.667
	5	5.887	8.0	97.729	115.116
	50	19.267	4.0	334.858	467.922
28	1	2.360	1.0	29.120	35.446
	5	8.830	2.0	124.654	144.735
	50	29.700	2.0	416.006	460.653
182	1	2.433	1.0	20.805	22.296
	5	10.837	1.0	103.038	112.045
	50	33.650	1.0	413.352	473.973
Female					
1	1	0.985	2.0	8.338	8.395
	5	4.033	1.0	47.117	48.032
	50	19.167	4.0	282.128	360.764
28	1	1.072	1.0	10.229	10.313
	5	8.657	1.0	61.759	62.981
	50	35.067	1.0	594.528	1686.159
182	1	1.700	1.0	13.665	13.999
	5	9.060	1.0	79.409	83.362
	50	44.700	2.0	614.127	779.527

AR306388 Toxicokinetic Summary After Oral Administration of APD356 to Male and Female Rats on Days 1, 28 and 182.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)	AUC _(0-inf) (hr·µg/mL)
Male					
1	1	No TK ¹	-	-	-
	5	0.042	0.5	0.080	0.113
	50	0.206	0.5	1.159	1.365
28	1	No TK	-	-	-
	5	0.025	0.5	0.052	0.071
	50	0.376	0.5	1.386	2.077
182	1	No TK	-	-	-
	5	0.062	1.0	0.123	0.149
	50	0.825	0.5	4.551	4.591
Female					
1	1	No TK	-	-	-
	5	0.021	1.0	0.091	0.130
	50	0.074	0.3	0.991	1.698
28	1	No TK	-	-	-
	5	0.024	1.0	0.062	0.088
	50	0.285	0.5	2.256	2.393
182	1	No TK	-	-	-
	5	0.032	0.3	0.086	0.120
	50	0.496	0.5	4.160	4.350

¹No TK: incomplete AR306388 concentration-time profiles prevented toxicokinetic parameter determinations.

Safety Margins for lorcaserin based on AUC in rats:

NOAEL of 5 mg/kg was based on liver histopath findings in the 6-month rat study..

Species	Dose, mg/kg	AUC _{0-t} µg.h/ml	Animal to human dose exposure ratio based on AUC
26-Week Rat Study with a 4-Week Recovery	1	M: 0.27, F:0.389	<1
	5	M: 1.53, F: 2.9	M: 1.5, F: 2.8
	50	M: 22.7, F: 37.7	M: 22.2 F: 37
MRHD: 10 mg BID		1.02	

Safety Margins for M1 metabolite (APD244208, sulfamate metabolite), based on AUC:

Species	Dose, mg/kg	AUC _{0-t} µg.h/ml	Animal to human dose exposure ratio based on AUC
26-Week Rat Study with a 4-Week Recovery	1	M: 22.2, F: 13.9	M1: 4, F: 2.6
	5	M: 112, F: 83.3	M: 21, F: 15
	50	M: 473, F: 779	M: 87, F: 144
MRHD: 10 mg BID		5.422	

Stability and Homogeneity

The stability studies showed lorcaserin solutions were stable for 3 months at room temperature or stressed temperature (40C). Solution concentrations were within 91.1% to 104.5% of the target solution concentrations.

Study Title: A 52-Week Toxicity Study of APD356 Administered by Nasogastric Intubation to Cynomolgus monkeys with a 4-Week Recovery Period.

Key Findings:

- There were 2 deaths in HD (1 F: Day15; 1 M: Day64) due to apparent gavage error. These two animals had frequent emesis and deaths may have been due to aspiration of dose or stomach content. In 13-WK study, there were also two deaths (1 F at 125 mg/kg, 1 M at 75 mg/kg) of unknown cause.
- Dose-related clinical signs consisted of decreased activity, emesis, hunched appearance, tremors, and emesis which were absent during recovery. Monkeys at 125 mg/kg displayed greater incidence of tremor (females only) and emesis (both gender)
- Incidence of seizure was noted in 1 HD male 20 min post dose on Day 1. There were no more seizure in this male after administration of diazepam, IV
- Dose-related decrease in food consumption and some decrease in body weight at doses \geq 50 mg/kg. There was no change in BW or BW gain at 2 and 10 mg/kg.
- There was no drug-related change in cardiovascular parameters in monkeys
- Significant decrease erythroid parameters (Hgb, Hct and RBC) in HD monkeys was associated with compensatory increase in reticulocytes at 125 mg/kg
- Iorcaserin reduced serum lipids (LDL, HDL and Cholesterol) at \geq 50 mg/kg
- There was a mild increase in ALT in HD monkeys likely due to mild hepatic lipidosis
- Urinalysis found presence of WBC in 1 F at 50 mg/kg and 3 F at 125 mg/kg two of which had protein and cellular casts suggesting renal damage
- Although there were no notable differences in organ weights, the absolute kidney weights appeared to be unchanged or slightly heavier even though they had lower BWs. The relative organ /BW weight for kidney, liver and heart were increased in HD females.
- There was a dose-dependent increase in the incidence of renal tubular regeneration at all doses but more frequently at 10, 50 and 125 mg/kg. The incidence of renal tubular degeneration (minimal to moderate) was only observed at 125 mg/kg, while cellular casts were seen at doses \geq 50 mg/kg suggesting that drug-related renal histopathology was Iorcaserin related but more notable at doses \geq 10 mg/kg (regeneration) with degeneration limited to 125 mg/kg where both regeneration and degeneration occurred. Renal findings were not reversed at the end of the 4-week recovery phase in MD and HD monkeys.
- As a 5HT_{2C} agonist, there was no evidence of valvulopathy although some questionable findings were noted in couple of drug treated monkeys such as aortic intima fibrosis (1 M at 50 mg/kg) and atrial epicardial inflammation (1 F at 125 mg/kg)
- Minimal bone marrow lymphoid nodules in 1 male at 50 and 125 mg/kg
- Incidence of liver necrosis in 1 HD male in the main study and 1 HD recovery female
- Minimal to mild thyroid follicular degeneration at 10 mg/kg (2 M) and 50 mg/kg (1 M, 1 F) and at 125 mg/kg (1 F). Similar findings were noted in 2 HD females in the recovery phase of the study.

- Ovarian follicular cysts at ≥ 10 mg/kg (1/4, 1/4 and 2/4 at 10, 50 and 125 mg/kg)
- Minimal to mild thyroid follicular degeneration at 10 mg/kg (2 M) and 50 mg/kg (1 M, 1 F) and at 125 mg/kg (1 F). Similar findings were noted in 2 HD females in the recovery phase of the study
- There were no gender differences in PK or difference between the two lorcaserin formulations
- Repeated dosing with APD356 (lorcaserin) resulted in up to 1.8 fold increase in exposure. Lower exposure at the end of the study (10 to 40%) at 125 mg/kg was likely due emesis
- The exposure of the two metabolites, M1 (APD244208) and APD306388 (M5) increased with increase in dose and duration of the study.
- The M1 metabolite was 15 to 44 fold greater than parent drug in monkeys.
- PK studies in rats had reported a 13 fold increase in brain exposure to lorcaserin than plasma. Based on higher brain exposures, safety margins based on plasma exposures alone will not be adequate for CNS signals (tremors, convulsions...) unless the exposure ratio between brain and plasma in humans is the same as those in rats.
- The 2 mg/kg (AUC 0.54 to 0.961 $\mu\text{g}\cdot\text{h}/\text{ml}$) was considered NOAEL since most renal findings occurred at ≥ 10 mg/kg. At NOAEL, the clinical exposure was near unity (10 mg BID, 1.02 $\mu\text{g}\cdot\text{hr}/\text{ml}$)

Study No: TX04039 ((b) (4) # VAV00016)
Amendment # 0032, Vol # 9 and page # 1-1615

Conducting laboratory and location: (b) (4)
(b) (4) Analytical parts of the study were done by (b) (4)
(b) (4) for serological screening of animals, ECG by (b) (4) and
plasma concentrations by (b) (4)

Date of study initiation: Oct 5, 2004

GLP compliance: Yes

QA- Report: Yes (x) No ()

METHODS: cynomolgus monkeys (4-6/sex/group) were treated with lorcaserin for 52-week by nasogastric route (prepared weekly in distilled water, 5 ml/kg). Gavage tubes were flushed with 3 ml of tap water. Two monkeys from the control and mid high dose (MHD) and HD groups were allowed to recover for 4 weeks. For the first 91 days animals were given anhydrous lorcaserin. From Day 92 to Day 364, hemihydrate lorcaserin was used. The PK analysis found the anhydrous to provide similar exposure to hemihydrate lorcaserin. Drug solution concentrations were within $\pm 10\%$ of the target concentration. Animals were fasted overnight before sample collections at the end of the study.

Group No.	Number of Males/Females	Dose Level (mg/kg/day)	Number Sacrificed:	
			Week 53	Week 57
1	6/6	0 (control)	4/4	2/2
2	4/4	2	4/4	-
3	4/4	10	4/4	-
4	6/6	50	4/4	2/2
5	6/6	125	4/4*	2/2

*One male (Animal 60) and one female (Animal 25) from Group 5 died due to dosing accidents. The male died on Day 364 and data from this animal are included with the other terminal sacrifice animals; the female died on Day 16 and was replaced.

Dosing: 0, 2, 10, 50 and 125 mg/kg

Species/strain: Cynomolgus monkeys (*Macaca fascicularis*) from [REDACTED] (b) (4)

Age: 3.4 to 5.6 years for males and 2.8 to 4.8 years for females

Weight: 2.8 to 4.7 kg males and 2.5 to 3.6 kg females

Satellite groups used for toxicokinetics or recovery: Recovery animals

Drug, lot#, radiolabel, and % purity: 04P0222 (Day 1-91) and 04L018 (Day 92-264), 99.2%

Formulation/vehicle: distilled water, 5 ml/kg

OBSERVATIONS AND TIMES:

Clinical signs: Twice daily

Body weights: weekly

Food and Water consumption: daily first week, weekly there after

Ophthalmoscopy: Pre-dose and on Week 13, 26, 39 and 52 (4-6 hrs postdose)

ECG: pre-dose and during WK 13, 26, 39 and 51 (4-6 hrs post dose) using Lead I, II, III, aVR, aVL and aVF. No ECG during recovery.

Hematology: Standard hematology evaluations before treatment and post dose during Week 13, 26, 39 and 52 from overnight fasted animals

Clinical chemistry: Standard clinical chemistry from serum samples collected prior to treatment and on Week 13, 26, 39 and 52 of the treatment after an overnight fast

Urinalysis: Urine samples before treatment on Week 4 and 13

Gross pathology: All animals after an overnight fast

Organ weights: Standard list. Liver samples were obtained for total CYP content determination

Histopathology: Standard histopath on all controls and mid high dose (MHD, 50 mg/kg) and HD (125 mg/kg) animals and suspected tissues from in LD (2 mg/kg) to MD (10 mg/kg) groups. In addition heart, liver and seventh rib bone marrow samples were collected. The liver sample was used for CYP450 enzyme analysis. Furthermore, glycogen and fat content of the liver samples were determined. Bone marrow analysis was later determined unnecessary. Due to potential drug-induced valvulopathy, comprehensive cardiac histopathology was performed. Histology slides were reviewed by study pathologist, [REDACTED] (b) (6) and peer reviewed and agreed by the second pathologist, [REDACTED] (b) (6). The interpretation of the kidney findings in monkeys by the study pathologists were challenged by the

sponsor. The sponsor enlisted two external renal pathologists to review new set of renal slides prepared from the same or new renal tissue blocks. These slides were not reviewed by the original study pathologists. Furthermore, since the slides were labeled as group 1 and 5 (read first) and groups 2, 3 and 4 (read second), they were more not blinded as the sponsor claims since every pathologist knows that group 1 and 5 represent control and high dose group, respectively. The two renal pathologists were

(b) (6)

Due to potential valvulopathy concern with lorcaserin, additional heart tissue sectioning was made to examine the heart valves in the 12-month monkey study (see table below for additional sectioning of the heart).

Cardiac Evaluation - Histopathology	
Region Examined	Sections Processed*
Atrium	Left and right free wall parallel to long axis of heart
Ventricle	Left and right free wall parallel to long axis of heart
Atrioventricular Septum	Midsection to include left and right endocardial surfaces; section parallel to the long axis of the heart
Atrioventricular (Mitral and Tricuspid) Valves	Left and right – section through the valve cusp closest to atrioventricular septum to include septal muscle
	Left and right – section through the valve cusp closest to atrioventricular free wall to include free wall muscle
	Both sections parallel to the long axis of the heart
Aortic Valve	Two sections (two valve cusps) through the long axis of the cusp
Pulmonic Valve	Two sections through the long axis of the valve cusp
* Regions may have been combined in the same tissue section in an effort to preserve morphology	

Toxicokinetics: Blood samples (0.5 ml) were collected into heparinized tubes on Day 1 (0.5, 1, 2, 4, 6, 8 and 24 hrs), Week 4 (-0.5, 1, 2, 4, 6, 8 and 24 hr), Day 91 (-0.5, 0.5, 1, 2, 4, 6, 8 and 24 hr post dose of anhydrous formulation) Day 91 (-0.5, 0.5, 1, 2, 4, 6, 8 and 24 hr post dose of hemihydrate formulation) and on week 51 (-0.5, 0.5, 1, 2, 4, 6, 8 and 24 hrs post dose. Plasma concentrations of lorcaserin and APD244208 were measured by

(b) (4) while the analysis of APD306388 was conducted by (b) (4)

Statistics: Standard statistical analysis methods using SAS were applied: Shapiro-Wilkes test for normality, Dunnett’s test for pair comparison with control and Kruskal-Wallis non-parametric ANOVA was used for group differences.

RESULTS:Mortality:

- Two deaths: 1 male (#60, Day 365) and 1 female (#25, Day 16) treated with 125 mg/kg of lorcaserin died due to gavage error. Male monkey (#60) died within minutes of the dose. This animal was found lying down several times on the bottom of the cage until approached at the end of the study. This behavior was not observed in other animals. The female monkey (#25) died shortly after drug administration also on Day 16. This animal was replaced by another (#8) since the death occurred early in the study. In both cases, the histological data support gavage error. It is not clear why gavage error occurred in both instances in the high dose group. It is not clear if emesis or tremor or any thing else had contributed to intubation error.
- In the earlier 3-month study the previous study, there were also several deaths: 1 HD female on Day 3, right after gavage (on Day 2 this animal had elevated ALT, AST and LD); 1 male (# 2145) treated with 75 mg/kg was found dead on Day 43. According to the sponsor, this animal was difficult to intubate during that day and had severe emesis. No adverse clinical signs or any histological abnormalities were noted, with cause of death still undetermined. The role of lorcaserin in causing sudden deaths in both animals in the 3-month study could not be ruled out since there were no clear signs of lung injury. It is highly possible that the deaths were drug-induced serotonin toxicity related.

Clinical signs:

- The most common dose-related signs were emesis, tremor, decreased activity/lethargy, sleepy and tired appearance, droopy eyes, (≥ 10 mg/kg),
- Orange discoloration of skin noted only in the treated monkeys (≥ 2 mg/kg) was attributed to poor grooming.
- Dose-related increase in emesis (1-3 hrs post dose, especially in the MD males and both sexes at HD), at ≥ 10 mg/kg. The incidences of emesis at lower doses were similar to controls. Emesis did not occur during recovery period.
- Dose-related increase in incidence of hunched appearance at ≥ 10 mg/kg
- Notable increases in incidences of tremors were noted with increase in lorcaserin in both sexes. The incidences of emesis were greater in animals treated with 75 and 125 mg/kg of lorcaserin.
- One incidence of seizure was noted on Day 1 in 1 HD male (20 min post dose). The veterinarian found the animal to be recumbent with continued seizure every 3-5 seconds thus treated with diazepam intravenously. There were no incidence of seizure on the following days and animal was considered fit to stay in the study.
- Incidence of periorbital swelling and dilated pupils was more common at 50 and 125 mg/kg, although were also rarely noted in controls
- Most of the clinical signals such as emesis and tremor in the 12-month study were also observed in the 13-WK monkey study. These clinical signs generally disappeared after the cessation of dosing.

Intergroup Comparison of Notable Clinical Observations of (Days 1-364)								
Group Dose Size	Gender	Animal	Low Food Consumption	Decreased Activity*		Total Hunched Appearance†	Total Emesis†	Total Tremors†
				Routine a.m./p.m.	Postdose 1 hr/2hr/3 hr			
Group 1 (0 mg/kg/day) N = 12	Male	37	2	-	-	1	3	-
		41	2	5	3	1	1	6
		43	-	8	9	1	-	-
		46	5	8	7	4	3	-
		58	-	-	-	-	1	-
	59	1	2	3	-	2	-	
	Female	4	1	-	-	-	-	-
		6	12	62	28	6	4	2
		13	3	24	11	1	6	3
		20	-	-	-	-	1	-
27		-	1	-	4	-	-	
30	1	4	3	-	3	2	1	
Group 2 (2 mg/kg/day) N = 8	Male	34	5	13	11	1	2	-
		35	-	1	3	-	3	5
		47	-	11	2	-	1	-
		50	1	-	1	1	3	-
	Female	1	4	-	1	-	2	-
		22 [^]	9	1	4	1	4	7
		23	24	4	1	1	-	-
		31	9	-	1	-	-	-
Group 3 (10 mg/kg/day) N=8	Male	39	19	43	39	-	-	-
		49	1	2	22	1	1	1
		53	11	2	3	2	2	-
		57	8	32	92	9	-	-
	Female	2 [^]	25	3	20	6	4	17
		11	1	-	3	-	2	-
		18	20	62	42	55	4	-
		21	4	14	19	1	2	-
Group 4 (50 mg/kg/day) N = 12	Male	36 [^]	4	42	53	9	7	2
		42	3	10	29	1	15	-
		45	6	34	48	6	1	2
		51	-	8	21	-	7	1
		52	6	56	74	2	1	-
		55	6	2	8	1	3	-
	Female	5	6	65	43	27	2	4
		14	14	-	2	2	2	-
		17	8	78	89	5	1	-
		24 [^]	66	17	32	2	4	-
		26 [^]	35	78	103	102	1	12
		29	-	2	15	-	4	1
Group 5 (125 mg/kg/day) N = 12	Male	33	-	-	2	2	31	-
		38	3	25	38	9	7	1
		40 [^]	53	59	68	62	152	18
		54	30	46	66	6	19	3
		56	-	4	13	-	12	2
	60 [^]	14	125	134	301	36	19	
	Female	3 [^]	17	6	34	16	17	-
		8 [^]	37	161	144	234	11	12
		10	20	17	36	21	20	18
		15 [^]	72	27	37	45	18	33
		16	18	119	132	70	1	99
		19 [^]	11	6	14	1	4	2

* Included observations of decreased activity and other descriptors of decreased activity

† Total of routine and postdose observations

[^] Animals examined by a veterinarian for low food consumption and/or weight loss and provided dietary supplements

- = not observed

Body weight:

- The mean body weight in females at ≥ 50 mg/kg were lower than controls. Lorcaseerin had no effect on BW of female monkeys at 2 and 10 mg/kg (see table below)
- No notable effect on BW was noted in males although BW gain of both male and female monkeys were lower at ≥ 50 mg/kg
- The decrease in body weight was associated with decrease in food intake in females

- The HD animals had greater incidence of emesis (both sexes) and tremor (females).
- Tremors and emesis did not occur during recovery phase.
- There was no difference in the BW of lorcaserin treated and controls during recovery

Percent Increase in mean BW at the end of the study relative to day 1

Percent Increase in Group Mean Body Weight, Days 1-364*					
	Group 1 (0 mg/kg/day)	Group 2 (2 mg/kg/day)	Group 3 (10 mg/kg/day)	Group 4 (50 mg/kg/day)	Group 5 (125 mg/kg/day)
Male	50.2±15.3	47.9±20.7	43.6±8.3	30.7±13.4	34.7±12.7
Female	46.0±6.8	27.7±14.4	36.7±32.8	11.3±2.5	3.8±4.2

* Calculated as the mean of the percent change from individual Day 364 body weight compared to the Day -1 individual body weight for each group

Food and Water Intake:

- There was an apparent decrease in appetite in treated monkeys. Some animals were given fruit to supplement calories. It wasn't clear after that if monkeys wouldn't eat expecting fruit or had reduced appetite and would eat only tasty treats. It should be noted that lorcaserin decreased activity in a dose dependent manner. Since BW was reduced only at high doses of lorcaserin with clinical signs of hypoactivity, hunched back suggests that decrease in appetite may have been partially due to ill effect of the drug and hence the weight loss.

Dietary Supplementation				
Group (Dose)	Animal	Sex	Days of Therapy	Percentage Weight Gain**
Group 1 (0 mg/kg/day)	None	NA	NA	NA
Group 2 (2 mg/kg/day)	22	Female	80-96	6.9
Group 3 (10 mg/kg/day)	2	Female	53-92	24
Group 4 (50 mg/kg/day)	24	Female	59-96	11.5
	26	Female	54-99	11.1
	36	Male	69-98	45.7
Group 5 (125 mg/kg/day)	3	Female	80-97	11.5
	8*	Female	11-97	0.0
	15	Female	10-35; 44-143	0.0
	19	Female	94-113	3.0
	40	Male	54-107	50.0
	60*	Male	58-92; 182-189	26.1

* Therapy also included subcutaneous administration of lactated Ringer's solution.

** Calculated as the percent change of the Day 364 body weight compared to the Day -1 body weight

ECG:

- There were no treatment related changes in cardiovascular parameters (i.e. ECG) monitored 4 to 6 hr post dose. Since the Tmax was generally between 3 and 5 hrs, the ECG analysis was acceptable.
- In in-vitro studies (hERG and Purkinje fiber assays) lorcaserin significantly prolonged action potential duration at 90% (ADP90) in isolated canine Purkinje fiber assay at 30 μ M (6.96 μ g/ml) but had no effect on ADP60 (3, 10 and 30 μ M). In hERG channel study, lorcaserin significantly inhibited hERG (IKr) current at all concentrations in a dose-dependent manner with IC50 =14 μ M (3.25 μ g/ml). The concentrations at which these findings were observed are significantly higher than the anticipated peak plasma levels in humans (Cmax of 85 ng/ml at 20 mg/kg). Based on the nonclinical data thus far lorcaserin is unlikely to prolong action potential in humans.

Ophthalmoscopy: Nothing abnormal

Hematology:

- Significant decline in Hgb, hematocrit and RBC in HD monkeys week 39 and 52. The decreases were associated with increase in reticulocytes
- There was no change in coagulation parameters

Urinalysis:

- White blood cells were noted in urine of 1 female at 50 mg/kg and 3 females treated with 125 mg/kg.
- 2 of the HD females also had protein and cellular casts in the renal tubules
- 1 HD female had protein cast during recovery
- The increase in incidence of WBC was consistent with drug-related inflammation, renal tubular degeneration and regeneration noted in these groups.

Clinical chemistry:

- Reduced serum lipids (LDL, HDL and cholesterol) at several time points were noted \geq 50 mg/kg. The slight decrease at 2 and 10 mg/kg was not consistent at all time intervals.
- Serum triglycerides were generally lower at WK 13, 26 and 39 and 52 at 2, 10 and 50 mg/kg. Serum triglycerides for some reason were increased at 125 mg/kg at WK 52.
- Mild increase in ALT was seen in most groups including controls, however only the HD animals had histological correlates (mild hepatic lipidosis) with the increase in ALT.

Fold Change in Individual ALT Values							
Group	Monkey No.	Gender	Week 13 ¹	Week 26 ¹	Week 39 ¹	Week 52 ¹	Histologic Correlate
1	6	Female	0.6	3.5	1.3	1.3	none
2	50	Male	1.7	2.2	2.1	1.8	none
	22	Female	3.2	2.3	3.2	1.1	hepatic lipidosis
3	18	Female	1.2	4.9	3.1	4.2	hepatic lipidosis
4	29	Female	1.5	1.8	1.9	0.8	hepatic lipidosis
5	3	Female	3.5	3.8	4.0	2.3	none
	16	Female	1.7	1.1	1.5	1.3	hepatic lipidosis

¹ Values represent the fold change versus respective prestudy values

Significant changes in serum chemistry at WK 52 are shown below:

Serum Chemistry		0	2 mg/kg	10 mg/kg	50 mg/kg	125 mg/kg
Glucose, %	M	-	-	-		45%
LDL, %	M	-	-	-	- 37.9%	- 47%
	F	-	-	-		- 31%
HDL, %	M	-	-	-		- 26%
	F	-	-	-	- 33%	- 51%
Trig, %	M	-	-	-	- 13.2%	↑ 58%
	F	-	-	-	- 26%	↑ 36%
Cholesterol, %	M	-	-	-		- 29%
	F	-	-	-		- 37 %

-- Not significantly different from controls

Organ Weight:

- Although there were no notable differences in organ weights, the kidney weight of both HD male and female monkeys were relatively larger considering that they generally (female) weighed less than controls.
- The relative organ to body weight was increased for liver, kidney and heart in HD females. The increase in relative kidney weight might have been due to drug-related increase in inflammation /renal tubular degeneration/regeneration. The increase in liver weight correlated with hepatic lipidosis.

Absolute organ weight at the end of 12-month monkey study

Organ weights		Control	2 mg/kg	10 mg/kg	75 mg/kg	125 mg/kg
Body weight, kg	M	5.03	5.2	4.88	4.28	4.4
	F	3.65	3.30	3.80	2.88	2.70
Kidney, g	M	17.08	15.69	14.32	15.97	17.11
	F	12.36	12.39	12.56	12.03	14.56
Liver, g	M	95.9	84.1	76.97	76.9	93.8
	F	72.7	67.0	77.6	58.9	67.5
Heart, g	M	16.25	17.86	15.96	15.21	13.86
	F	11.52	12.07	12.60	8.83	10.10
Brain, g	M	69.87	65.35	66.44	67.74	66.34
	F	60.73	59.67	62.20	63.38	56.73

Gross Pathology:

- Kidney of one female at 50 mg/kg was small in size
- No differences in the recovery animals

Histopathology:

- Minimal to moderate drug-related increase in incidence of tubular epithelial degeneration of renal cortex (6/8) at 125 mg/kg. There was also an incidence at 10 and 50 mg/kg in male monkeys
- Minimal to moderate dose-related increase in renal tubular cortical epithelial regeneration (0/8, 1/8, 2/8, 3/8 and 7/8 at 0, 2, 10, 50 and 125 mg/kg, respectively) with slight increase in mitotic figures in area's of regenerating tubules at high dose. There were no notable renal findings in the control monkeys.
- Drug-related increase in incidence of cellular casts in renal tubules at \geq 50 mg/kg (M) and at 125 mg/kg in females also suggesting renal injury
- There were also incidences of glomerular sclerosis, tubular epithelial vacuolation and atrophy in some of the monkeys treated with lorcaserin.
- The renal tubular injury was not reversed during 1 month recovery. The incidence of tubular degeneration and regeneration and cellular casts (minimal to mild) were noted in recovery male and female monkeys at 50 and 125 mg/kg. In the 13-week monkey study, minimal tubular regeneration was noted in 1 monkey at 125 mg/kg. The renal finding was evaluated by a peer review pathologist. The sponsor had also requested the assistance of two additional renal pathologists to review newly prepared kidney slides from Group 1 and 5. The two renal pathologists concluded that the renal findings were not drug-related. The reviewer agrees with the original study pathologist and considers renal findings to be drug-related for several reasons: 1) the new pathologists were given new slides from both old and newly prepared tissue blocks, 2) the external pathologists can hardly be considered blinded since they were instructed to read slides from group 1 and 5 first (control and HD), 4) neither groups appeared to have reviewed the same slides, thus new slides could have been different from the original slides, and 5) the external pathologists were clinicians, not veterinary pathologists.
- Non-selective 5HT agonists are know to cause valvulopathy by supposedly activation 5HT_{2b} receptors in the heart. In this study, there was no evidence of valvulopathy in the monkeys. The significance of some minor findings such as mild incidence of aortic intima fibrosis in 1 male at 50 mg/kg and fibrosis of hear epicardium in 1 male at 2 mg/kg and mild atrial epicardial inflammation in 1 female at 125 mg/kg is not clear. Myocardial fibrosis was also noted in 1 HD male during recovery phase of the study.
- Bone marrow minimal lymphoid nodules in 1 male at 50 and 125 mg/kg
- An incidence of liver necrosis (focal, minimal) in 1 male at 125 mg/kg in the main study and in 1 female (125 mg/kg) during the recovery phase
- Minimal to mild thyroid follicular degeneration at 10 mg/kg (2 M) and 50 mg/kg (1 M, 1 F) and at 125 mg/kg (1 F). Similar findings were noted in 2 HD females in the recovery phase of the study

- Ovarian follicular cysts were observed in a dose related manner with lorcaserin (1/4, 1/4 and 2/4 at 10, 50 and 125 mg/kg, respectively treated animals but were reversible during the recovery.
- The NOAEL was considered to be 2 mg/kg since most renal findings were observed at doses greater than 10 mg/kg.

Histopathology of notable organs in the 12-month monkey study with lorcaserin

Notable Histopath Findings			Lorcaserin doses, mg/kg				
			0	2	10	50	125
Kidney	Tubular degeneration, focal /multifocal	M			1mi	1mi	2mi,1ml
		F					3mi,1md
	Tubular regeneration, focal/multifocal	M			1ml	1mi,1ml	1mi,2ml
		F		1mi	1mi	1 mi	1mi,1ml,1md
	Cast cellular, focal/multifocal	M				1mi	2mi
		F					2mi
	Glomerularsclerosis, focal/multifocal	M			1ml	1mi	
		F				1mi	
	Vacuolation, tubular epithelium	M			1mi		
		F		1ml			
Atrophy, tubular epithelium	M			1ml			
Swelling, tubular epithelium	M				1mi		
	F				1mi		
Cortex, focal scar	F					1mi	
Aorta, fibrosis, intima, focal	M				1ml		
Heart	Fibrosis, epicardium, diffuse	M		1ml			
	Inflammation, atrial epicardium, focal	F				1ml	
	Hypertrophy, epicardial	F			1mi,1ml, 1md		
Bone marrow, nodule, lymphoid	M				1mi	1mi	
	F				1mi		
Brain, necrosis, arteriole, focal	F					1ml	
Liver	Vacuolation	F		1mi			
	Necrosis, focal	M				1mi	
Skeletal muscle, necrosis, myofiber, multifocal	M		1mi				
Thyroid, degeneration, follicular, focal/multifocal	M			2mi	1mi		
	F				1mi	1ml	
Ovarian follicular cysts	F			1	1	2	
Histopath after 1 month recovery in control, MD (50 mg/kg) and HD (125 mg/kg)							
Renal tubular Regeneration, focal /multifocal	M				1mi	1ml	
	F				1mi	1mi	
Renal tubular degeneration, multifocal	M					1mi	
Renal tubular, epithelia, atrophy focal/multifocal	M				1mi	1mi	
	F				1mi	1mi	
Renal, cast, cellular multifocal	M	-	-			1mi	
Renal, cast, cellular multifocal	M	-	-			1mi	
Glomerular atrophy focal/multifocal	M	-	-		1ml		
Heart, fibrosis, myocardium, multifocal	M					1Mi	

Liver, necrosis, focal	F	-	-	-		1mi
Thyroid, degeneration, follicular, multifocal						2mi

M=male, F= female, mi=minimal, ml= mild, md=moderate

The study pathologist and peer review pathologist's statement on renal pathology findings in the 12-month lorcaserin study in monkeys

Histologic Findings considered due to APD-356 administration were identified in the kidneys of animals given 2, 10, 50, and 125 mg/kg/day of APD-356 and in the ovaries of females given 10, 50, and 125 mg/kg/day of APD-356. Test-article associated changes in the kidney were dose-proportional in incidence and severity and consisted of tubular degeneration, regeneration, cellular casts, and an increase in protein casts. Minimal to mild multifocal degeneration of the tubular epithelium in the cortex was seen in 0/8, 0/8, 0/8, 0/8, and 6/8 animals given 0, 2, 10, 50, or 125 mg/kg/day of APD-356 respectively. Tubular degeneration affected the epithelium of cortical tubules and consisted of scattered tubules with attenuated epithelium, sloughing of damaged cells, single cell necrosis, inflammatory cell infiltration, and increased mitotic figures. Minimal to moderate, local or multifocal tubular regeneration was identified in 0/8, 1/8, 2/8, 3/8, and 7/8 animals given 0, 2, 10, 50, or 125 mg/kg/day of APD-356, respectively. Tubular regeneration affected the epithelium of cortical tubules and consisted of scattered tubules lined by more numerous but smaller epithelial cells with large nuclei and basophilic cytoplasm. There was a slight increase in mitotic figures in areas of regenerating tubules of the highest dose group. Some affected tubules were distended with protein casts, while a few others contained detached epithelial cells mixed with a few inflammatory cells (cellular casts). The surrounding interstitium sometimes contained a mononuclear inflammatory cell infiltrate. In all cases, affected tubules were intermixed with many morphologically unremarkable tubules. Minimal, focal or multifocal protein casts were found in four control animals in this study; occasional scattered protein casts are a commonly observed background change in cynomolgus monkeys at this facility. Intratubular protein casts in one animal given 10 mg/kg/day and 3 animals given 125 mg/kg/day APD-356 were graded mild because there were more present in the section that are usually seen, and therefore considered test article related. Cellular casts, not commonly seen as background, occurred in 0/8, 0/8, 0/8, 1/8, and 4/8 animals given 0, 2, 10, 50, or 125 mg/kg/day of APD-356 respectively, and were therefore considered test article related. Tubular degeneration and regeneration, along with protein and cellular casts, indicate damage to renal tubular epithelium. The renal tubular damage did not result in altered serum chemistry parameters, reflecting the large reserve capacity of the kidney and the multifocal minimal to mild nature of the histologic changes. An additional change in the kidney, termed glomerulosclerosis, was observed in 3 animals (17, 45, 49) given 10 or 50 mg/kg/day APD-356 and had an uncertain relationship to test article administration. Glomerulosclerosis consists of fibrotic or scarred glomeruli and minimal glomerulosclerosis (one or very few affected glomeruli) is sometimes seen as an incidental background change in cynomolgus monkeys at this facility. Two animals (49, 17) had a mild degree of glomerulosclerosis that was associated with minimal swelling and hyaline droplet degeneration of some tubular epithelium. The changes in the tubular epithelium probably indicate excess albumin resorption. The increased severity of glomerulosclerosis in these two animals suggests a possible relationship to APD-356 administration, but the change was infrequent and not dose proportional, rendering the relationship uncertain.

Histopathology of notable tissues at necropsy, Day 365

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
Study: AVA00016

Observations: Neo-Plastic and Non Neo-Plastic	MALES					FEMALES				
	0 mg/kg	2 mg/kg	10 mg/kg	50 mg/kg	125 mg/kg	0 mg/kg	2 mg/kg	10 mg/kg	50 mg/kg	125 mg/kg
Removal Reasons: All of those SELECTED	4	4	4	4	4	4	4	4	4	4
Number of Animals on Study :	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Number of Animals Completed:	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(3)	(4)
ADRENAL;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	4	4	4	4	3	4	4	4	3	4
Congestion; diffuse.....	0	0	0	0	1	0	0	0	0	0
Ectopic Tissue.....	0	0	0	0	0	0	0	0	1	0
AORTA;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	4	4	4	3	4	4	4	4	4	4
Fibrosis; intima; focal.....	0	0	0	1	0	0	0	0	0	0
BRAIN;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	3	1	2	3	3	4	2	2	3	1
Infiltrate, Mononuclear Cell; choroid plexus; focal.....	0	1	0	0	0	0	0	0	0	1
Infiltrate, Mononuclear Cell; choroid plexus; multifocal.....	0	0	0	0	0	1	1	0	0	0
Infiltrate, Mononuclear Cell; parenchyma; perivascular; multifocal.....	0	1	0	0	1	0	0	0	0	2
Infiltrate, Mononuclear Cell; meninges; focal.....	1	0	0	0	0	0	0	0	0	1
Infiltrate, Mononuclear Cell; meninges; multifocal.....	0	1	2	0	1	0	1	1	1	0
Necrosis; arteriole; focal.....	0	0	0	0	0	0	0	0	0	1
Mineralization; meninges; focal.....	0	0	0	0	0	0	0	0	0	1
HEART;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	1	0	1	1	2	0	2	1	0	1
Hypertrophy; myofiber; multifocal.....	0	0	0	0	0	0	0	1	0	0
Hypertrophy; epicardial adipose tissue; diffuse.....	0	0	0	0	0	0	1	2	4	0
Infiltrate, Mononuclear Cell; myocardium; diffuse.....	3	3	3	3	2	4	1	2	4	3
Fibrosis; epicardium; focal.....	0	1	0	0	0	0	0	0	0	0
Granuloma; epicardial adipose tissue; multifocal.....	0	0	0	0	0	1	1	1	0	0
Inflammation, Chronic; epicardium; atrium; focal.....	0	0	0	0	0	0	0	0	0	1
Hemorrhage; epicardial adipose tissue; focal.....	0	1	0	0	0	0	0	0	0	0
ILEOCECAL VALVE;										
Examined.....	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(2)	(0)	(0)
Within Normal Limits.....	0	0	0	0	0	0	0	0	0	0
Hemorrhage; focal.....	0	0	0	0	0	0	0	1	0	0
Hemorrhage; multifocal.....	0	0	0	0	0	0	0	1	0	0
KIDNEY;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	0	0	0	0	0	0	1	0	1	0
Scar; cortex; focal.....	0	0	0	0	0	0	0	0	0	1
Mineralization; papilla; multifocal.....	2	1	2	2	1	2	1	1	2	0
Mineralization; tubules; cortex; focal.....	0	0	0	0	0	0	0	0	0	1
Mineralization; tubules; cortex; multifocal.....	0	1	0	0	0	0	0	0	0	0
Infiltrate, Mononuclear Cell; multifocal.....	4	3	4	2	4	3	3	4	1	4
KIDNEY; (continued)										
Regeneration; tubular epithelium; cortex; focal.....	0	0	0	1	0	1	0	0	0	4
Regeneration; tubular epithelium; cortex; multifocal.....	0	0	1	2	2	0	0	1	1	0
Glomerular sclerosis; focal.....	0	0	0	1	0	0	0	0	0	0
Glomerular sclerosis; multifocal.....	0	0	1	0	0	0	0	0	1	0
Congestion.....	0	0	0	0	1	0	0	0	0	0
Cast, Cellular; focal.....	0	0	0	1	0	0	0	0	0	2
Cast, Cellular; multifocal.....	0	0	0	0	0	1	0	0	0	0
Cast, Protein; focal.....	0	1	0	0	1	0	1	0	0	0
Cast, Protein; multifocal.....	0	1	1	1	4	3	2	1	1	4
Vacuolation, Cytoplasm; tubular epithelium; cortex; multifocal.....	0	0	1	0	0	0	1	0	0	0
Atrophy; tubular epithelium; cortex; multifocal.....	0	0	1	0	0	0	0	0	0	0
Degeneration; tubular epithelium; cortex; multifocal.....	0	0	0	0	3	0	0	0	0	3
Degeneration, Hyaline Droplet; tubular epithelium; cortex; multifocal.....	0	0	1	0	0	0	0	0	1	0
Swelling; tubular epithelium; cortex; multifocal.....	0	0	1	0	0	0	0	0	1	0
LIVER;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	3	3	4	2	2	1	1	2	2	1
Infiltrate, Mononuclear Cell; parenchyma; multifocal.....	1	0	0	0	1	0	1	0	1	1
Vacuolation, Cytoplasm; hepatocyte; diffuse.....	0	0	0	0	0	0	0	1	0	0
Necrosis; hepatocyte; focal.....	0	0	0	0	1	0	0	0	0	0
Necrosis; hepatocyte; focal.....	0	0	0	0	1	0	1	0	0	0
Lipidosis; diffuse.....	0	1	0	1	0	0	0	1	1	0
Lipidosis; focal.....	0	0	0	1	0	1	2	1	0	2
Lipidosis; multifocal.....	0	0	0	0	0	0	0	0	0	0
Lipidosis; centrilobular; diffuse.....	0	0	0	0	0	0	0	0	0	1
Lipidosis; centrilobular; multifocal.....	0	0	0	0	0	1	0	0	0	0
Proliferation, Bile Ductule; multifocal.....	0	0	0	0	0	1	0	0	0	0
Extramedullary Hematopoiesis.....	0	0	0	0	0	0	0	1	0	0
LUNG;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	4	2	4	4	2	3	3	4	3	2
Hemorrhage; focal.....	0	0	0	0	1	0	0	0	0	0
THYROID;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	1	3	2	1	2	2	2	3	2	2
Cyst; follicular; focal.....	1	0	0	0	0	0	0	0	0	0
Infiltrate, Mononuclear Cell; focal.....	0	1	0	1	1	0	0	1	0	0
Infiltrate, Mononuclear Cell; multifocal.....	0	0	1	0	0	2	0	0	0	1
Ectopic Tissue, Thymus.....	2	0	0	2	1	0	2	0	1	2
Degeneration; basophilic; follicle; focal.....	0	0	0	1	0	0	0	0	0	0
Degeneration; basophilic; follicle; multifocal.....	0	0	2	0	0	0	0	0	1	1
URINARY BLADDER;										
Examined.....	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits.....	4	4	4	4	3	3	4	4	4	4
Infiltrate, Mononuclear Cell; focal.....	0	0	0	0	0	1	0	0	0	0
Calculus.....	0	0	0	0	1	0	0	0	0	0

Histopathology of notable tissues at the end of recovery (Day 393)

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
Study: AVA00016

Observations: Neo-Plastic and Non Neo-Plastic	----- MALES -----			----- FEMALES -----		
	0	50	125	0	50	125
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Removal Reasons: All of those SELECTED						
	2	2	2	2	2	2
Number of Animals on Study :	(2)	(2)	(1)	(2)	(2)	(2)
Number of Animals Completed:						
HEART:						
Examined.....	(2)	(2)	(2)	(2)	(2)	(2)
within Normal Limits.....	1	1	0	0	2	0
Infiltrate, Mononuclear Cell; multifocal	1	1	1	2	0	2
Fibrosis; myocardium; multifocal	0	0	1	0	0	0
KIDNEY:						
Examined.....	(2)	(2)	(2)	(2)	(2)	(2)
within Normal Limits.....	0	0	0	1	1	0
Mineralization; papilla; multifocal	0	0	2	0	0	1
Mineralization; tubules; cortex; focal	1	0	0	0	0	0
Infiltrate, Mononuclear Cell; multifocal	1	1	1	0	1	2
Regeneration; tubular epithelium; cortex; focal	0	1	0	0	0	0
Regeneration; tubular epithelium; cortex; multifocal	0	0	1	0	1	1
Cast, Cellular; multifocal	0	0	1	0	0	0
Cast, Protein; multifocal	0	2	1	1	1	2
Atrophy; glomerular tuft; multifocal	0	1	0	0	0	0
Atrophy; tubular epithelium; cortex; focal	0	0	0	0	1	0
Atrophy; tubular epithelium; cortex; multifocal	0	1	1	0	0	1
Degeneration; tubular epithelium; cortex; multifocal	0	0	1	0	0	0
LIVER:						
Examined.....	(2)	(2)	(2)	(2)	(2)	(2)
within Normal Limits.....	2	2	2	2	2	1
Necrosis; hepatocyte; focal	0	0	0	0	0	1
THYMUS:						
Examined.....	(2)	(2)	(2)	(2)	(2)	(2)
within Normal Limits.....	0	1	0	1	2	0
Involution	2	1	1	1	0	1
Cyst	2	1	2	1	0	1
Hemorrhage; focal	0	0	1	0	0	0
Hemorrhage; multifocal	0	1	0	0	0	0
THYROID:						
Examined.....	(2)	(2)	(2)	(2)	(2)	(2)
within Normal Limits.....	1	1	1	0	2	0
Cyst; follicular	0	1	1	0	0	0
Infiltrate, Mononuclear Cell; focal	0	0	0	0	0	1
Infiltrate, Mononuclear Cell; multifocal	0	0	0	1	0	0
Ectopic Tissue, Thymus	1	0	0	1	0	1
Degeneration; basophilic; follicle; multifocal	0	0	0	0	0	2

Toxicokinetics:

- Monkeys were treated with 2, 10, 50, and 125 mg/kg of lorcaserin for 1 year and evaluated at several time intervals (Day 1, 22, 91 for anhydrous and Day 92 and 358 for hemihydrate lorcaserin)
- There was no notable gender differences in lorcaserin parameters in monkeys
- Lorcaserin plasma exposure increased in a linear manner in monkeys.
- There was no significant difference in lorcaserin when the anhydrous formulation on Day 91 (Day 1-Day 91) was switched to hemihydrate formulation on Day 92 (Day 92 –Day 358)
- Repeated administration of lorcaserin increased parent exposure by 1 to 1.8 fold relative to Day 1 at 2 and 50 mg/kg. The exposure at 125 mg/kg was 10 to 40% lower on Day 358 relative to Day 1
- Lorcaserin was metabolized to APD244208 (M1) and APD306388 in monkeys.
- The AUC exposure to APD244208 was 20 to 60 fold greater than the parent drug
- The AUC exposure to APD306388 was 1 to 3 fold greater than the parent drug

APD356 Toxicokinetic Summary after Oral Administration of APD356 to Male Monkeys on Day 1, 22, 91, 92, and 358.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)
Male				
1	2	0.184	2.1	0.961
	10	0.723	3.5	6.735
	50	2.082	4.8	29.391
	125	5.068	8.2	80.698
22	2	0.115	1.3	0.944
	10	0.835	3.5	7.841
	50	2.813	3.0	33.615
	125	4.250	5.0	60.213
91	2	0.189	1.8	1.382
	10	0.953	3.8	7.922
	50	3.563	4.3	38.052
	125	5.880	3.3	68.384
92	2	0.135	1.5	1.212
	10	0.971	2.5	7.962
	50	3.270	4.0	39.047
	125	4.648	4.5	59.579
358	2	0.141	2.5	1.006
	10	0.791	3.8	7.930
	50	3.327	5.3	43.603
	125	3.943	4.5	50.916

APD356 Toxicokinetic Summary after Oral Administration of APD356 to Female Monkeys on Day 1, 22, 91, 92, and 358.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)
Female				
1	2	0.116	2.0	0.547
	10	0.767	1.1	4.472
	50	1.644	5.4	22.954
	125	4.228	10.9	58.501
22	2	0.209	1.0	0.688
	10	0.817	1.0	4.610
	50	2.780	3.2	29.904
	125	5.257	4.8	68.674
91	2	0.446	1.3	0.990
	10	0.720	1.5	4.653
	50	2.622	3.8	29.319
	125	6.067	7.0	88.195
92	2	0.142	2.3	0.666
	10	0.557	2.0	4.020
	50	2.808	4.0	35.537
	125	6.220	9.3	95.053
358	2	0.136	1.5	0.598
	10	0.615	2.3	4.500
	50	2.850	4.2	31.390
	125	5.370	3.0	51.091

AR244208 Toxicokinetic Summary after Oral Administration of APD356 to Male Monkeys on Days 1, 22, 91, 92 and 358.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)
Male				
1	2	2.623	6.0	38.994
	10	17.600	4.0	235.264
	50	49.783	6.3	750.524
	125	82.617	8.7	1433.588
22	2	3.443	4.0	47.395
	10	23.600	3.5	279.566
	50	61.267	4.3	824.690
	125	104.600	4.7	1619.193
91	2	4.845	2.8	52.643
	10	26.575	4.0	270.423
	50	69.067	4.3	895.393
	125	113.917	4.3	1606.620
92	2	3.430	3.0	44.760
	10	23.775	3.5	261.450
	50	58.783	5.3	842.719
	125	100.517	3.8	1305.613
358	2	5.013	3.0	56.415
	10	21.950	3.3	275.946
	50	62.283	4.7	903.901
	125	105.550	3.7	1461.326

AR244208 Toxicokinetic Summary after Oral Administration of APD356 to Female Monkeys on Days 1, 22, 91, 92 and 358.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)
Female				
1	2	2.755	3.0	32.896
	10	22.000	2.8	226.242
	50	39.950	6.3	613.806
	125	76.817	12.0	1261.963
22	2	3.998	2.3	37.333
	10	28.275	1.8	273.938
	50	61.367	3.7	831.831
	125	99.583	5.3	1587.358
91	2	3.955	2.3	39.432
	10	28.200	2.0	262.772
	50	53.583	4.0	721.639
	125	108.667	6.3	1767.742
92	2	3.515	3.5	37.424
	10	20.625	3.3	228.553
	50	53.067	5.0	765.067
	125	101.917	6.3	1721.917
358	2	4.575	1.5	39.321
	10	27.525	2.5	269.227
	50	56.317	5.2	771.220
	125	114.533	3.5	1581.971

AR306388 Toxicokinetic Summary after Oral Administration of APD356 to Female Monkeys on Days 1, 22, 91, 92 and 358.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)
Female				
1	2	0.271	1.1	0.871
	10	1.497	0.6	4.239
	50	2.212	4.5	24.111
	125	4.832	4.8	59.498
22	2	0.440	1.0	1.222
	10	1.485	1.0	4.977
	50	4.192	2.8	38.564
	125	10.137	3.7	123.887
91	2	0.335	1.3	0.935
	10	1.293	1.3	5.242
	50	3.012	2.8	29.495
	125	13.037	6.0	150.882
92	2	0.364	1.3	1.261
	10	0.969	1.3	4.987
	50	3.710	3.5	39.676
	125	11.922	6.7	162.625
358	2	0.771	0.5	1.756
	10	1.670	1.0	6.137
	50	3.598	3.2	38.659
	125	19.698	2.7	134.243

AR306388 Toxicokinetic Summary after Oral Administration of APD356 to Male Monkeys on Days 1, 22, 91, 92 and 358.

Day	Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)
Male				
1	2	0.183	1.5	0.904
	10	1.287	0.8	7.980
	50	3.550	4.3	41.258
	125	10.958	4.9	101.765
22	2	0.367	1.0	1.423
	10	2.067	1.3	9.275
	50	6.005	2.7	62.778
	125	13.450	2.3	157.489
91	2	0.533	1.0	1.551
	10	1.584	1.3	9.834
	50	6.682	3.3	70.394
	125	15.767	3.3	154.431
92	2	0.297	1.3	1.362
	10	1.670	2.3	9.896
	50	5.923	2.8	62.201
	125	14.225	2.5	126.048
358	2	0.635	0.8	1.816
	10	2.483	1.8	12.472
	50	7.040	2.3	68.988
	125	15.833	2.2	169.732

Safety Margins for lorcaserin in monkeys:

The NOAEL dose of 2 mg/kg was based on notable renal regeneration at ≥ 10 mg/kg. Due to notable renal tubular regeneration at ≥ 10 , Based on notable renal tubular regeneration, a

Species	Dose, mg/kg	AUC _{0-t} µg.h/ml	Animal to human dose exposure ratio based on AUC
52-Week Monkey Study with a 4-Week Recovery	2 (NOAEL)	M:1, F:0.6	~ 0.8
	10	M:7.9, F: 4.5	6
	50	M:43.6, F:31.1	37
	125	M:50.9, F:51.1	50
MRHD: 10 mg BID		1.02	

Safety Margins for M1 metabolite (APD244208, sulfamate metabolite) in monkeys:

Species	Dose, mg/kg	AUC _{0-t} µg.h/ml	Animal to human dose exposure ratio based on AUC
52-Week Monkey Study with a 4-Week Recovery	2 (NOAEL)	47.9	~ 9
	10	272.4	50
	50	837.4	154
	125	1521.6	281
MRHD: 10 mg BID		5.422	

Stability and Homogeneity

The stability studies showed lorcaserin solutions were stable for 3 months at room temperature or stressed temp (40°C). Solution concentrations were within 91.1% to 104.5% of the target solution concentrations.

7 Genetic Toxicology

Mutagenicity of lorcaserin was tested using Ames assay. Salmonella strains (TA98, TA100, TA 1535, TA1537) and E.coli (WP2 uvrA) at lorcaserin concentrations ranging from 25 to 5000 µg/plate were tested with and without S9 mix. Lorcaserin was not mutagenic in Ames test in presence or absence of S9 mix.

In the preliminary human lymphocyte chromosomal aberration test, samples were incubated for 3 hr (with and without) and 20 hr (without S9). In the confirmatory test, the 3 hr incubation was conducted with and without S9 and the 20 hr incubation without S9. Lorcaserin concentration used in the confirmatory assay did not include lorcaserin doses that increased mitotic index up to 50% (i.e. 250 and 275 µg/ml). Overall, lorcaserin did not cause chromosomal aberration in CHO cells at non cytotoxic concentrations up to 37.5 µg/ml without S9 mix and 225 µg/ml with S9mix. A statistically significant increase in endoreduplication was observed at some doses. The significance of the endoreduplication is not clear. The increase is thought to be related to cytotoxicity since it occurred at doses that caused severe reduction in dividing cells. Of note, the sulfated M1 metabolite would not be generated by incubation with S9, so the *in vitro* genotoxicity of M1 was not assessed. However, M1 is generated by rats *in vivo*, so the *in vivo* genotoxicity assessment would adequately address the M1 metabolite.

The clastogenicity of lorcaserin was evaluated in the rat bone-marrow erythrocyte micronucleus assay (62.5, 125 and 250 mg/kg, oral gavage). Lorcaserin doses greater than 250 mg/kg has been shown to be toxic in rats. No evidence of bone-marrow toxicity was observed. There were no toxicologically significant increases in MNPCE in any drug-treated animals, and exposure to the parent and the sulfated M1 metabolite was adequate. LORCASERIN was non-genotoxic in the oral mouse bone-marrow micronucleus test when tested up to the maximum dose level (250 mg/kg).

8 Carcinogenicity

Study title: A 2 year carcinogenicity study of APD356 given by oral a gavage to mice

Study no.: TX0507 ((b) (4) study number 900-062)
 Study report location: Arena Pharmaceuticals, Inc.
 Conducting laboratory and location: (b) (4)
 Date of study initiation: June 29, 2006 (completed on May 8, 09)
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: #06P0031 (97.5%), #06P0044 (96.3%)
 CAC concurrence: Yes

Key Study Findings

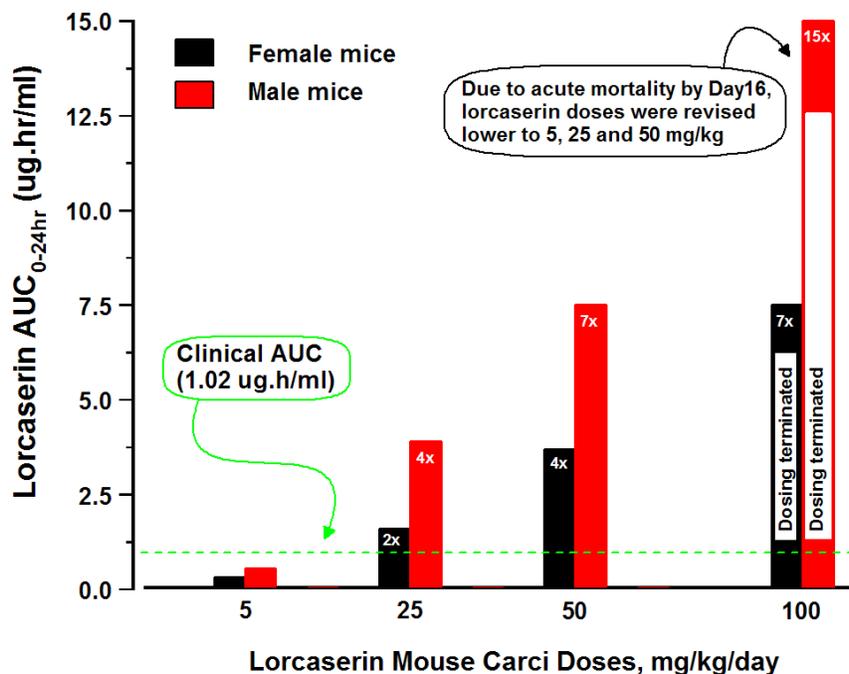
Mouse (65/sex/group) study was initiated with 25, 50 and 100 mg/kg of lorcaserin, however due to excessive and unexpected increase in incidence of mortality during the first 16 days of the study (22 total), lorcaserin doses were revised lower to 5, 25 and 50 mg/kg (5 ml/kg) on Day 19 (concurred with eCAC) and additional 10 mice /sex were added to the control and high dose group (75/sex) by the sponsor. Most of the deaths occurred in the first 2 days at 100 mg/kg (8F, 2M, replenished). The cause of death was not determined. Lower doses of lorcaserin were introduced on Day 19 until the end of the 2-year study.

Lorcaserin Dose	Sex	Controls	5 mg/kg	25 mg/kg	50 mg/kg
Survival rate	M	41%	37%	28%	37%
	F	35%	32%	38%	33%

There was no treatment-related effect on survival rate after revision to lower doses of lorcaserin. All causes of death/moribundity during the course of the 2-year study were of the type typically seen in this type of study in mice. No clear or definitive treatment-related effects were noted on clinical findings (including masses), food consumption, or on macroscopic and microscopic evaluations during the study. Although possible slight treatment-related decreases in mean body weight were noted in males, primarily at 25 and 50 mg/kg, when compared to controls, there were no food consumption correlates and no similar effects in female animals, which showed slight increases at 50 mg/kg, making a relationship to treatment unclear.

The only safety concern in the study was the unexpected number of deaths after the initial dose of 100 mg/kg (7 to 15x the clinical dose) which remains unresolved. Prior studies had shown minimal incidence of mortality in the first few days with lorcaserin (up to 250 mg/kg in the 13-week study) and nearly no deaths at doses up to 350 mg/kg in the 2-week study. Whether deaths were due to sensitivity of some mice to the sudden high lorcaserin exposure is not clear. It is highly possible that the remaining mice in the study could have tolerated the 100 mg/kg since 50 mg/kg did not significant impact the survival rate. The role of convulsion can not be ruled out due to significant lorcaserin accumulation in the brain (25x greater than plasma).

In conclusion, once daily oral administration of APD356 to mice for 2 years at new reduced dose levels of 5, 25, and 50 mg/kg were well tolerated and APD356 did not produce any evidence of a carcinogenic effect in mice. Since the survival was the same among control and revised low doses up to 50 mg/kg, the only potential concern remains to be the steep increase in death at the initial HD dose of 100 mg/kg.



Adequacy of Carcinogenicity Study: Yes

Appropriateness of Test Models: Yes

Evaluation of Tumor Findings:

There were no significant drug-related tumors in mice. The incidence of mammary adenocarcinoma were similar among groups in female mice (2/75, 1/65, 1/65 and 4/75 in control, 5, 25 and 50 mg/kg, respectively). With exposure at top dose of 50 mg/kg in female and male mice being 4 and 7x the clinical dose of 10 mg BID on AUC basis, lorcaserin exposure appeared to be low in mice thus absence of pharmacodynamic effect and neoplasia is not unexpected.

Methods

The study was initiated with 25, 50 and 100 mg/kg of lorcaserin but had to be revised lower due to high incidence of mortality (unknown cause). The revised doses of lorcaserin to 5, 25 and 50 mg/kg were administered to mice for 2-year. The unexpected sharp rise in mortality at the initial dose of 100 mg/kg (7.5x-15x the clinical dose) suggests a steep dose response with respect to death in mice

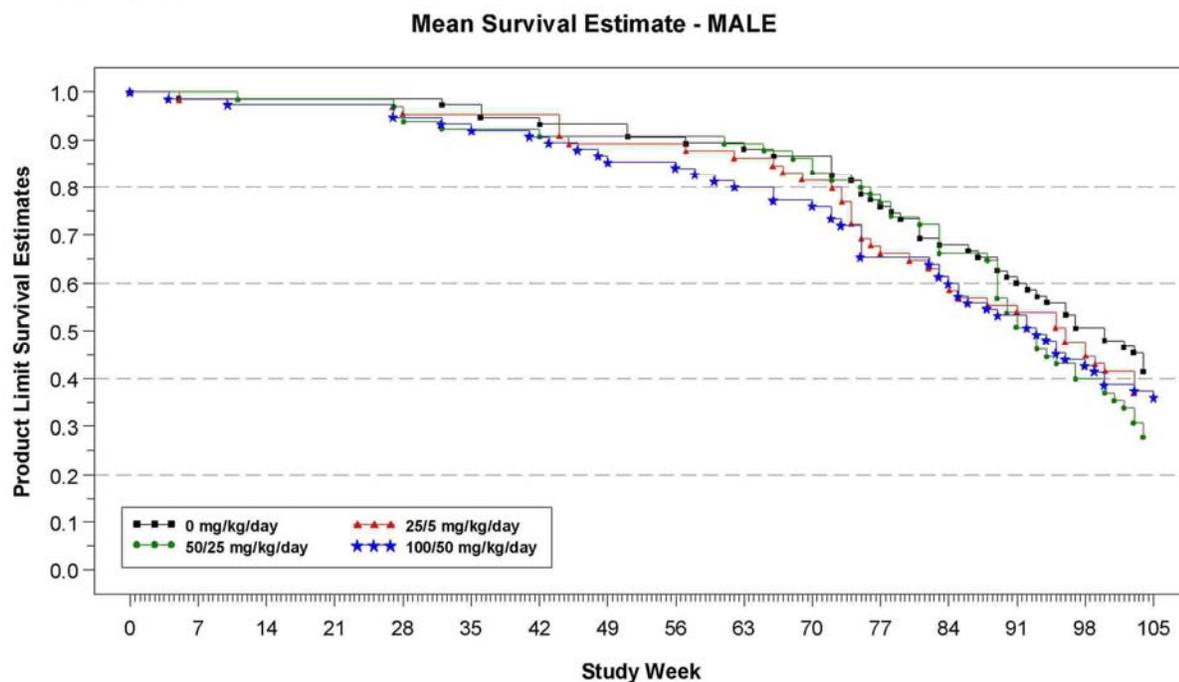
Doses: 5, 25 and 50 mg/kg
 Frequency of dosing: Daily
 Dose volume: 5 ml/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: water
 Basis of dose selection: MTD (mortality at higher doses \geq 250 mg/kg)
 Species/Strain: Crl:CD1® (Icr) mice
 Number/Sex/Group: 75/sex/control and HD and 65/sex/LD and MD
 Age: 6 weeks of age
 Animal housing: Housed individually in stainless steel wire mesh cages
 Paradigm for dietary restriction: Add lib water and food
 Dual control employed: One control group
 Interim sacrifice: No
 Satellite groups: Yes (27/sex/treatment group and 6/sex/control)
 Deviation from study protocol: Lower dose adjustment due to mortality at 100 mg/kg
 Diet: Certified Rodent Chow #5002, Nutrition Internat. Inc.

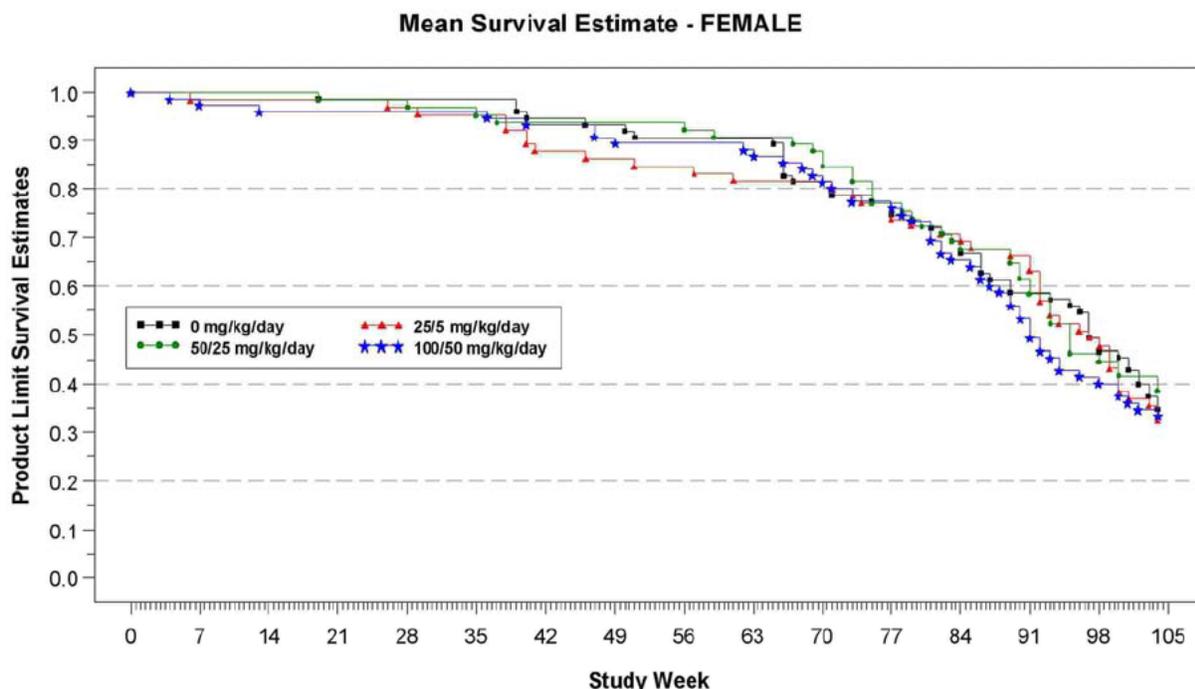
Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
<u>Main Study</u>			
1	0	75	75
2	25 ^a /5 ^b	65	65
3	50 ^a /25 ^b	65	65
4	100 ^a /50 ^b	65	65
4	50	10	10
<u>Toxicokinetics^c</u>			
5	0	6	6
6	25 ^a /5 ^b	27	27
7	50 ^a /25 ^b	27	27
8	100 ^a /50 ^b	27	27
<u>Sentinels</u>			
9	NA	15	15
^a Days 1 to 16 ^b Day 19 to termination ^c Three extra animals/sex/group NA - Not applicable			

Observations and Results

Mortality

- Due to significant drug-related mortality within the first 2-weeks of study at 100 mg/kg (8F, 2M within 2 days to total of 21 deaths in 16 days) lorcaserin doses were reduced to 5, 25 and 50 mg/kg on Day 19. Additional 10 mice/sex were added to the control and new HD groups (75/sex for control and HD). It should be noted that mice had tolerated as much as 250 mg/kg with few deaths for as long as 13-weeks and up to 350 mg/kg with no deaths in a 2-week study.
- The cause of death early in the study that lead to dose reduction was not determined. Whether convulsion played a role is not clear but since only one incidence of convulsion was noted in a male at 100 mg/kg on Day1, convulsion may not have been the culprit but clearly highly sensitive mice were more responsive. It is possible that surviving mice at 100 mg/kg could have tolerated this dose for the remainder of the study since there were no findings at the reduced dose of 50 mg/kg.
- The cause of death after dose reduction was also generally unknown in most of the mice that died before necropsy (11/75, 11/65, 11/65 and 22/75 males and 14/75, 13/65, 15/65 and 16/75 females). Interestingly, deaths of 3 female mice at 50 mg/kg (HD) were attributed to mammary tumor (D 603, D603, D624). Although mammary tumors (adenocarcinoma) were identified in 2 controls (D457 and D678), 1 LD (D680) and 1 MD (D661) and 1 HD female (D661) female, their deaths were not attributed to mammary tumors. Some of these animals had lung adenocarcinoma (secondary) to other tumors including mammary tumor (1 C and 3 HD) suggesting that secondary lung tumors were not limited to mammary adenocarcinoma alone.
- The incidence of mortality and survival rate (%) was similar among groups at the end of the 2-year study.
- Serological analysis was negative suggesting that infections had no role in the study outcome.





(b) (4)
Study Number 900-062
A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Probable Cause of Death - MALE				
Cause of Death	0 mg/kg/day	25/5 mg/kg/day	50/25 mg/kg/day	100/50 mg/kg/day
Number of Animals	75	65	65	75
Summary of Animal Disposition				
died during blood collection	0	1	0	0
died prior to euthanasia	1	0	1	1
euthanized <i>in extremis</i>	7	5	12	5
found dead	36	35	34	42
terminal necropsy	31	24	18	27
Cause of Death				
accidental injury	1	0	0	0
amyloidosis	3	2	5	3
brain tumor	1	1	1	0
chronic progressive nephropathy/uremia	1	1	0	0
dosing error	3	2	1	0
fibrosarcoma/fibroma	0	0	2	0
fibrous histiocytoma	2	1	2	0
heart failure/atrial thrombus	4	1	1	2
hemangiosarcoma/hemangioma	0	2	1	1
hemorrhage	0	0	1	0
inflammation/septicemia	2	4	1	4
leukemia	1	0	0	0
liver inflammation/necrosis	1	0	0	0
liver tumor	0	1	2	2
lung inflammation/necrosis	0	0	1	0
lung tumor	3	1	2	1
lymphoid tumor	4	2	3	2
mesothelioma	0	0	1	0
polyarteritis	1	1	0	1
probable dosing error	0	0	1	1
skin inflammation/necrosis	4	4	3	2
skin tumor	0	0	0	1
testes carcinoma, interstitial cell	0	0	1	0
undetermined	11	11	15	22
urogenital inflammation/obstruction/calculi	2	7	3	6

(b) (4)
 Study Number 900-062
 A 2 Year Carcinogenicity Study of APD366 Given by Oral Gavage to Mice

Summary of Probable Cause of Death - FEMALE				
Cause of Death	0 mg/kg/day	25/5 mg/kg/day	50/25 mg/kg/day	100/50 mg/kg/day
Number of Animals	75	65	65	75
Summary of Animal Disposition				
died prior to euthanasia	0	0	1	1
euthanized <i>in extremis</i>	14	8	5	4
found dead	35	35	34	45
missing from cage - presumed dead	0	1	0	0
terminal necropsy	26	21	25	25
Cause of Death				
accidental injury	0	1	0	0
adrenal gland tumor	1	0	0	0
amyloidosis	4	3	3	3
chronic progressive nephropathy/uremia	7	5	3	4
dosing error	4	1	2	3
encephalitis	0	1	0	0
fibrosarcoma/fibroma	0	0	0	1
fibrous histiocytoma	0	0	0	1
gastrointestinal tumor	1	0	0	0
heart endocarditis, valvular vegetative	1	2	0	1
heart failure/atrial thrombus	1	1	2	1
histiocytic sarcoma	4	2	2	2
large intestine, rectum prolapse	0	0	0	1
leukemia	0	0	1	0
liver tumor	0	0	1	0
lung tumor	0	3	0	1
lymphoid tumor	4	6	6	8
mammary tumor	0	0	0	3
ovarian cyst/hemorrhage	1	1	2	0
ovaries abscess	1	0	1	1
ovary tumor	1	1	0	1
schwannoma	0	0	0	1
skin inflammation/necrosis	3	0	0	1
skin, subcutis abscess	0	1	0	0
spinal cord tumor	1	0	0	0
undetermined	14	13	15	16
urogenital inflammation/obstruction/calculi	0	1	0	0
uterus inflammation/necrosis	0	0	1	1
uterus tumor	1	1	1	0

The number of dead mice at the end of 52 weeks in the TK satellite groups:

Lorcaserin Dose	Sex	Controls (H ₂ O)	5 mg/kg	25 mg/kg	50 mg/kg
Number of Dead mice in TK groups	M	0/6	6/27	10/27	11/27
	F	3/6	4/27	5/27	6/27

Clinical Signs:

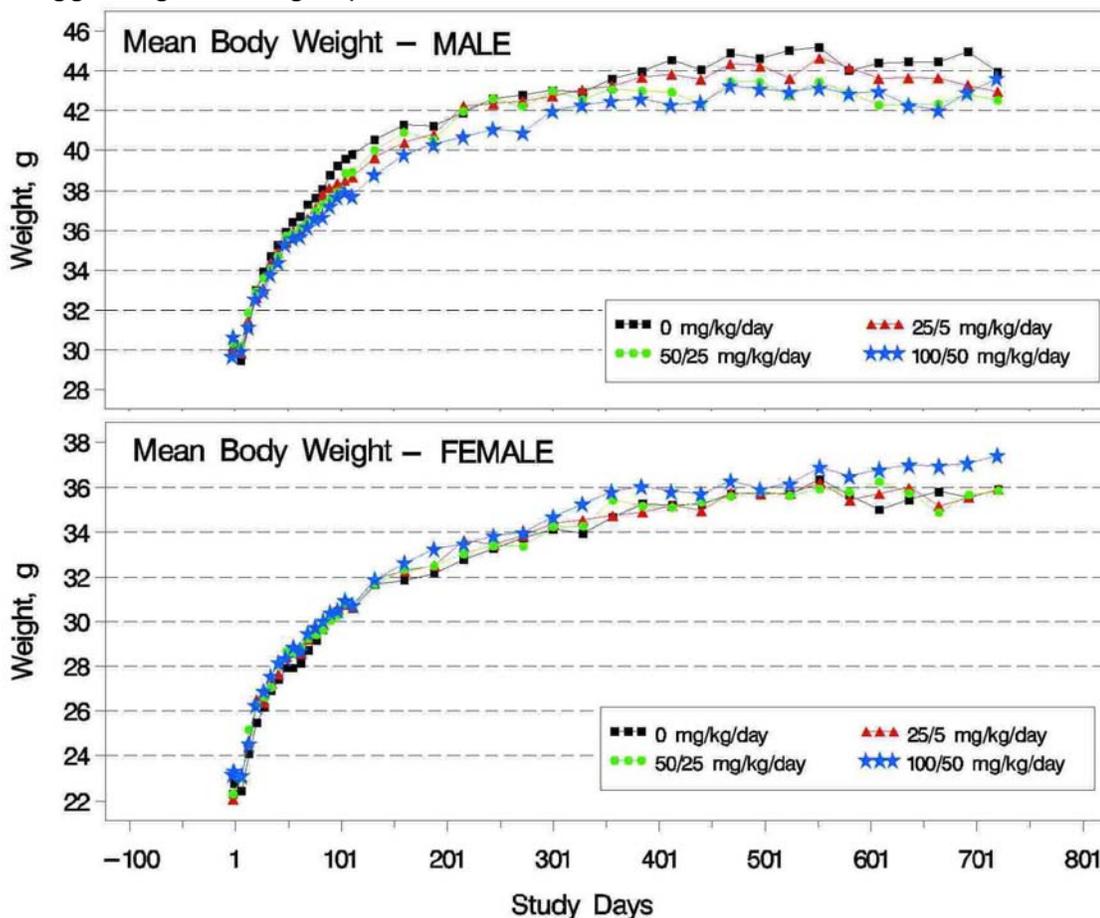
- Except for minor clinical signs noted below, there were no notable clinical signs of toxicity to suggest what was causing the deaths during the first two weeks. Clinical signs post week 2 were also minor.
- During the first week of when the full dose of lorcaserin (25, 50 and 100 mg/kg) was administered, clinical signs were minimal in both male (1 M at 100 mg/kg with an episode of clonic convulsion) and female mice (no convulsion). Although there were notable deaths in the HD mice, there was no notable clinical sign to

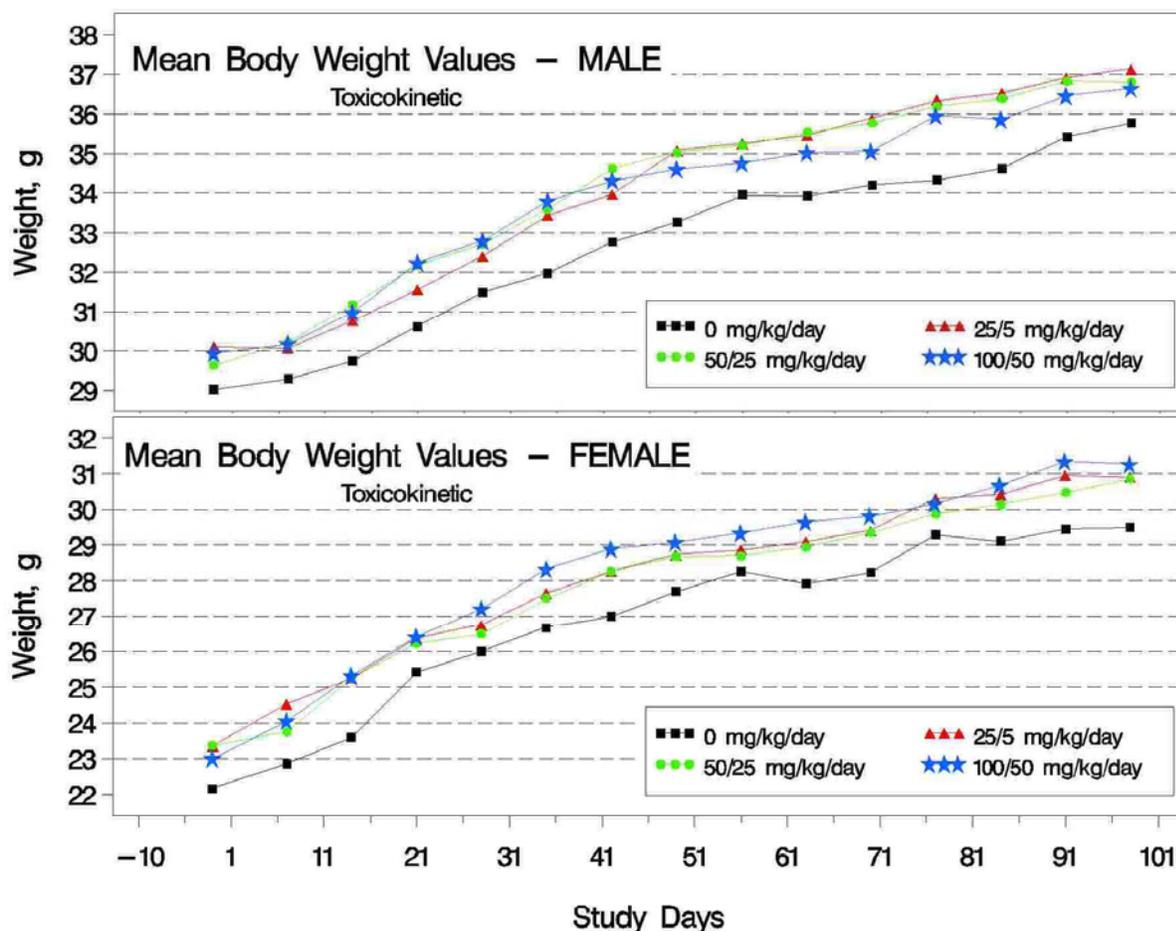
suggest that lorcaserin was not tolerable due to convulsion, significant decreased activity.

- Following dose reduction to (5, 25 and 50 mg/kg), the clinical signs from week 2 to 105 appeared to be similar among groups in both males and female mice in with regards to decreased activity, tremor and external appearance. The slightly higher incidence of decrease activity in control females was not considered significant (18/75 mice in control vs. 10/75 mice in HD females).
- Two episodes of convulsion were noted in 1 female at 5 mg/kg during week 2 to week 105.

Body Weights

- There was no significant overall drug effect on BW in mice. The BW in MD (25 mg/kg) and HD (50 mg/kg) males tended to be lower while in the HD females the BW tended to be higher than concurrent controls.
- In the TK mice, the BW in the HD males and females appeared to be higher than controls but since the starting weight in HD mice were slightly higher, there was no effect on BW in TK animals.
- The absence of drug effect on BW in the 2-year mouse study is reasonable since the exposure to lorcaserin doses of 5, 25 and 50 mg/kg were 0.1x, 3x and 7x the clinical dose in males and 0.1x, 2x and 4x the clinical dose in females suggesting that drug exposure was insufficient.



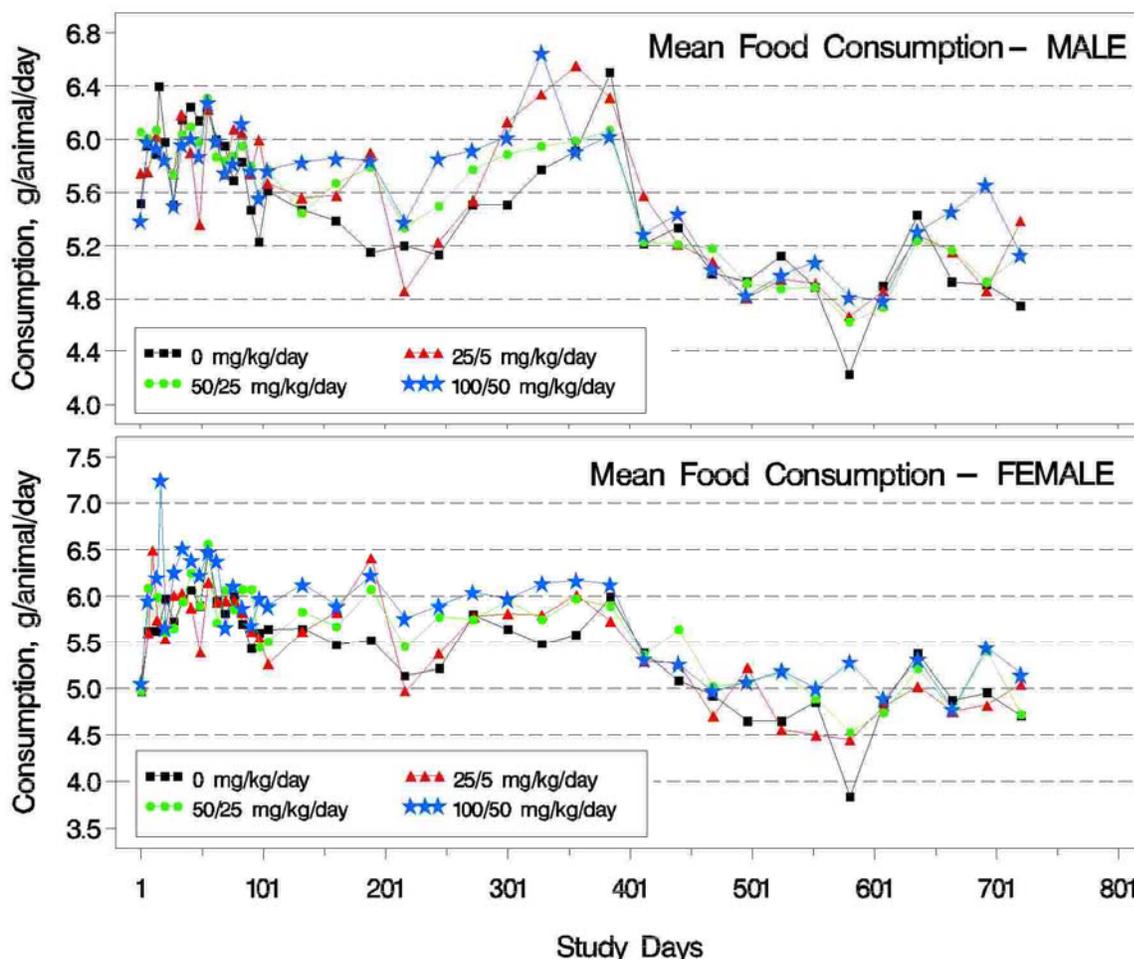


Percent Differences in Mean Body Weight (g)								
Dose Level mg/kg/day	Male				Female			
	0*	5	25	50	0*	5	25	50
Day 7	29.48	↑1.3	↑2.4	↑1.6	22.44	↑2.9	↑2.7	↑3.1 ^a
Day 21	33.01	↓1.1	↓0.2	↓1.2	25.47	↑4.2 ^a	↑3.1	↑3.1
Day 91	38.79	↓1.6	↓3.0	↓4.0 ^b	30.31	↑0.1	↓0.9	↑0.4
Day 189	41.23	↓1.0	↓1.7	↓2.2	32.18	↑1.1	↑1.0	↑3.4
Day 273	42.78	↓0.7	↓1.4	↓4.3 ^a	33.75	↑0.4	↓1.1	↑0.7
Day 385	43.95	↓0.6	↓2.2	↓3.0	35.27	↓1.0	↓0.3	↑2.2
Day 553	45.18	↓1.2	↓3.8	↓4.5	36.39	↓0.5	↓1.2	↑1.4
Day 721	43.92	↓2.2	↓3.1	↓0.6	35.91	↑0.1	↓0.1	↑4.2

* Control value is expressed in grams.
^aSignificantly different from control; (p<0.05)
^bSignificantly different from control; (p<0.01)

Feed Consumption:

- There was no consistent increase in food intake, however, at several time points food intake in lorcaserin treated male and female mice were higher than control. The occasional increase in food intake may explain the increase in BW in female mice.
- The absence of decrease BW and food intake and low exposure multiple in male and female mice suggests that lorcaserin doses were not high enough to produce the desired pharmacological effect of appetite suppression in mice.
- Serological health screen evaluations were negative thus there was no evidence that any external pathogen had played any role.



Gross Pathology

- There were no notable lorcaserin related gross findings in either sex.
- Tissues with notable gross findings in control and lorcaserin treated mice are shown in table below for reference purpose.

(b) (4) Study Number 900-062
 A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Macroscopic Observations - MALE

Tissue Observation	Severity	Terminal								
		0		25/5		50/25		100/50		
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	
Number of Animals Examined		44	31	41	24	47	18	48	27	
kidneys										
abscess	- moderate	0	0	1	0	0	0	0	0	
cyst		3	9	3	9	3	5	3	7	
	- minimal	0	1	0	0	0	0	0	1	
	- mild	2	5	2	9	3	4	2	4	
	- moderate	1	1	1	0	0	1	1	2	
	- severe	0	2	0	0	0	0	0	0	
dilatation, pelvic		4	3	3	1	1	0	3	0	
	- mild	3	2	1	1	0	0	0	0	
	- moderate	1	0	1	0	1	0	3	0	
	- severe	0	1	1	0	0	0	0	0	
discoloration, green	- moderate	0	0	0	0	1	0	0	0	
discoloration, tan	- moderate	0	0	0	0	1	0	0	0	
enlarged		0	1	2	0	2	0	3	0	
	- mild	0	1	1	0	1	0	1	0	
	- moderate	0	0	1	0	0	0	2	0	
	- severe	0	0	0	0	1	0	0	0	
fluid, red	- moderate	0	0	1	0	0	0	0	0	
focus/foci, black	- mild	1	1	0	0	0	0	0	0	
focus/foci, tan	- mild	0	0	0	0	1	0	0	0	
irregular surface		3	1	2	1	2	0	1	0	
	- mild	2	1	1	0	2	0	0	0	
	- moderate	1	0	1	1	0	0	1	0	
not identified	- no grade	0	0	1	0	0	0	0	0	

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 A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Macroscopic Observations - MALE

Tissue Observation	Severity	Terminal								
		0		25/5		50/25		100/50		
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC	
Number of Animals Examined		44	31	41	24	47	18	48	27	
large intestine, cecum										
distended with gas		0	0	0	0	0	0	2	0	
	- mild	0	0	0	0	0	0	1	0	
	- moderate	0	0	0	0	0	0	1	0	
liver										
cyst	- severe	0	0	0	0	0	1	0	0	
discoloration, black	- moderate	0	0	1	0	0	0	0	0	
discoloration, tan		0	0	1	1	0	0	0	1	
	- mild	0	0	1	0	0	0	0	0	
	- moderate	0	0	0	1	0	0	0	1	
enlarged		3	2	2	0	1	0	1	1	
	- mild	1	2	0	0	1	0	0	0	
	- moderate	2	0	2	0	0	0	1	1	
focus/foci, tan	- mild	0	0	0	0	0	0	1	0	
focus/foci, yellow	- moderate	0	0	0	1	0	0	0	0	
irregular surface		0	0	1	0	0	0	0	1	
	- minimal	0	0	1	0	0	0	0	0	
	- moderate	0	0	0	0	0	0	0	1	
mass	- present	3	2	4	8	4	3	3	1	
nodule	- present	2	0	0	2	0	0	0	1	
lung										
cyst	- mild	0	0	0	0	1	0	0	0	
discoloration, red	- mild	0	0	2	0	0	0	0	0	
discoloration, tan		0	1	0	1	0	0	0	0	
	- mild	0	1	0	0	0	0	0	0	
	- moderate	0	0	0	1	0	0	0	0	
discoloration, white	- moderate	0	0	0	1	0	0	0	0	
focus/foci, red	- mild	1	0	0	0	1	0	0	0	
focus/foci, white	- moderate	0	0	1	0	0	0	0	0	
mass	- present	4	3	0	2	3	1	1	0	
nodule		3	2	2	7	4	2	1	8	
	- present	3	2	2	7	4	2	1	7	
	- mild	0	0	0	0	0	0	0	1	

DOS - Died or euthanized on study
 SNC - Scheduled necropsy

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Macroscopic Observations - MALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		44	31	41	24	47	18	48	27
lymph node, cervical									
enlarged		0	0	0	0	0	0	2	0
	- mild	0	0	0	0	0	0	1	0
	- severe	0	0	0	0	0	0	1	0
not identified	- no grade	0	0	0	0	0	0	1	0
lymph node, hepatic									
discoloration, red	- mild	0	0	0	0	0	0	1	0
not identified	- no grade	2	0	2	7	4	3	2	1
penis									
enlarged	- severe	0	0	0	0	0	0	0	1
extended		3	0	4	0	4	1	4	2
	- no grade	3	0	3	0	3	1	4	2
	- mild	0	0	1	0	1	0	0	0
seminal vesicles									
enlarged		11	17	10	15	16	13	15	18
	- mild	4	2	1	3	5	2	5	0
	- moderate	5	9	4	8	8	7	7	11
	- severe	2	6	5	4	3	4	3	7
mass	- present	0	0	0	0	1	0	0	0
small	- moderate	1	0	0	0	0	0	0	0
urinary bladder									
calculus/calculi	- moderate	1	0	0	0	0	1	0	0
discoloration, red	- mild	1	0	0	0	0	0	0	0
discoloration, tan	- mild	0	0	0	1	0	0	0	0
distended with urine		8	2	7	3	7	3	15	4
	- mild	1	1	2	0	1	2	1	1
	- moderate	5	1	2	3	3	0	8	3
	- severe	2	0	3	0	3	1	6	0
swollen/thickened	- mild	0	0	0	0	2	0	0	0

(b) (4) Study Number 900-062

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Macroscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
cavity, abdominal									
adhesion		0	0	1	0	0	0	4	0
	- mild	0	0	1	0	0	0	1	0
	- moderate	0	0	0	0	0	0	2	0
	- severe	0	0	0	0	0	0	1	0
distended with fluid	- moderate	1	0	0	0	0	0	0	0
fluid, clear		0	0	0	0	2	0	0	0
	- mild	0	0	0	0	1	0	0	0
	- moderate	0	0	0	0	1	0	0	0
fluid, red		4	0	2	0	1	0	2	1
	- mild	2	0	1	0	1	0	2	1
	- moderate	2	0	1	0	0	0	0	0
foreign material	- mild	0	0	0	0	0	0	1	0
kidneys									
cyst		0	2	1	0	1	0	1	0
	- mild	0	1	0	0	1	0	1	0
	- moderate	0	1	1	0	0	0	0	0
dilatation, pelvic		3	0	2	0	0	0	0	0
	- mild	2	0	1	0	0	0	0	0
	- moderate	1	0	1	0	0	0	0	0
discoloration, black	- moderate	0	0	0	0	0	0	1	0
discoloration, tan		0	0	0	0	1	1	0	2
	- mild	0	0	0	0	0	0	0	1
	- moderate	0	0	0	0	1	1	0	1
discoloration, white	- moderate	0	0	1	0	0	0	0	0
enlarged		0	0	0	0	0	1	1	0
	- mild	0	0	0	0	0	0	1	0
	- moderate	0	0	0	0	0	1	0	0
focus/foci, red	- mild	1	0	0	0	0	0	0	0
focus/foci, tan	- mild	0	0	0	0	0	1	0	0
irregular surface		2	0	3	0	3	0	1	0
	- mild	0	0	1	0	1	0	0	0
	- moderate	2	0	1	0	2	0	1	0
	- severe	0	0	1	0	0	0	0	0
small	- severe	0	0	1	0	1	0	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Macroscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
liver									
discoloration, brown	- mild	0	0	0	0	1	0	0	0
discoloration, red	- moderate	0	1	0	0	0	0	0	0
discoloration, tan		0	0	3	0	0	0	0	0
	- mild	0	0	1	0	0	0	0	0
	- moderate	0	0	2	0	0	0	0	0
enlarged		2	0	0	0	1	0	2	1
	- mild	0	0	0	0	1	0	1	1
	- moderate	0	0	0	0	0	0	1	0
	- severe	2	0	0	0	0	0	0	0
focus/foci, red		0	0	0	2	0	0	0	0
	- mild	0	0	0	1	0	0	0	0
	- moderate	0	0	0	1	0	0	0	0
focus/foci, tan		0	1	1	1	1	0	0	0
	- mild	0	1	1	1	0	0	0	0
	- moderate	0	0	0	0	1	0	0	0
irregular surface mass	- moderate	1	0	0	0	0	0	0	0
	- present	0	0	2	0	1	1	0	2
nodule	- present	0	0	0	0	0	0	1	0
lung									
discoloration, red	- moderate	0	0	0	0	0	0	2	0
discoloration, tan	- mild	1	0	0	0	0	1	0	0
discoloration, white		1	0	0	0	1	0	0	0
	- mild	1	0	0	0	0	0	0	0
	- moderate	0	0	0	0	1	0	0	0
focus/foci, brown	- mild	0	0	0	0	1	0	0	0
focus/foci, red	- mild	0	0	0	0	1	0	0	0
focus/foci, tan	- mild	0	0	0	0	0	0	1	1
focus/foci, white		1	0	0	1	2	0	1	2
	- mild	1	0	0	1	2	0	1	1
	- moderate	0	0	0	0	0	0	0	1
mass	- present	0	1	2	0	1	2	2	0
nodule	- present	1	0	1	1	2	1	1	1

(b) (4)

Study Number 900-062

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Macroscopic Observations - FEMALE

Tissue Observation	Severity	Terminal							
		0		25/5		50/25		100/50	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		49	26	43	21	40	25	50	25
uterus with cervix									
discoloration, green	- moderate	0	0	1	0	0	0	0	0
discoloration, red	- mild	0	0	0	0	1	0	0	0
enlarged		13	17	17	10	14	14	19	14
	- minimal	0	0	0	0	0	1	0	0
	- mild	2	4	2	2	3	5	2	2
	- moderate	11	5	9	6	7	5	10	7
	- severe	0	8	6	2	4	3	7	5
mass	- present	1	0	0	2	1	1	1	1
swollen/thickened	- moderate	0	0	0	0	0	0	1	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy

Histopathology

Peer Review

The histopathology evaluations at [REDACTED] (b) (4) for male and female mice were performed by [REDACTED] (b) (6) [REDACTED] respectively.

The slides were peer reviewed by [REDACTED] (b) (6) [REDACTED]. The peer review pathologist evaluated all slides and all diagnoses from 10% of randomly selected control (male and female) and HD male and female mice. Differences in the opinions were resolved and agreement was reached on diagnosis.

Neoplastic findings

- There were no drug-related increases in incidence of any specific neoplastic tumor in mice treated with 5, 25 and 50 mg/kg of lorcaserin relative to controls.
- The incidence of mammary adenocarcinoma were similar among groups in females (2/75, 1/65, 1/65 and 4/75 in control, 5, 25 and 50 mg/kg, respectively).
- There was no difference in this tumor among female mice.
- Overall, the mouse carcinogenicity study carried out with lorcaserin doses up to 50 mg/kg (2.5 to 7x the clinical dose of 10 mg BID) was negative for neoplastic lesions in mice.
- Incidence of more notable neoplastic findings in males and female mice are shown in tables below.

The incidences of notable neoplastic lesions are shown in tables below for reference.

(b) (4)
 Study Number 900-062
 A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - MALE

Tissue Diagnosis	Terminal											
	0 mg/kg/day			25/5 mg/kg/day			50/25 mg/kg/day			100/50 mg/kg/day		
	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day
adipose tissue	(13)			(4)			(12)			(6)		
adipose tissue, brown, perirenal	(0)			(0)			(0)			(1)		
adrenal glands	(75)			(63)			(64)			(74)		
adenoma, subcapsular cell, benign, primary	3	1119 1132 1151	S 730 S 733 S 733	5	2107 2120 2130 2146 2162	D 582 S 730 S 733 D 664 S 733	1	3141	S 730	3	4145 4149 4161	D 652 S 733 S 733
fibrosarcoma, malignant, secondary	0			0			1	3152	D 575	0		
pheochromocytoma, benign, primary	0			0			1	3154	D 617	0		
aorta	(75)			(65)			(65)			(75)		
mesothelioma, malignant, secondary	0			0			1	3135	E 709	0		
bone, rib	(0)			(1)			(0)			(0)		
bone, sternum	(75)			(65)			(65)			(75)		
carcinoma, bronchiolar alveolar, malignant, secondary	0			0			1	3108 ^f	D 623	0		
mesothelioma, malignant, secondary	0			0			1	3135	E 709	0		
brain	(75)			(65)			(65)			(75)		
astrocytoma, malignant, primary	1	1140	D 499	1	2131	E 308	0			0		
meningioma, benign, primary	0			0			1	3111	E 663	0		
cavity, abdominal	(0)			(0)			(1)			(1)		
cavity, cranial	(0)			(1)			(0)			(0)		
cavity, thoracic	(0)			(0)			(1)			(0)		
mesothelioma, malignant, primary	0			0			1	3135	E 709	0		
coagulating glands	(75)			(64)			(64)			(74)		
adenoma, benign, primary	0			0			0			1	4147	S 733

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
 No. - Number () - Total number examined
^fReplacement animal

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - MALE

Tissue Diagnosis	0 mg/kg/day			Terminal 25/5 mg/kg/day			50/25 mg/kg/day			100/50 mg/kg/day		
	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day
ears	(2)			(0)			(0)			(1)		
epididymides	(75)			(64)			(65)			(75)		
sarcoma, undifferentiated, malignant, primary	2	1122 1165	S 730 S 733	0			0			0		
harderian glands	(75)			(64)			(65)			(75)		
adenocarcinoma, malignant, primary	0			0			1	3129	S 730	0		
adenoma, benign, primary	3	1110 1154 1165	D 502 E 722 S 733	3	2129 2138 2149	S 733 S 733 D 668	1	3153	S 733	4	4145 4159 4162 4164	D 652 S 733 S 733 D 489
heart	(75)			(65)			(65)			(75)		
carcinoma, bronchiolar alveolar, malignant, secondary	0			0			1	3108 ^f	D 623	0		
fibrous histiocytoma, malignant, secondary	1	1123	D 525	0			0			0		
mesothelioma, malignant, secondary	0			0			1	3135	E 709	0		
kidneys	(75)			(65)			(65)			(75)		
adenoma, tubular cell, benign, primary	0			0			1	3147	E 629	0		
fibrosarcoma, malignant, secondary	0			0			1	3152	D 575	0		
lacrimal glands, exorbital	(75)			(65)			(65)			(74)		
fibrous histiocytoma, malignant, secondary	0			0			0			1	4126	E 334
large intestine, cecum	(75)			(65)			(65)			(75)		
liver	(75)			(65)			(65)			(75)		
adenoma, hepatocellular, benign, primary	4	1136 1145 1156 1161	D 605 S 733 D 567 D 530	11	2110 2114 2115 2123 2124 2128 2129 2130 2138 2145 2150	S 729 S 730 D 616 S 730 S 730 S 733 S 733 S 733 S 733 D 522 S 733	4	3141 3151 3154 3156	S 730 S 733 D 617 S 733	3	4127 4148 4161	D 586 S 733 S 733
carcinoma, hepatocellular, malignant, primary	1	1151	S 733	3	2113 2124 2141	S 729 S 730 E 716	3	3108 ^f 3123 3150	D 623 E 674 D 725	4	4103 4133 4158 4161	D 689 D 697 S 733 S 733

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
 No. - Number () - Total number examined
^fReplacement animal

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - MALE

Tissue Diagnosis	Terminal											
	0 mg/kg/day			25/5 mg/kg/day			50/25 mg/kg/day			100/50 mg/kg/day		
	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day
lung	(75)			(65)			(65)			(75)		
adenoma, bronchiolar alveolar, benign, primary	8	1108 1130 1141 1142 1150 1162 1171 1172	D 696 D 727 S 733 S 733 D 722 S 733 S 729 S 729	10	2106 2107 2114 2122 2123 2124 2128 2134 2138 2146	S 729 D 582 S 730 D 469 S 730 S 730 S 733 D 684 S 733 D 664	4	3118 3123 3135 3155	D 698 E 674 E 709 S 733	8	4102 4108 4131 4143 4152 4162 4163 4166	S 729 S 729 D 522 S 733 S 733 S 733 S 733 S 729
carcinoma, bronchiolar alveolar, malignant, primary	8	1111 1112 1127 1128 1137 1146 1166 1172	D 633 S 729 D 539 D 548 S 733 D 630 D 673 S 729	4	2119 2141 2161 2165	S 730 E 716 D 560 S 733	2	3108 ^f 3136	D 623 D 649	1	4135	D 685
carcinoma, hepatocellular, malignant, secondary	0			1	2141	E 716	0			0		
fibrosarcoma, malignant, secondary	0			0			1	3152	D 575	0		
fibrous histiocytoma, malignant, secondary	1	1123	D 525	0			0			1	4126	E 334
mesothelioma, malignant, secondary	0			0			1	3135	E 709	0		
lymph node, axillary	(1)			(1)			(2)			(0)		
lymph node, cervical	(0)			(0)			(0)			(2)		
lymph node, mandibular	(74)			(64)			(63)			(73)		
fibrosarcoma, malignant, secondary	0			0			1	3144	D 674	0		
lymph node, mandibular	(74)			(64)			(63)			(73)		
fibrous histiocytoma, malignant, secondary	1	1123	D 525	0			0			0		
lymph node, mediastinal	(5)			(1)			(3)			(0)		
carcinoma, bronchiolar alveolar, malignant, secondary	1	1127	D 539	0			1	3108 ^f	D 623	0		
lymph node, renal	(1)			(0)			(1)			(1)		
multicentric neoplasm	(9)			(6)			(8)			(5)		
hemangioma, benign, multicentric	2	1141 1155	S 733 S 733	1	2132	E 661	2	3101 3151	S 729 S 733	1	4175	S 729
hemangiosarcoma, malignant, multicentric	3	1115 1130 1162	S 729 D 727 S 733	2	2133 2157	D 594 D 574	2	3115 3161	D 623 E 697	1	4122	D 659
leukemia, malignant, multicentric	1	1134	D 357	0			0			0		
lymphoma, malignant, multicentric	4	1124 1130 1163 1166	D 698 D 727 D 672 D 673	3	2124 2134 2136	S 730 D 684 D 719	4	3102 3114 3137 3154	D 617 S 729 D 656 D 617	3	4110 4154 4157	D 458 D 621 S 733

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
 No. - Number () - Total number examined
^fReplacement animal

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - MALE

Tissue Diagnosis	Terminal											
	0 mg/kg/day			25/5 mg/kg/day			50/25 mg/kg/day			100/50 mg/kg/day		
	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day
parathyroid glands	(50)			(36)			(36)			(43)		
adenoma, benign, primary	1	1165	S 733	0			0			0		
penis	(3)			(4)			(5)			(7)		
pituitary gland	(74)			(62)			(64)			(73)		
adenoma, pars distalis, benign, primary	0			1	2141	E 716	0			0		
preputial glands	(0)			(1)			(3)			(3)		
skeletal muscle, biceps femoris	(75)			(65)			(65)			(75)		
fibrous histiocytoma, malignant, secondary	0			0			0			1	4126	E 334
skin	(75)			(65)			(65)			(75)		
skin, subcutis	(4)			(5)			(5)			(3)		
fibrosarcoma, malignant, primary	0			0			2	3144 3152	D 674 D 540	0		
fibrous histiocytoma, malignant, primary	2	1123 1128	D 525 D 456	2	2124 2143	S 730 D 659	2	3132 3133	E 471 D 491	1	4126	E 334
small intestine, duodenum	(75)			(65)			(65)			(75)		
sarcoma, undifferentiated, malignant, primary	1	1120	S 730	0			0			0		
spleen	(75)			(65)			(65)			(75)		
fibrous histiocytoma, malignant, secondary	1	1123	D 525	0			0			0		
stomach, glandular	(75)			(65)			(65)			(75)		
adenoma, benign, primary	0			0			1	3143	S 730	0		
testes	(75)			(65)			(65)			(75)		
adenoma, interstitial cell, benign, primary	1	1118	S 730	0			1	3130	D 725	1	4107	S 729
testes	(75)			(65)			(65)			(75)		
carcinoma, interstitial cell, malignant, primary	0			0			1	3107	D 635	0		
thymus gland	(61)			(58)			(61)			(64)		
carcinoma, bronchiolar alveolar, malignant, secondary	1	1146	D 630	0			1	3108 ^f	D 623	0		
fibrous histiocytoma, malignant, secondary	1	1123	D 525	0			0			0		
mesothelioma, malignant, secondary	0			0			1	3135	E 709	0		
thyroid gland	(75)			(63)			(65)			(75)		
adenoma, follicular cell, benign, primary	0			0			0			1	4155	E 404
carcinoma, follicular cell, malignant, primary	0			1	2120	S 730	0			0		
urinary bladder	(75)			(65)			(65)			(75)		
mesenchymal tumor, benign, primary	1	1118	S 730	1	2162	S 733	0			0		

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 No. - Number () - Total number examined
^fReplacement animal

A 2 Year Carcinogenicity Study of APD356 Given by Oral Gavage to Mice

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - FEMALE

Tissue Diagnosis	Terminal											
	0 mg/kg/day			25/5 mg/kg/day			50/25 mg/kg/day			100/50 mg/kg/day		
	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day
adipose tissue	(0)			(1)			(0)			(0)		
adrenal glands	(75)			(64)			(65)			(75)		
adenocarcinoma, malignant, secondary	1	1226	E 708	1	2255	D 673	0			0		
adenoma, subcapsular cell, benign, primary	1	1273	S 729	1	2249	S 733	1	3207	S 729	1	4212	S 729
carcinoma, subcapsular cell, malignant, primary	0			0			1	3228	S 733	0		
pheochromocytoma, malignant, primary	1	1263	D 605	0			0			0		
aorta	(75)			(63)			(65)			(74)		
adenocarcinoma, malignant, secondary	0			1	2255	D 673	0			0		
bone, femur	(75)			(64)			(65)			(75)		
osteoma, benign, primary	0			0			0			1	4271 ^f	D 680
brain	(75)			(64)			(65)			(75)		
oligodendroglioma, malignant, primary	1	1257	D 577	0			0			0		
cavity, abdominal	(0)			(1)			(0)			(3)		
harderian glands	(75)			(64)			(65)			(75)		
adenoma, benign, primary	2	1274 1275	S 729 E 573	2	2227 2248	D 687 S 733	2	3214 3245	S 730 D 579	4	4211 4236 4239 4250	S 729 S 733 D 643 E 461
heart	(75)			(64)			(65)			(75)		
adenocarcinoma, malignant, secondary	0			1	2255	D 673	0			0		
large intestine, colon	(75)			(64)			(65)			(75)		
carcinoma, bronchiolar alveolar, malignant, secondary	0			1	2255	D 673	0			0		
large intestine, rectum	(75)			(64)			(65)			(75)		
leiomyoma, benign, primary	1	1275	E 573	0			0			0		
liver	(75)			(64)			(65)			(75)		
adenoma, hepatocellular, benign, primary	1	1213	D 675	1	2222	D 684	1	3240	S 733	3	4223 ^f 4253 4262	S 730 S 733 D 604
carcinoma, hepatocellular, malignant, primary	0			0			1	3254	D 637	0		
schwannoma, malignant, secondary	0			0			0			1	4229	D 479
lung	(75)			(64)			(65)			(75)		
adenocarcinoma, malignant, secondary	2	1226 1229	E 708 D 678	1	2255	D 673	0			2	4214 ^f 4271 ^f	D 627 D 680

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
 No. - Number () - Total number examined
^fReplacement animal

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - FEMALE

Tissue Diagnosis	Terminal											
	0 mg/kg/day			25/5 mg/kg/day			50/25 mg/kg/day			100/50 mg/kg/day		
	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day
lung	(75)			(64)			(65)			(75)		
adenoma, bronchiolar alveolar, benign, primary	3	1210 1230 1247	D 666 D 623 S 730	6	2205 2211 2217 2254 2255 2259	S 729 D 727 S 729 S 733 D 673 E 394	8	3211 3216 3219 3227 3234 3235 3254 3262	D 586 D 386 E 698 S 730 S 733 D 648 D 637 D 661	5	4206 4218 4223 ^f 4227 4244	D 487 S 729 S 730 S 730 S 733
carcinoma, bronchiolar alveolar, malignant, primary	2	1201 1270	S 729 S 729	4	2207 2209 2252 2255	D 698 D 700 D 538 D 673	3	3209 3243 ^f 3251	S 729 S 733 D 490	2	4231 ^f 4259	D 579 D 568
granulosa cell tumor, malignant, secondary	0			1	2219	S 729	0			0		
luteoma, malignant, secondary	0			0			0			1	4250	E 461
sarcoma, stromal, malignant, secondary	0			1	2253	S 733	0			0		
lymph node, mediastinal	(3)			(3)			(5)			(5)		
adenocarcinoma, malignant, secondary	0			0			0			2	4214 ^f 4271 ^f	D 627 D 680
lymph node, mesenteric	(75)			(64)			(63)			(73)		
adenocarcinoma, malignant, secondary	0			1	2255	D 673	0			0		
mammary gland	(74)			(63)			(65)			(75)		
adenocarcinoma, malignant, primary	2	1205 1229	E 421 D 603	1	2209	D 680	1	3262	D 661	4	4214 ^f 4245 4253 4271 ^f	D 603 D 603 S 733 D 624
multicentric neoplasm	(15)			(13)			(15)			(15)		
hemangiosarcoma, malignant, multicentric	1	1225	S 729	0			0			0		
leukemia, malignant, multicentric	0			0			1	3206	D 130	0		
lymphoma, malignant, multicentric	8	1204 1222 1234 1235 1238 1254 1256 1261	D 461 D 676 E 684 S 730 S 730 E 719 S 733 S 733	9	2205 2235 2237 2238 2239 2245 2249 2256 2265	S 729 S 730 D 595 D 670 D 649 D 621 S 733 D 698 D 687	11	3209 3228 3233 3236 3250 3252 3254 3259 3262 3263 3264	S 729 S 733 D 723 D 484 D 244 D 628 D 637 D 631 D 661 E 257 S 733	12	4212 4216 4219 4230 4235 4236 4238 4239 4247 4248 ^f 4261 4270	S 729 D 669 D 695 D 641 S 730 S 733 D 658 D 643 D 561 D 599 S 733 E 714
sarcoma, histiocytic, malignant, multicentric	6	1211 1215 1240 1244 1253 1255	D 273 D 553 E 701 D 621 S 733 D 496	4	2204 2207 2222 2251	D 640 D 698 D 684 S 733	3	3230 3246 3251	D 408 S 733 D 490	3	4231 ^f 4240 4241	D 579 D 657 S 733

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
 No. - Number () - Total number examined
^fReplacement animal

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - FEMALE

Tissue Diagnosis	Terminal											
	0 mg/kg/day			25/5 mg/kg/day			50/25 mg/kg/day			100/50 mg/kg/day		
	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day	No. with Tumor	Animal No.	Fate/ Day
skin, subcutis	(3)			(5)			(4)			(4)		
fibrous histiocytoma, malignant, primary	0			0			0			1	4205 ^f	E 601
small intestine, jejunum	(75)			(64)			(65)			(75)		
adenocarcinoma, malignant, primary	0			0			0			1	4218	S 729
thyroid gland	(74)			(64)			(65)			(75)		
adenoma, c-cell, benign, primary	0			0			0			1	4261	S 733
adenoma, follicular cell, benign, primary	0			0			0			1	4273	S 729
uterus with cervix	(75)			(64)			(65)			(75)		
adenoma, benign, primary	1	1234	E 684	0			0			0		
leiomyoma, benign, primary	3	1245 1246 1257	D 599 D 320 D 577	2	2214 2263	S 729 S 733	3	3226 3238 3239 ^f	S 730 S 733 D 665	6	4209 4222 4228 4232 4269 4275	D 566 D 589 S 730 S 730 S 729 D 629
leiomyosarcoma, malignant, primary	0			0			0			1	4241	S 733
uterus with cervix	(75)			(64)			(65)			(75)		
polyp, stromal, benign, primary	5	1214 1238 1239 1258 1271	S 729 S 730 S 730 S 733 E 673	7	2209 2217 2218 2233 2234 2241 2259	D 700 S 729 S 729 E 643 S 730 D 726 E 394	10	3207 3208 3214 3224 3238 3247 3248 3249 3251 3261	S 729 S 729 S 730 S 730 S 733 S 733 D 696 D 519 D 490 S 733	8	4216 4237 4243 ^f 4244 4251 4260 4263 4274	D 669 S 733 D 618 S 733 D 510 S 733 S 733 S 729
sarcoma, stromal, malignant, primary	1	1238	S 730	5	2202 2209 2214 2250 2253	D 566 D 700 S 729 E 352 S 733	5	3207 3208 3217 3232 3248	S 729 S 729 S 730 D 520 D 696	0		
schwannoma, malignant, primary	0			0			2	3217 3244	S 730 S 733	1	4229	D 479
vagina	(75)			(63)			(65)			(75)		
sarcoma, stromal, malignant, secondary	0			1	2214	S 729	0			0		

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
 No. - Number () - Total number examined
^fReplacement animal

Non Neoplastic findings

- There were no treatment-related histopath findings in mice treated with 5, 25 and 50 mg/kg of lorcaserin.
- There were no notable drug-related changes in heart tissue including valves or renal pathology in mice treated with lorcaserin doses up to 50 mg/kg.

Tissue Observation	Severity	0 mg/kg/day		25/5 mg/kg/day		50/25 mg/kg/day		100/50 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
FEMALE									
heart		(49)	(26)	(43)	(21)	(40)	(25)	(50)	(25)
cardiomyopathy	- minimal	4	2	3	0	0	1	9	4
endocarditis, valvular vegetative	- severe	1	0	2	0	0	0	1	0
MALE									
heart		(44)	(31)	(41)	(24)	(47)	(18)	(48)	(27)
carcinoma, bronchiolar alveolar, malignant, secondary		0	0	0	0	1	0	0	0
cardiomyopathy		18	21	11	7	10	12	15	7
	- minimal	17	19	9	6	8	8	12	7
	- mild	1	2	2	1	2	4	3	0
endocarditis, valvular vegetative		0	0	2	0	0	0	1	0
	- mild	0	0	0	0	0	0	1	0
	- moderate	0	0	2	0	0	0	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

- The absence of any notable microscopic finding in mice is reasonable since the top dose of 50 mg/kg was 3.6 to 7x the clinical dose.
- Overall, there were no notable treatment related non-neoplastic/histopath findings in mice treated with lorcaserin up to 50 mg/kg.

Toxicokinetics:

Blood samples (predose, 0.5, 1, 2, 4, 8, 12 and 24 hrs) were collected from nonfasted mice via orbital sinus after CO₂ anesthesia at week 52 of treatment (TK satellite animals). In addition to concentrations of lorcaserin (ADP356), levels of two common metabolites M1 (lorcaserin sulfamate, AR244208) and M5 (N-carbamoyl glucuronide, AR306388) were measured.

- Lorcaserin AUC at 5, 25 and 50 mg/kg were 0.5x, 3.8 and 7.3x the clinical dose in males and 0.3x, 1.5 and 3.6x the clinical dose of 10 mg BID based on AUC.
- Lorcaserin was rapidly absorbed from the GI tract.
- Lorcaserin exposure was dose-proportional and linear although plasma exposure was lower in female mice than male mice. The AUC in male mice was 1.7 to 2.4x the AUC in female mice.
- The t_{1/2} in male and female mice was 2.37 hr to 3.01 hr, respectively
- APD356 was rapidly absorbed from the gastrointestinal tract.
- Both M1 (AR244208) and M5 (AR306388) metabolites were formed at all doses in mice.
- Exposure to sulfamate metabolite (M1, APD244208) was 41 to 93x greater than lorcaserin suggesting significant and rapid metabolism of lorcaserin to M1 in mice.

- The metabolism of lorcaserin to M5 was relatively limited. The exposure to M5 was nearly equal to or half of the parent drug. However, since exposure to M5 was about 4 fold greater than human M5 exposure; the safety of M5 was considered covered by mouse study.

Safety Margins for lorcaserin (ADP356) drug based on AUC determined at WK 52:

Species	Dose, mg/kg	AUC ₀₋₂₄ µg.h/ml	Animal to human dose exposure ratio based on AUC
Mouse carci study TK	5	M:0.55, F:0.32	M:0.5, F:0.3
	25	M:3.9, F: 1.6	M:3.8, F: 1.5
	50	M:7.5 F:3.7	M:7.3, F: 3.6
MRHD: 10 mg BID		1.02	

Safety Margins for the M1 metabolite (APD244208, lorcaserin sulfamate), based on AUC determined at WK 52:

Species	Dose, mg/kg	AUC ₀₋₂₄ µg.h/ml	Animal to human dose exposure ratio based on AUC
Mouse carci study TK	5	M:59.7, F: 74.4	M:13, F:16
	25	M:223, F: 217	M:50, F:46.5
	50	M:308, F:345	M:66, F: 74
M1 metabolite		4.66	

Safety Margins for the M5 metabolite (APD306388), based on AUC determined in mice at Wk 52:

Species	Dose, mg/kg	AUC ₀₋₂₄ µg.h/ml	Animal to human dose exposure ratio based on AUC
Mouse carci study TK	5	M:0.134, F: 0.184	M:0.2 F:0.25
	25	M:1.84, F: 1.21	M:2.4 F: 1.6
	50	M:3.42, F:3.23	M:4.5 F: 4.2
M5 metabolite		0.765	

**Toxicokinetic Parameters of APD356, AR244208, and AR306388
after Oral Dosing of APD356 for 52 Weeks.**

Compound	APD356 Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)	AUC _(0-inf) (hr·µg/mL)	
Male	APD356	5	0.149	0.500	0.546	0.566
		25	0.814	0.500	3.90	UD ^a
		50	1.51	1.00	7.50	7.53
	AR244208	5	10.8	0.500	59.7	61.2
		25	25.4	0.500	223	UD
		50	42.7	0.500	308	319
	AR306388	5	0.0983	0.500	0.134	0.178
		25	0.590	0.500	1.84	UD
		50	1.00	0.500	3.42	3.47
Female	APD356	5	0.0841	0.500	0.319	0.341
		25	0.702	0.500	1.64	UD
		50	0.890	1.00	3.72	3.77
	AR244208	5	14.4	0.500	74.4	75.2
		25	39.8	0.500	217	221
		50	49.1	0.500	345	356
	AR306388	5	0.100	0.500	0.184	0.201
		25	0.431	0.500	1.21	UD
		50	0.824	0.500	3.23	3.26

^a UD = Undefined; parameter not determined due to uninteruptible terminal phase

Stability and Homogeneity

The concentrations of lorcaserin in the solutions administered to mice were within 92.2 to 107% to the target concentrations. The solutions were stable and homogenous (98 to 100% of the label claim). Analysis of the vehicle control samples did not present any lorcaserin.

Study title: A 2-Year carcinogenicity study of APD356 given by oral gavage to rats

Study no.: Arena# TX05071 (b) (4) #900-063)
 Study report location: eCTD format,
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Feb 21, 2006 (completed Jan 30, 09)
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: 06P0031 and 06P0044, 96.3% (w/w)
 CAC concurrence: Yes

Key Study Findings:

Adequacy of Carcinogenicity Study

The rat carci study appeared to be adequate and acceptable. Lorcaserin significantly increased mortality at all doses in females due to mammary tumors and in HD males due to various types of tumors. Lorcaserin doses of 10, 30 and 100 mg/kg had no apparent effect on BW in female rats, however, the BW of male rats at 100 mg/kg was reduced by as much as 28% at the end of the study. The weight loss effect was not considered a toxicological response since lorcaserin is designed to suppress appetite via 5HT_{2C} receptors in the brain with a consequent decrease BW. Therefore, since deaths were caused by tumors and weight loss in the HD males is a combination of pharmacological activity of lorcaserin and higher tumor burden, the top dose of 100 mg/kg did not exceed the maximum tolerated dose (MTD) and the rat study was accepted as valid by the reviewer and eCAC (see appendix A).

Appropriateness of Test Models:

SD rats are standard rodent model used in carcinogenicity assessment.

Evaluation of Tumor Findings:

The sponsor and the FDA statistician, Dr. Jackson analyzed the incidences of tumors in rats. Dr. Jackson analysis was in general in agreement with the sponsors' analysis. There was a significant dose-dependent increase in incidence of several tumors in lorcaserin treated rats. Two primary tumors of interest were mammary adenocarcinoma and fibroadenoma in female and males and brain astrocytoma in males. The incidence of mammary fibroadenoma alone and combined with adenocarcinoma (analyzed by the sponsor and FDA) was significantly increased at all doses of lorcaserin in females. The combined incidence of fibroadenoma and adenocarcinoma was significantly increased at the MD and HD males. The safety margins are provided in table below.

Species	Dose, mg/kg	Lorcaserin, AUC, µg.h/ml	Safety margins based on AUC (rat/human)
104-week Rat Carci Study	5	M:4.78, F:6.86	M:4.7, F:6.7
	25	M:16.9, F: 24.1	M:16.6, F: 24
	50	M:55.9 F:83.8	M:55, F: 82
Clinical Dose: 10 mg BID		1.02	

Other tumors primarily identified male rats were mammary fibroadenoma, skin, thyroid, liver, and schwannoma. The incidences of tumors with statistical significance for trend and pair wise comparisons are listed in tables below.

Neoplastic tumors in male rats treated with lorcaserin ^a
(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in male rats		Lorcaserin dose, mg/kg				Dose Response
		0	10	30	100	
Brain	astrocytoma	1	0	4 ND	8 ^b p=0.0019	< 0.0001
	mixed glioma	0	0	0	1	NS
	reticularis	0	1	0	1	NS
	granular cell	0	0	1	1	NS
	oligodendroglioma	1	0	0	0	NS
	combined	2	1	5	20	
Liver	hepatocellular carcinoma	1	3	2	4	NS
	hepatocellular adenoma	1	1	2	6 p=0.030	p=0.033
	combined	2	4	4	10 p=0.0048	p=0.0012
Lung,	carcinosarcoma, sec.	0	1	1	0	NS
	fibrosarcoma, sec.	0	0	0	1	
	osteosarcoma, sec.	0	0	1	0	
	mesothelioma, sec.	1	1	0	0	
Mammary	adenocarcinoma,	0	0	2	2 NS	p=0.046
	fibroadenoma	0	1	4 NS	6 NS	p=0.0001
	combined	0	1	6 p=0.0131	8 p=0.0009	p< 0.0003
Pituitary, adenoma, pars distalis, benign		32	22 NS	22 NS	15 p=0.0003	p=0.0005
Skin, subcutis	benign fibroma	3	7 NS	11 p=0.017	17 p<0.0001	p<0.0001
	fibrosarcoma	1	0	0	0	
	combined	4	7	11	17	
Skin	squamous cell carcinoma,	0	0	4 NS	5 p=0.014	p=0.003
	papilloma	1	0	1	2	NS
	combined	1	0	5	7	
Schwannoma, all sites		0	0	2	9 p<0.0037	p<0.0001
Schwannoma, Subcutis alone			0	1	5 p=0.047	p=0.0053
Thyroid, follicular cell adenoma, benign		0	5 p=0.02	4 NS	8 p=0.0011	p=0.0035

^a Statistical analysis and p values in the table were provided by the FDA statistician, Dr. Jackson

^b One of the astrocytomas in the HD males was reclassified as infarct due to lymphocytic leukemia in an amendment to the NDA.

Neoplastic tumors in female rats treated with lorcaserin ^a
(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in female rats		Lorcaserin dose, mg/kg				Dose Response
		0	10	30	100	
Brain	astrocytoma	0	2	0	1	NS
	mixed glioma	0	0	0	1	NS
	combined	0	2	0	2	
Lung	carcinoma, second.	0	4	9	6	NS
	carcinosarcoma, second	0	0	0	1	NS
	Combined	0	4	9	7	
Mammary	carcinosarcoma	0	0	0	1	NS
	adenocarcinoma	28	34 NS	35 p=0.02	60 p<0.0001	p < 0.0001
	fibroadenoma	20	47 p<0.0001	53 p<0.0001	45 p<0.0001	p < 0.0001
	combined	40	56 p<0.0001	61 p<0.0001	71 p<0.0001	p<0.0001
Skin,	fibrosarcoma, subcutis	0	0	0	2	p=0.067
Pituitary	adenoma, pars distalis, benign	50	46 (NS)	31 (p=0.001)	20 (p<0.0001)	p<0.0001
	carcinoma, pars distalis	0	2	0	0	NS
	combined	50	48	31	20	
Thyroid, adenoma follicular	2	2	4	3	p=0.03	
Uterus with cervix, benign glandular polyp	0	0 NS	3 NS	3 NS	p=0.003	

^a The statistical analysis and p values in the table were provided by the FDA statistician, Dr. Mathew Jackson.

NS = not significant

The ECAC discussed the findings of the rat study and concluded that the following statistical and numerical increases in tumors were related to treatment with lorcaserin:

Males

Brain: Astrocytoma at HD. Numerical, non-statistically significant increase in astrocytoma at mid-dose also considered drug-related.

Liver: Hepatocellular adenoma and carcinoma combined, at HD.

Mammary: Adenocarcinoma and fibroadenoma combined, at MD & HD.

Skin, subcutis: Fibroma at MD & HD

Skin: Squamous Carcinoma at HD. Numerical, non-statistically significant increase in squamous carcinoma at MD also considered drug-related.

Schwannoma (all sites) at HD. Numerical, non-statistically significant increase at the MD also considered drug-related.

Thyroid: Follicular cell adenoma at HD.

Females

Mammary: Adenocarcinoma + fibroadenoma at LD, MD, HD

Note that for females, statistical significance was achieved at all doses for fibroadenoma and at the mid-dose for adenocarcinoma.

Methods

Doses: 0, 10, 30 and 100 mg/kg (C, LD, MD & HD)
 Frequency of dosing: single oral dose/day
 Dose volume: 5 ml/kg
 Route of administration: oral gavage
 Formulation/Vehicle: water
 Basis of dose selection: 3-month rat tox study
 Species/Strain: CD® [CrI:CD®(SD)] rats
 Number/Sex/Group: 65/sex/C, LD, MD and 75/sex/HD
 Age: 6 weeks plus acclimation
 Animal housing: individually in stainless steel cages
 Paradigm for dietary restriction: ad lib food (Lab Diet #5002) and water
 Dual control employed: No
 Interim sacrifice: No (TK animals were sacrificed after 1 year)
 Satellite groups: TK rats (6, 15, 15 & 15 rats/sex for C, LD,MD and HD, respectively)

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study			
1	0	65	65
2	10	65	65
3	30	65	65
4	100	75	75
Toxicokinetic			
5	0	6	6
6	10	15	15
7	30	15	15
8	100	15	15

Deviation from study protocol:

Deviations from the protocol were generally minor except for the following changes.

- Due to significant incidence of mammary and brain tumors, TK animals were also evaluated for incidence of mammary and astrocytoma and reported in the bimonthly interim reports. The histopath evaluations from the TK animals were not provided in final NDA report.
- Remaining pooled plasma from zero time TK samples (Week 52) were used to analyze Thyroid Stimulation Hormone (TSH).
- Pituitary and mammary tissues from TK animals were evaluated with immunohistochemistry to detect prolactin positive staining cells. To do so, some of the TK animals were allowed additional 3 (M) to 4 (F) weeks of lorcaserin treatment (post Week 52) to allow determination of estrous cycle and blood levels hormones prolactin (males and females) and estradiol (females) during diestrus. These rats were also necropsied during diestrus (Week 55 and 56) with macroscopic and microscopic evaluation. However, according to the sponsor the “results of these evaluations were not submitted” with the NDA.
- Brain immunohistochemistry was also performed in rats with brain tumors (n=20) using three different stains to investigate astrocytoma lineage
- One HD female (4255) was accidentally and prematurely killed on Day 467.
- A minor addition to the study protocol was bimonthly updates following the initial 15-day safety report of high incidence of mammary tumor related deaths in female rats (WK 55). Mammary tumor-related death in HD females occurred as early as WK 42.

Observations and Results**Mortality**

- There was a significant increase in drug-related mortality in both male and female rats. The most affected groups were the HD rats. The incidence of death/euthanasia in extremis in control, LD, MD and HD were 15.4%, 21.5%, 23.1% and 48%, respectively, while the incidence of death/euthanasia in the control, LD, MD and HD females were 66.2%, 75.4%, 69.2% and 94.7%, respectively. The rate of mortality was increased after week 61 in males. In females, deaths due to mammary tumors started rising after week 42.
- The number of deaths in TK animals at week 56 were highest among treated with 30 and 100 mg/kg. (C:0F,1M; LD:1M,1F; MD 4M,1F; HD:1M, 5F).
- Serological evaluation of rats at 6, 12 and 18 months (b) (4) found no evidence of microbial infections suggesting that dose-related increase in deaths were not caused by any commonly known rodent microorganisms.
- The most frequently cited cause of death in HD males were brain tumor (5/71), fibroma (4/71), alveolar lipidosis/histocytosis (5/71), mammary tumor (2/71), malignant schwannoma (5/71) and skin tumors (5/71).
- The most common cause of death in HD females was mammary tumor (68/75). Mammary tumor also caused death in control (15/65), LD (31/65) and MD (43/65) females. All probable cause of deaths in rats are listed below:

(b) (4) Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Probable Cause of Death - MALE				
Cause of Death	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals	65	65	65	75
Summary of Animal Disposition				
died after dosing	1	0	0	0
died prior to euthanasia	0	0	1	1
euthanized <i>in extremis</i>	10	14	15	36
found dead	32	35	29	34
terminal necropsy	22	16	20	4
Cause of Death				
adipose tissue tumor	1	2	0	3
bone tumor	0	0	1	0
brain tumor	2	0	4	5
cardiomyopathy	0	0	0	1
chronic progressive nephropathy/uremia	2	5	0	0
dosing error	1	0	0	1
fibrosarcoma/fibroma	2	1	1	4
fibrous histiocytoma	0	1	0	1
heart failure/atrial thrombus	1	0	0	0
heart inflammation/necrosis	0	1	0	0
histiocytic sarcoma	0	4	2	1
inflammation/septicemia	0	1	1	0
kidney inflammation/necrosis	0	0	0	1
kidney tumor	1	0	0	0
leukemia	1	0	1	2
liver inflammation/necrosis	0	0	1	0
liver tumor	0	1	1	0
lung lipoidosis, alveolar	0	0	0	1
lymphoid tumor	2	0	1	1
mammary tumor	0	0	1	2
mesothelioma	0	2	0	1
pituitary tumor	12	6	6	6
polyarteritis	1	0	0	0
sarcoma, type undetermined	0	1	1	1
schwannoma	0	0	1	5
skin inflammation/necrosis	0	0	2	2
skin tumor	0	0	2	5
skin, subcutis lymphangiosarcoma	1	0	0	0
spleen leiomyosarcoma	0	0	0	1
testes carcinoma, rete testis	0	0	0	1
undetermined	14	23	18	23
urinary bladder leiomyosarcoma	0	0	1	0
urogenital inflammation/obstruction/calculi	2	1	0	2
zymbals gland tumor	0	0	0	1

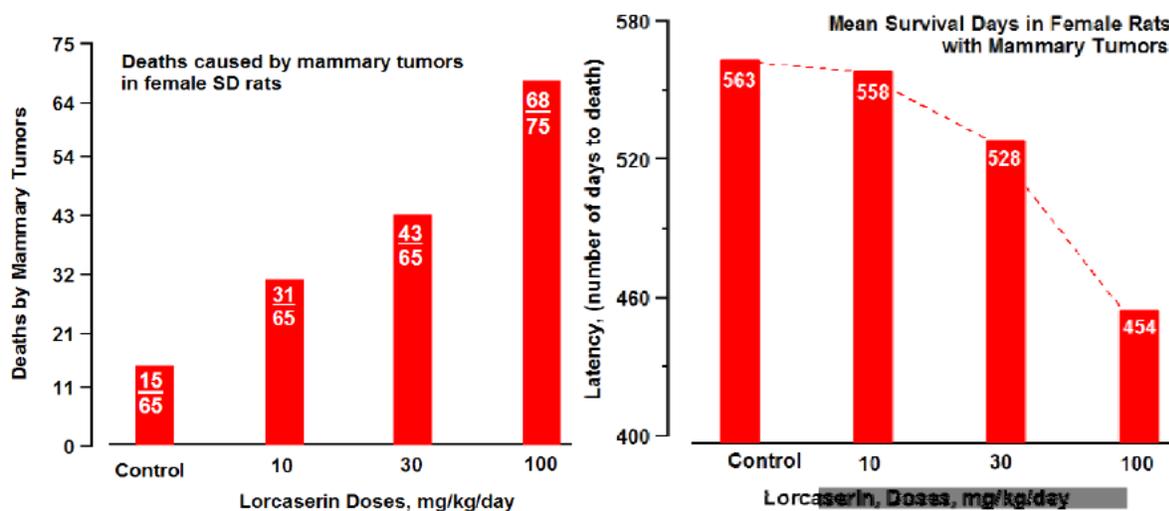
(b) (4) Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Probable Cause of Death - FEMALE				
Cause of Death	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals	65	65	65	75
Summary of Animal Disposition				
died prior to euthanasia	0	0	0	1
euthanized <i>in extremis</i>	34	36	43	61
found dead	8	17	17	13
terminal necropsy	23	12	5	0
Cause of Death				
brain tumor	0	2	1	0
kidneys hydronephrosis, bilateral	1	0	0	0
kidneys nephropathy, chronic progressive	1	0	1	0
kidneys pyelonephritis, bilateral	0	1	0	1
leukemia	1	0	0	0
lymphoid tumor	0	2	0	0
mammary tumor	15	31	43	68
pituitary tumor	15	13	10	2
skin hemangiosarcoma	0	0	1	0
spleen sarcoma, undifferentiated	0	0	0	1
stomach, glandular sarcoma, undifferentiated	0	1	0	0
undetermined	9	3	2	3
uterus inflammation/necrosis	0	0	1	0
zymbals gland tumor	0	0	1	0

Deaths in female SD rats due mammary tumors

Lorcaserin Dose, mg/kg	0	10	30	100
Number of death due to mammary tumors in female rats				
Number of animals per group	65	65	65	75
Due to fibroadenoma	2	9	14	10
Due to adenocarcinoma and or fibro	13	22	29	50
Combined	15	31	43	68

The figure below shows the total number of deaths and the number of days (latency) female rats survived in each treatment group. There were nearly twice as many dead female rats in the LD group than control.



The number of deaths by all causes compared to those caused by mammary tumor in female rats (Fig a). The number of deaths by all causes in male and female rats (Fig b).

Figure a

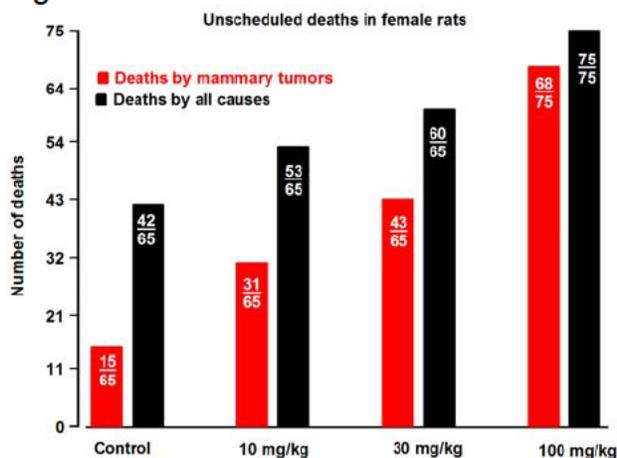
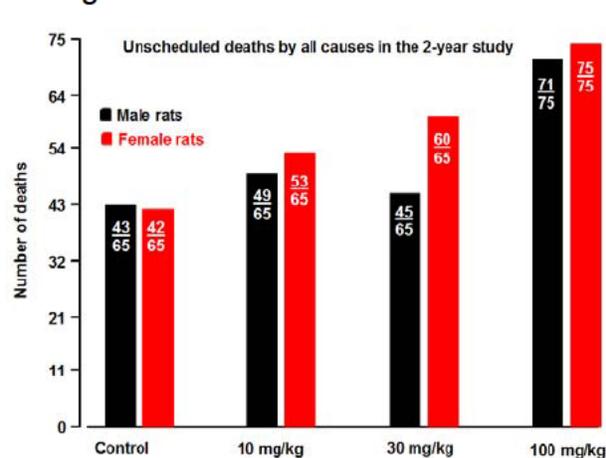


Figure b



Lorcaserin resulted in numerous tumors in both male and female rats. These tumors occurred much earlier in lorcaserin treated rats. The first appearance of nodules in lorcaserin treated female was 11 to 13 weeks earlier than controls while in males they occurred 10 to 23 weeks earlier than control males.

Lorcaserin Dose, mg/kg	0	10	30	100
First tumor appearance, Weeks				
Female rats	33	24	20	20
Male rats	50	40	38	27

The number of female rats with single or multiple cites of fibroadenoma and adenocarcinoma and their combination in the same animal are shown in table below. With increase in dose there was more tumor cites in each animal. The number of animals with both tumor types increased with increase in lorcaserin dose.

Dose Lorcaserin, mg/kg	0	10	30	100
Number necropsied	65	65	65	75
Adenocarcinoma				
# Adenocarcinoma, single	19	13	22	27
# Adenocarcinoma, multiple	9	21	13	33
# any Adenocarcinoma	28	34	35	60
Fibroadenoma				
# Fibroadenoma, single	16	15	9	12
# Fibroadenoma, multiple	4	32	45	33
# any Fibroadenoma	20	47	53	45
Fibroadenoma and adenocarcinoma				
# Fibro + Adeno in same animal	18	24	25	29

Note

Two HD female rats (**4202 and 4212**) with axillary mass had no evidence of mammary tumor in the histopathology report. The cause of death in rat# 4202 with a mass 1-3.9 mm in size was not determined. However, the death of rat #4212 with a small nodule in the axillary area was attributed to mammary tumor. The histopathology findings in the two animals remain unexplained. See next pages for more detail.

HD female Rat #4202 was euthanized in extremis on Day 344 with 2-3.9 cm mass in the axillary region. The eventual cause of death was not determined. The review of the histopath by this reviewer found no tumors of any type and only mild mammary hyperplasia in the histopath report.

Individual Clinical Findings - FEMALE

Group, Animal Number		Week(s) Sign Present
<u>100 mg/kg/day</u>		
4202	No Abnormalities Detected	2-12, 16-30, 44-50
	Hair sparse, Forefoot/left	13-15
	Hair sparse, Forefoot/right	13-15
	Nodule, 1-5 mm, Axillary region/right	31-34
	Nodule, 5-20 mm, Axillary region/right	35, 43
	Nodule, >20 mm, Axillary region/right	36-42

Individual Mass Findings - FEMALE

Group, Animal Number		Week(s) Sign Present
<u>100 mg/kg/day</u>		
4202	No Masses Detected	2-43
	Mass 1, Small 1-1.9 cm, Axillary region/right	44-47
	Mass 1, Ulcerated, medium 2-3.9 cm, Axillary region/right	48-50

HD female Rat #4212 was also euthanized in extremis on Day 516. Clinical observations at different time intervals had identified a 1-5 mm nodules in the right axillary region with ulcerated mass of 1-5 mm nodule in the anogenital region. In the histopath evaluation, mammary tumor was considered as the cause of death and yet there was no mammary tumor except for moderate lobular mammary hyperplasia.

Individual Clinical Findings - FEMALE

Group, Animal Number		Week(s) Sign Present
<u>100 mg/kg/day</u>		
4212	No Abnormalities Detected	2-15, 18-45, 51-69
	Activity decreased	74
	Hair sparse, Forefoot/left	16-17
	Hair sparse, Forefoot/right	16-17
	Material around nose, Red	74
	Nodule, 1-5 mm, Anogenital region	46-50
	Nodule, 1-5 mm, Axillary region/right	70-74
	Thin	74

Individual Mass Findings - FEMALE

Group, Animal Number		Week(s) Sign Present
<u>100 mg/kg/day</u>		
4212	No Masses Detected	2-47
	Mass 1, Large \geq 4 cm, Anogenital region	67-73
	Mass 1, Medium 2-3.9 cm, Anogenital region	59, 61-66
	Mass 1, Small 1-1.9 cm, Anogenital region	48-58, 60
	Mass 1, Ulcerated, large \geq 4 cm, Anogenital region	74

Individual Animal Data Record: Pathology - FEMALE
Terminal

Group, Animal Number	Fate	Tissue	Observations
<u>100 mg/kg/day</u>			
4212	E	Macroscopic adrenal glands liver lung lymph node, inguinal skin, subcutis	- enlarged, left, mild - discoloration, tan, multifocal, multiple lobes, moderate - cyst, clear, multiple, multiple lobes, mild - enlarged, bilateral, mild draining node for mass a. - mass, ulcerated, mass a, anogenital region, present corresponds to antemortem observation (mass 1) approximately 10.0 x 7.0 x 5.0 cm, red, cystic.
4212	E	spleen Microscopic adrenal glands aorta bone marrow, femur bone marrow, sternum bone, femur bone, sternum brain chordae tendineae, left chordae tendineae, right endocardium esophagus eyes eyes, optic nerves harderian glands lymph node, inguinal lymph node, mandibular lymph node, mesenteric mammary gland nerve, sciatic ovaries oviducts pancreas parathyroid glands peyers patch pituitary gland salivary gland, mandibular salivary gland, parotid salivary gland, sublingual skeletal muscle, biceps femoris skin skin, subcutis small intestine, duodenum small intestine, ileum small intestine, jejunum spinal cord, cervical spinal cord, lumbar spinal cord, thoracic spleen stomach, glandular stomach, nonglandular thymus gland thyroid gland tongue trachea ureters urinary bladder uterus with cervix vagina valve, aortic valve, left atrioventricular valve, pulmonic valve, right atrioventricular non-correlated macro observation Cause of Death	- enlarged, mild - angiectasis/cystic degeneration, focal cortical, bilateral, mild corresponds to macroscopic observation (adrenal glands - enlarged) - hematopoiesis, extramedullary, bilateral, mild - within normal limits - hyperplasia, granulocytic, moderate - hyperplasia, granulocytic, mild - within normal limits - hyperplasia, lymphocyte/plasmacyte, mild corresponds to macroscopic observation (lymph node, inguinal - enlarged) - hyperplasia, lymphocyte/plasmacyte, mild - macrophages, pigmented, mild - hyperplasia, lobular, moderate - within normal limits - corpus luteum absent, unilateral, no grade - corpus luteum decreased, unilateral, no grade - within normal limits - within normal limits - within normal limits - macrophages, pigmented, mild - hypertrophy/hyperplasia, mild - within normal limits - atrophy, mild - within normal limits - degeneration/regeneration, myofiber, mild - within normal limits - fibrosarcoma, malignant, primary, mortality-independent corresponds to macroscopic observation (skin, subcutis - mass a) - within normal limits - within normal limits - within normal limits - within normal limits - degeneration, axonal/myelin, minimal - degeneration, axonal/myelin, minimal - hematopoiesis, extramedullary, increased, moderate corresponds to macroscopic observation (spleen - enlarged) - macrophages, pigmented, mild - within normal limits - within normal limits - depletion, lymphoid, severe - macrophages, pigmented, mild - within normal limits - within normal limits - within normal limits - hyperplasia, transitional cell, bilateral, minimal - within normal limits - within normal limits - diestrus - within normal limits - within normal limits - within normal limits - within normal limits - lung - cyst - mammary tumor

E - Euthanized in extremis

The number of rats survived to necropsy is shown in the table below. Due to high incidence of mortality, all the surviving females at all doses and all surviving males at 100 mg/kg were necropsied on Day 697 (Week 100, 35 days earlier than scheduled). Control, LD and MD males were necropsied on Day 732 as scheduled.

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Dosage Level	Summary of Survival Estimates - MALE				Summary of Survival Estimates - FEMALE			
	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Study Interval (Week)	Deaths	Censored	Effective Sample Size
<u>0 mg/kg/day^z</u>								
	1-13	0	0	65.0	1-13	0	0	65.0
	14-26	0	0	65.0	14-26	1	0	65.0
	27-39	3	0	65.0	27-39	0	0	64.0
	40-52	3	0	62.0	40-52	0	0	64.0
	53-65	1	0	59.0	53-65	4	0	64.0
	66-78	8	0	58.0	66-78	9	0	60.0
	79-91	12	0	50.0	79-91	17	0	51.0
	92-104	16	0	38.0	92-104	11	23 ^{&}	22.5
	105	0	22 ^{&}	11.0				
<u>10 mg/kg/day</u>								
	1-13	1	0	65.0	1-13	0	0	65.0
	14-26	0	0	64.0	14-26	0	0	65.0
	27-39	1	0	64.0	27-39	0	0	65.0
	40-52	0	0	63.0	40-52	4	0	65.0
	53-65	3	0	63.0	53-65	2	0	61.0
	66-78	11	0	60.0	66-78	15	0	59.0
	79-91	14	0	49.0	79-91	17	0	44.0
	92-104	18	0	35.0	92-104	15	12 ^{&}	21.0
	105	1	16 ^{&}	9.0				
<u>30 mg/kg/day</u>								
	1-13	1	0	65.0	1-13	0	0	65.0
	14-26	1	0	64.0	14-26	0	0	65.0
	27-39	2	0	63.0	27-39	2	0	65.0
	40-52	1	0	61.0	40-52	5	0	63.0
	53-65	6	0	60.0	53-65	7	0	58.0
	66-78	4	0	54.0	66-78	10	0	51.0
	79-91	13	0	50.0	79-91	25	0	41.0
	92-104	17	0	37.0	92-104	11	5 ^{&}	13.5
	105	0	20 ^{&}	10.0				
<u>100 mg/kg/day^b</u>								
	1-13	0	0	75.0	1-13	0	0	75.0
	14-26	1	0	75.0	14-26	0	0	75.0
	27-39	0	0	74.0	27-39	2	0	75.0
	40-52	3	0	74.0	40-52	12	0	73.0
	53-65	10	0	71.0	53-65	21	0	61.0
	66-78	25	0	61.0	66-78	27	0	40.0
	79-91	21	0	36.0	79-91	12	0	13.0
	92-104	11	4 ^{&}	13.0	92-104	1	0 ^{&}	1.0

[&]This is the necropsy count

^zStatistically significant for overall test at p<0.05

^bSignificantly different from control; (p<0.01)

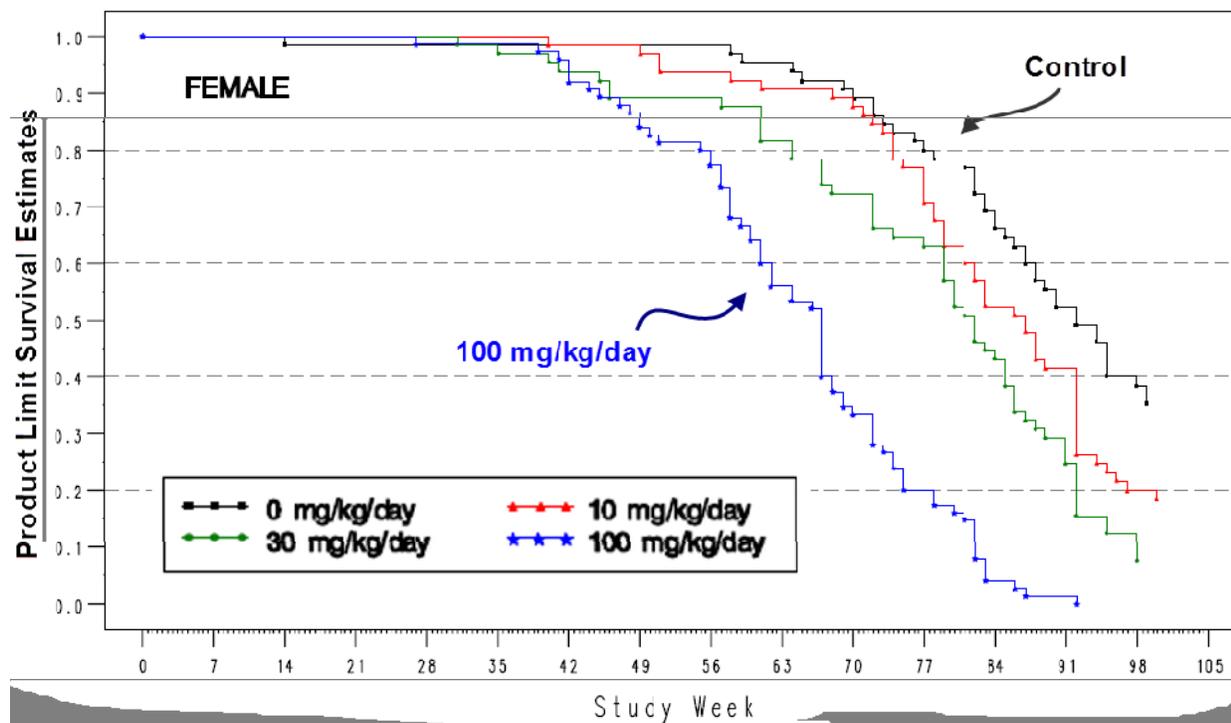
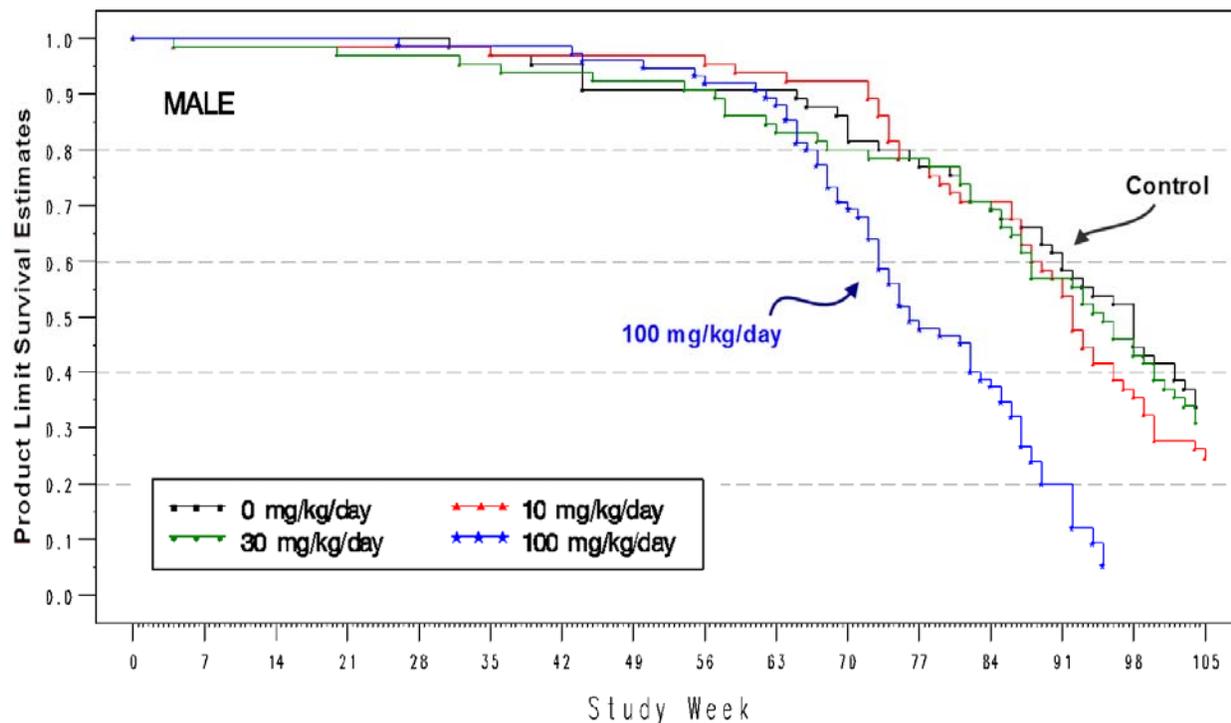
[&]This is the necropsy count

^zStatistically significant for overall test at p<0.05

^aSignificantly different from control; (p<0.05)

^bSignificantly different from control; (p<0.01)

Kaplan-Meier survival estimates in male and female rats treated with 10, 30 and 100 mg/kg of lorcaserin.



Clinical Signs

- Decreased activity in both males and female in all groups
- Clonic convulsion was more frequent in females (2x higher exposure than males) than males (2C male, 1 LD, 3 HD male, 1 LD female and 13 HD females).
- Tremors were noted in 1 LD and 5 HD males and 1 MD female
- Few rats displayed greater sensitivity to touch (M:1, 3, 1 and 6; F:0, 2, 0 and 0 in C, LD, MD and HD respectively)
- Salivation was significantly increased in MD and HD males and females which may have been due to taste (no change in BW in females)
- Although plantar/palmar ulceration was noted in the controls, there was a dose-related increase in incidence and severity of plantar/palmar ulceration in treated rats. The severity in HD was significant but the lower incidences of ulcerations were due to early and high mortality in female rats.
- Physical examination of the female rats found nodules as early as week 33 in control rats, week 24 in LD female and week 20 in MD and HD females suggesting that not only lorcaserin had hastened the formation of mammary tumors, higher doses were associated with more and larger nodules than control and lower doses of lorcaserin.
- Tumor nodules were found in males but the initial onset was delayed and at lower frequency (control at WK 50, LD at WK40, MD at WK 38 and HD at WK 27). In males there was no apparent difference in the number of nodules among groups except for slightly higher incidence in MD males. HD males may have been impacted by the high incidences of premature deaths.

Prominent clinical findings in male and female rats treated with lorcaserin

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Summary of Clinical Findings* - MALE				
Weeks 2 to 105				
Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Peilage/Skin				
Nodule, 5-20 mm	50/12	56/13	258/34	92/20
Nodule, >20 mm	0/0	6/2	13/3	2/1
Nodule, No size indicated	0/0	1/1	0/0	0/0
Ulcer plantar/palmar, 1-5 mm	125/13	215/24	191/24	256/22
Ulcer plantar/palmar, 5-20 mm	148/10	266/20	229/25	248/21
Ulcer plantar/palmar, >20 mm	118/6	139/11	483/18	416/19
Ulcer, 1-5 mm	0/0	6/2	0/0	0/0
Ulcer, 5-20 mm	0/0	4/2	0/0	0/0
Summary of Clinical Findings* - FEMALE				
Weeks 2 to 100				
Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Peilage/Skin				
Nodule, 1-5 mm	671/46	1041/57	945/56	730/68
Nodule, 5-20 mm	183/29	269/38	362/49	301/57
Nodule, >20 mm	9/4	11/5	42/14	98/27
Nodule, No size indicated	0/0	4/1	1/1	0/0
Ulcer plantar/palmar, 1-5 mm	12/2	178/17	92/13	57/11
Ulcer plantar/palmar, 5-20 mm	32/1	95/8	160/13	54/6
Ulcer plantar/palmar, >20 mm	0/0	23/1	80/7	28/7
Ulcer, 1-5 mm	0/0	4/2	0/0	0/0

*Number of times observed/Total number of animals affected

Regions, size and the number of masses observed (number of times observed/number of animals affected) in male and female rats are listed in tables below:

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Summary of Mass Findings* - MALE
 Weeks 2 to 105

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Alive at Start of Interval	65	65	65	75
Mass				
Abdominal region, Large >or=4 cm	0/0	0/0	4/2	0/0
Abdominal region, Medium 2-3.9 cm	0/0	0/0	12/3	5/1
Abdominal region, Small 1-1.9 cm	0/0	2/1	51/3	9/1
Abdominal region, Ulcerated, medium 2-3.9 cm	0/0	0/0	2/1	0/0
Abdominal region, Ulcerated, small 1-1.9 cm	0/0	0/0	1/1	0/0
Anogenital region, Small 1-1.9 cm	0/0	5/1	6/1	0/0
Anogenital region, Ulcerated, large >or=4 cm	0/0	0/0	2/1	0/0
Anogenital region, Ulcerated, medium 2-3.9 cm	0/0	4/1	0/0	1/1
Anogenital region, Ulcerated, small 1-1.9 cm	0/0	7/1	0/0	5/2
Axillary region/left, Large >or=4 cm	6/1	0/0	0/0	3/1
Axillary region/left, Mass > 10cm	1/1	0/0	0/0	0/0
Axillary region/left, Medium 2-3.9 cm	4/1	0/0	6/1	1/1
Axillary region/left, Small 1-1.9 cm	2/1	0/0	3/1	5/2
Axillary region/left, Ulcerated, large >or=4 cm	0/0	0/0	0/0	1/1
Axillary region/right, Large >or=4 cm	0/0	5/1	5/1	0/0
Axillary region/right, Mass > 10cm	0/0	0/0	3/1	0/0
Axillary region/right, Medium 2-3.9 cm	0/0	5/1	4/1	4/1
Axillary region/right, Small 1-1.9 cm	0/0	2/1	0/0	2/1
Cervical region, Large >or=4 cm	0/0	3/2	0/0	13/2
Cervical region, Mass > 10cm	0/0	3/1	0/0	1/1
Cervical region, Medium 2-3.9 cm	2/1	5/2	1/1	19/4
Cervical region, Small 1-1.9 cm	0/0	5/2	1/1	5/2
Cervical region, Ulcerated, medium 2-3.9 cm	6/2	0/0	0/0	2/1
Cervical region, Ulcerated, small 1-1.9 cm	11/1	0/0	0/0	0/0
Cranial region, Small 1-1.9 cm	0/0	0/0	6/2	1/1
Cranial region, Ulcerated, medium 2-3.9 cm	0/0	0/0	3/3	2/1
Cranial region, Ulcerated, small 1-1.9 cm	0/0	0/0	2/1	1/1
Dorsal surface, Large >or=4 cm	0/0	0/0	0/0	5/1
Dorsal surface, Mass > 10cm	0/0	0/0	0/0	1/1
Dorsal surface, Medium 2-3.9 cm	0/0	0/0	0/0	3/1
Dorsal surface, Small 1-1.9 cm	6/1	0/0	6/1	9/2
Dorsal surface, Ulcerated, large >or=4 cm	0/0	0/0	0/0	1/1
Face, Medium 2-3.9 cm	0/0	0/0	0/0	1/1
Face, Small 1-1.9 cm	0/0	0/0	0/0	3/1
Face, Ulcerated, medium 2-3.9 cm	0/0	0/0	0/0	1/1
Face, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	5/1
Hind limb/right, Large >or=4 cm	0/0	0/0	1/1	0/0
Inguinal region/left, Large >or=4 cm	3/1	0/0	0/0	0/0
Inguinal region/left, Mass > 10cm	3/1	0/0	0/0	0/0
Inguinal region/left, Medium 2-3.9 cm	0/0	0/0	1/1	0/0
Inguinal region/left, Small 1-1.9 cm	0/0	0/0	1/1	0/0
Inguinal region/right, Large >or=4 cm	0/0	3/1	7/1	8/1
Inguinal region/right, Medium 2-3.9 cm	0/0	5/1	5/1	2/1
Inguinal region/right, Small 1-1.9 cm	0/0	0/0	1/1	1/1
Inguinal region/right, Ulcerated, large >or=4 cm	0/0	1/1	3/1	3/1
Lumbar region, Large >or=4 cm	0/0	0/0	13/1	0/0
Lumbar region, Mass > 10cm	0/0	0/0	1/1	0/0
Lumbar region, Medium 2-3.9 cm	0/0	0/0	22/1	0/0
Sacral region, Large >or=4 cm	0/0	0/0	1/1	0/0
Sacral region, Mass > 10cm	0/0	0/0	1/1	0/0
Shoulder/left, Large >or=4 cm	0/0	0/0	0/0	7/1
Shoulder/left, Medium 2-3.9 cm	0/0	0/0	0/0	5/3
Shoulder/left, Small 1-1.9 cm	0/0	0/0	0/0	29/3
Shoulder/left, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	4/1
Shoulder/right, Large >or=4 cm	0/0	0/0	0/0	2/1
Shoulder/right, Medium 2-3.9 cm	0/0	0/0	0/0	16/1
Shoulder/right, Small 1-1.9 cm	0/0	0/0	0/0	3/1
Thoracic region, Large >or=4 cm	0/0	0/0	0/0	5/1
Thoracic region, Medium 2-3.9 cm	0/0	0/0	0/0	8/2
Thoracic region, Small 1-1.9 cm	0/0	0/0	1/1	10/4
Thoracic region, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	6/1
Ventral surface, Large >or=4 cm	0/0	2/1	1/1	0/0
Ventral surface, Medium 2-3.9 cm	0/0	4/1	4/1	0/0
Ventral surface, Small 1-1.9 cm	0/0	2/1	1/1	0/0

*Number of times observed/Total number of animals affected

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Summary of Mass Findings* - FEMALE
 Weeks 2 to 100

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Alive at Start of Interval	65	65	55	75
Mass				
Abdominal region, Large >or=4 cm	0/0	22/2	0/0	12/1
Abdominal region, Mass > 10cm	0/0	5/1	0/0	0/0
Abdominal region, Medium 2-3.9 cm	5/1	15/4	25/2	4/1
Abdominal region, Small 1-1.9 cm	18/1	9/3	4/1	4/1
Abdominal region, Ulcerated, medium 2-3.9 cm	3/1	0/0	0/0	0/0
Abdominal region, Ulcerated, small 1-1.9 cm	2/1	0/0	0/0	0/0
Anogenital region, Large >or=4 cm	18/3	36/4	75/9	29/7
Anogenital region, Mass > 10cm	6/3	6/3	2/2	0/0
Anogenital region, Medium 2-3.9 cm	54/9	82/10	61/10	36/9
Anogenital region, Small 1-1.9 cm	20/5	24/7	36/8	53/10
Anogenital region, Ulcerated, large >or=4 cm	4/2	2/1	6/2	12/6
Anogenital region, Ulcerated, medium 2-3.9 cm	6/2	0/0	1/1	1/1
Anogenital region, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	1/1
Axillary region/left, Large >or=4 cm	25/3	62/10	124/13	99/17
Axillary region/left, Mass > 10cm	2/2	3/2	2/2	0/0
Axillary region/left, Medium 2-3.9 cm	57/6	154/21	153/33	129/33
Axillary region/left, Small 1-1.9 cm	57/6	104/21	171/34	157/39
Axillary region/left, Ulcerated, large >or=4 cm	0/0	7/2	2/2	18/10
Axillary region/left, Ulcerated, medium 2-3.9 cm	0/0	1/1	9/5	31/10
Axillary region/left, Ulcerated, small 1-1.9 cm	0/0	6/1	12/3	10/4
Axillary region/right, Large >or=4 cm	9/2	118/14	135/17	133/19
Axillary region/right, Mass > 10cm	0/0	5/2	3/2	0/0
Axillary region/right, Medium 2-3.9 cm	73/8	126/21	212/35	149/29
Axillary region/right, Small 1-1.9 cm	63/8	154/25	144/32	144/39
Axillary region/right, Ulcerated, large >or=4 cm	4/1	3/1	8/3	22/8
Axillary region/right, Ulcerated, medium 2-3.9 cm	5/3	8/2	2/2	20/9
Axillary region/right, Ulcerated, small 1-1.9 cm	2/1	9/1	0/0	9/3
Cervical region, Large >or=4 cm	20/2	3/1	19/2	23/5
Cervical region, Mass > 10cm	6/1	0/0	0/0	0/0
Cervical region, Medium 2-3.9 cm	7/2	59/6	41/6	22/7
Cervical region, Small 1-1.9 cm	3/2	36/7	14/5	10/6
Cervical region, Ulcerated, large >or=4 cm	1/1	0/0	2/1	1/1
Cervical region, Ulcerated, medium 2-3.9 cm	0/0	0/0	1/1	0/0
Dorsal surface, Large >or=4 cm	0/0	1/1	0/0	2/1
Dorsal surface, Medium 2-3.9 cm	11/1	0/0	0/0	1/1
Dorsal surface, Small 1-1.9 cm	21/1	0/0	0/0	3/1
Dorsal surface, Ulcerated, large >or=4 cm	0/0	1/1	0/0	2/1
Face, Small 1-1.9 cm	0/0	4/1	0/0	0/0
Face, Ulcerated, medium 2-3.9 cm	0/0	1/1	0/0	0/0
Face, Ulcerated, small 1-1.9 cm	0/0	5/1	0/0	0/0
Hind limb/left, Medium 2-3.9 cm	0/0	0/0	0/0	8/1
Hind limb/left, Small 1-1.9 cm	0/0	0/0	0/0	12/2
Hind limb/right, Small 1-1.9 cm	0/0	0/0	0/0	8/1
Inguinal region/left, Large >or=4 cm	4/1	96/15	162/17	116/13
Inguinal region/left, Mass > 10cm	1/1	11/5	11/5	1/1
Inguinal region/left, Medium 2-3.9 cm	58/4	210/22	104/18	70/18
Inguinal region/left, Small 1-1.9 cm	13/3	53/18	99/23	74/19
Inguinal region/left, Ulcerated, large >or=4 cm	0/0	2/2	6/3	3/2
Inguinal region/left, Ulcerated, medium 2-3.9 cm	0/0	0/0	2/1	2/2
Inguinal region/left, Ulcerated, small 1-1.9 cm	1/1	0/0	1/1	4/3
Inguinal region/right, Large >or=4 cm	20/4	96/11	81/12	99/16
Inguinal region/right, Mass > 10cm	2/1	5/3	1/1	0/0
Inguinal region/right, Medium 2-3.9 cm	37/5	159/21	94/21	109/22
Inguinal region/right, Small 1-1.9 cm	23/4	74/16	59/14	68/22
Inguinal region/right, Ulcerated, large >or=4 cm	5/2	1/1	5/3	16/7
Inguinal region/right, Ulcerated, medium 2-3.9 cm	1/1	1/1	3/2	2/2

*Number of times observed/Total number of animals affected

(b) (4) Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

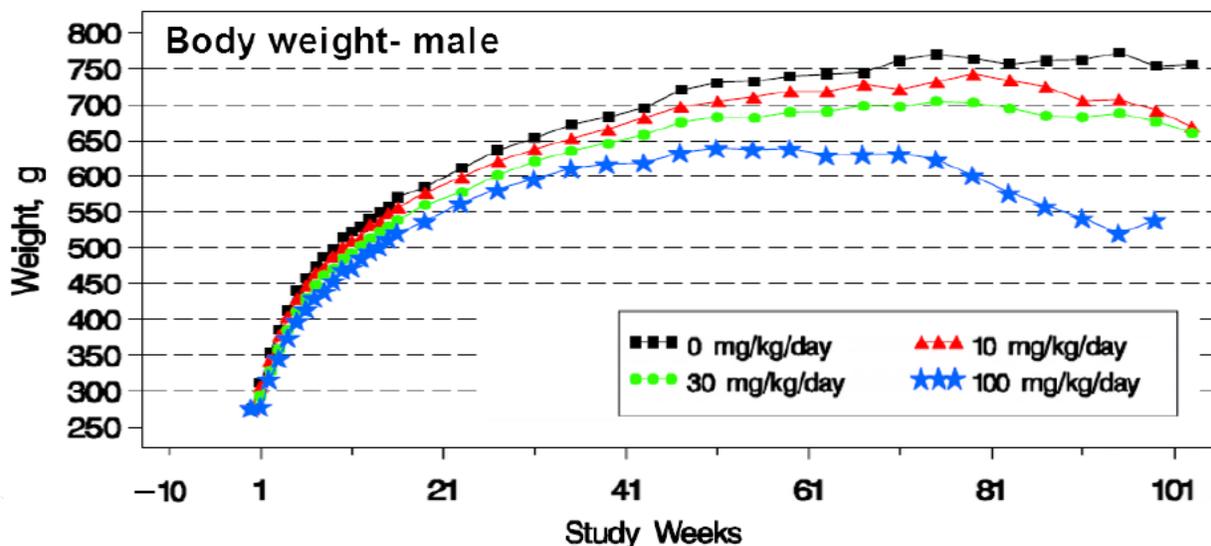
Summary of Mass Findings* - FEMALE
 Weeks 2 to 100

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Mass				
Inguinal region/right, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	1/1
Shoulder/left, Large >or=4 cm	0/0	2/1	0/0	0/0
Shoulder/left, Medium 2-3.9 cm	4/1	11/1	0/0	0/0
Shoulder/left, Small 1-1.9 cm	12/1	13/1	4/1	0/0
Shoulder/left, Ulcerated, large >or=4 cm	0/0	0/0	0/0	2/1
Shoulder/left, Ulcerated, medium 2-3.9 cm	8/1	0/0	0/0	1/1
Shoulder/left, Ulcerated, small 1-1.9 cm	0/0	0/0	0/0	1/1
Shoulder/right, Large >or=4 cm	0/0	14/1	5/1	3/1
Shoulder/right, Mass > 10cm	0/0	0/0	0/0	1/1
Shoulder/right, Medium 2-3.9 cm	0/0	2/1	3/1	6/1
Shoulder/right, Small 1-1.9 cm	0/0	5/1	3/1	6/1
Shoulder/right, Ulcerated, large >or=4 cm	0/0	0/0	0/0	1/1
Thoracic region, BI Large Ulcerated >4cm	0/0	0/0	0/0	1/1
Thoracic region, Large >or=4 cm	4/1	1/1	30/4	14/3
Thoracic region, Mass > 10cm	1/1	0/0	0/0	0/0
Thoracic region, Medium 2-3.9 cm	13/2	4/1	34/7	9/3
Thoracic region, Small 1-1.9 cm	9/3	5/1	13/5	7/4
Thoracic region, Ulcerated, large >or=4 cm	0/0	0/0	0/0	2/1
Thoracic region, Ulcerated, medium 2-3.9 cm	0/0	0/0	1/1	4/2
Thoracic region, Ulcerated, small 1-1.9 cm	0/0	0/0	3/1	9/2
Ventral surface, Small 1-1.9 cm	1/1	0/0	0/0	0/0
Ventral surface, Ulcerated, small 1-1.9 cm	1/1	0/0	0/0	0/0

*Number of times observed/Total number of animals affected

Body Weights

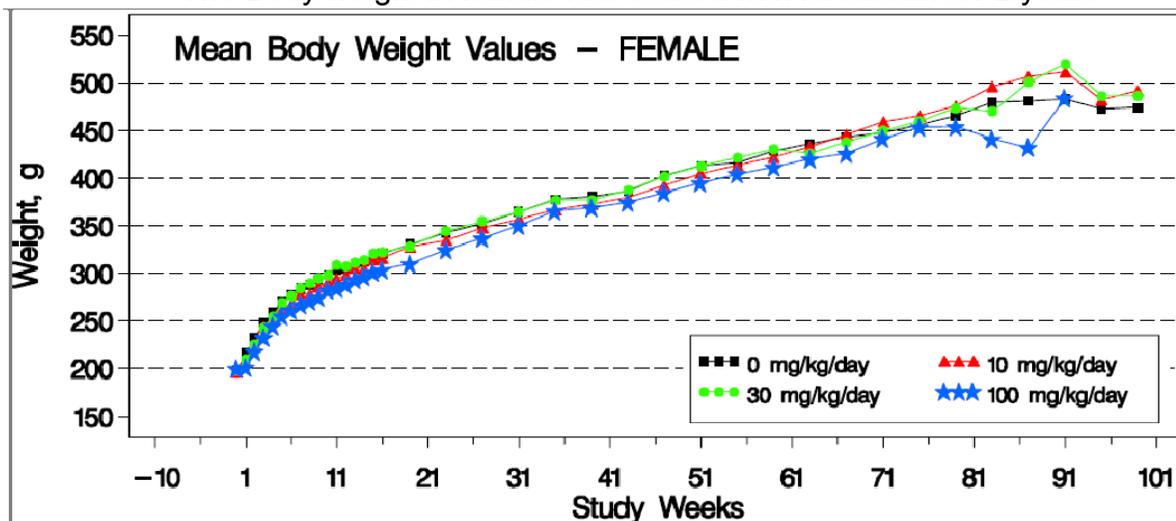
- There was a significant decrease in BW in males at 30 and 100 mg/kg (up to 28%, at 55x the clinical dose of 10 mg BID, based on AUC) in the main and TK animals.



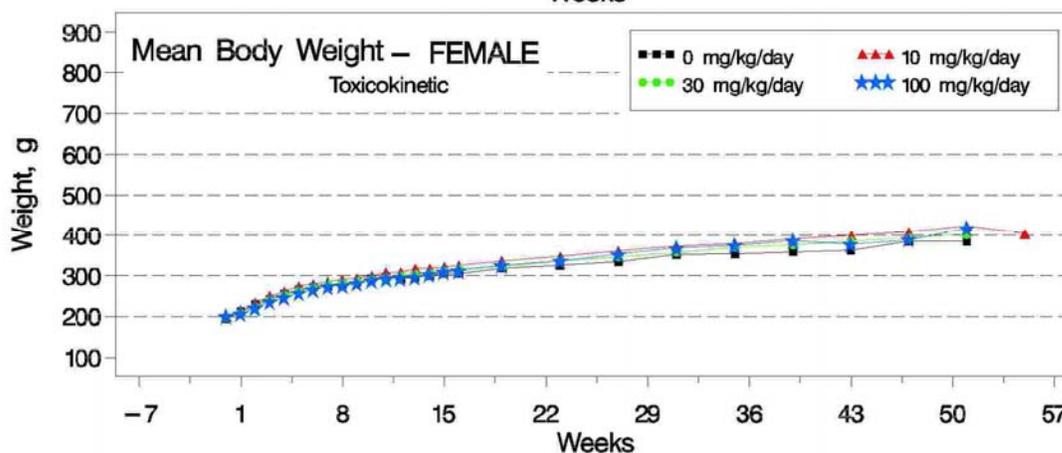
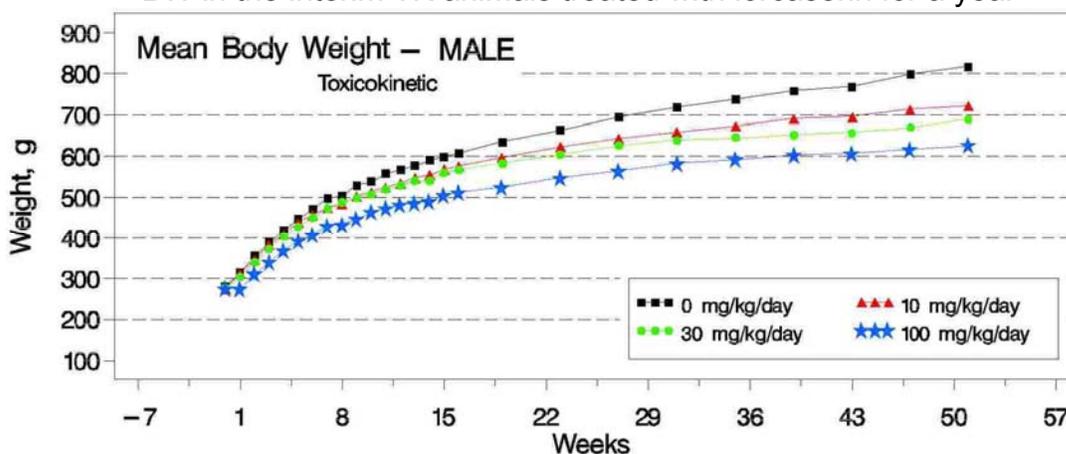
- There was no notable effect on BW in female rats. Although the female rats at 100 mg/kg had a lower BW trend, the BW at 10 and 30 mg/kg tend to be higher relative to the controls. There was no drug-related effect in females in the TK study.
- The decrease in BW in males suggests that lorcaserin was producing its anticipated pharmacological effect in males at ≥ 30 mg/kg but not in females.
- The lack of significant lorcaserin related effect on BW in female rats but lower BW trend in male rats is consistent with other studies in rats. Thus the potential

additive effect of large number of mammary tumors on BW of female rats was unlikely to be significant. It should be noted that the BW of TK female rats was also unaffected by lorcaserin. For some reason, lorcaserin pharmacodynamic effect in female rats is minimal in spite of higher plasma exposure than male rats. Whether there is a gender effect on brain lorcaserin levels is uncertain.

The Body weight in female rats treated with lorcaserin for 2 years

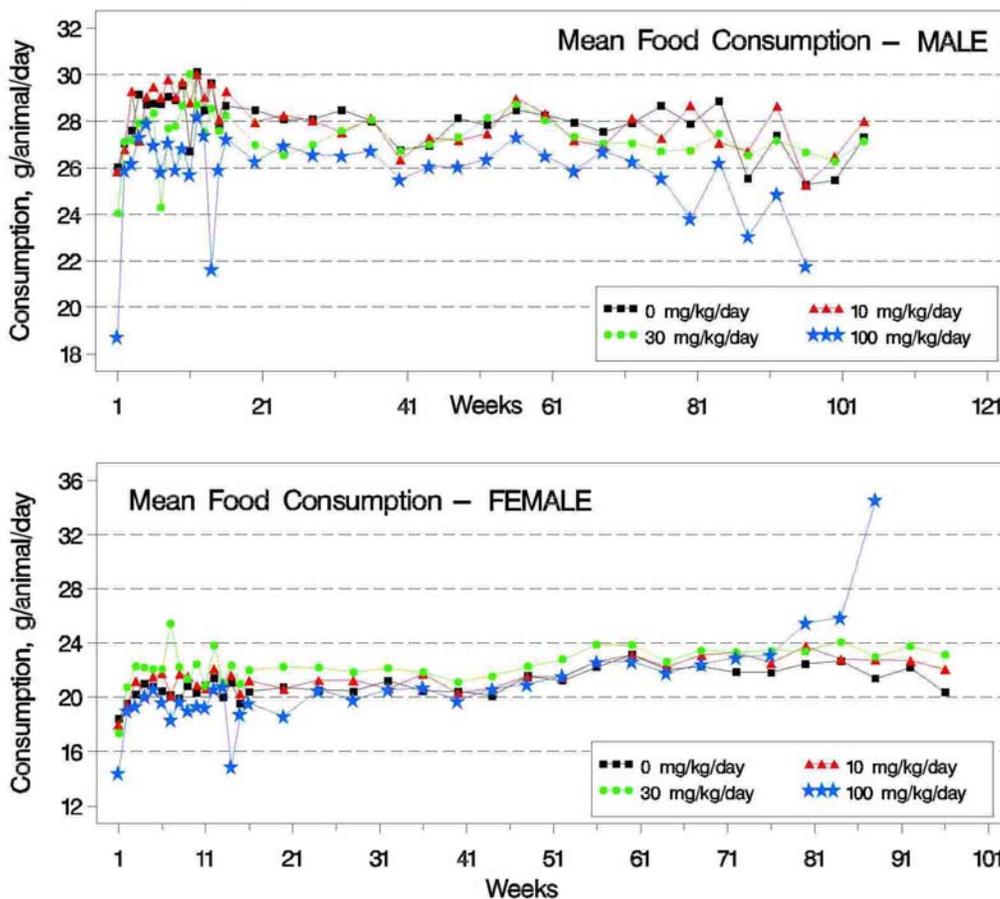


BW in the interim TK animals treated with lorcaserin for a year



Food Consumption

- There was a drug-related decrease in food intake in both sexes during WK 1 that was significant at ≥ 30 mg/kg in both sexes. However, the food intake returned to control levels with time more so in females. Food intake in males at 100 mg/kg tend to be lower than controls which corresponded to lower BW noted in HD males. Lack of drug effect on food intake in females corresponded with absence of change in BW noted earlier in female rats.



Serological Health Screen:

- Serum samples were collected from all animals for potential infections caused by common laboratory microorganisms listed in the table below at (b) (4) testing facility.
- All serological assays were negative suggesting that the high incidence of mammary and brain tumors were not influenced by other health issues (viral, microbial infections) in the 2-year rat study.

Name	Taxon	Report Abbrev.
Sendai virus	Parainfluenza	SEND
Pneumonia virus of mice	Paramyxo	PVM
Mouse encephalomyelitis	Picorna	GDVII
Kilham rat virus	Parvo	KRV
Toolan's H-1 virus	Parvo	H-1
Reovirus type 3	Reo	REO
Lymphocytic choriomeningitis virus	Arena	LCMV
Rat sialodacryoadenitis virus	Corona	SDAV
<i>Mycoplasma pulmonis</i>	<i>Mycoplasma</i>	MPUL

Neuroendocrine Hormone Assessment

Due to the high incidence of mammary tumors in lorcaserin treated female rats and in male rats, the potential role of 5HT_{2C} on neuroendocrine hormones such as serum prolactin, estradiol, TSH was investigated in several surviving TK animals. The sponsor also used immunohistochemistry staining methods to identify prolactin positive cells in the pituitary and mammary tissue in the TK animals maintained on lorcaserin for additional 3 (males) to 4 weeks (females). Only the summary data/description of the immunohistochemistry data was provided.

For serum prolactin levels, blood samples were collected from nonfasted TK rats between 9 to 11 AM from descending aorta under isoflurane anesthesia. In female rats blood samples were collected in diestrus. Estrus cycle was also determined by vaginal smears. The order of blood collection was randomized to reduce handling or time effect. The collected blood samples were centrifuged and divided into two aliquots and frozen at -70°C. One of the samples was shipped to (b) (4). Serum prolactin and estradiol in females and prolactin in males were measured per GLP guidelines. The second batch of samples was stored at (b) (4) for possible future analysis. The leftover serum from TK animals was used to determine Thyroid Stimulating Hormone (TSH) by a non-GLP method. The pituitary and mammary tissue Prolactin Labeling Index (PLI) was defined by the number of prolactin positive cells / number of mammary cells counted in the respective tissues. The number of animals used in the control, LD, MD and HD were 5, 14, 14 and 10 TK female rats at week 56.

Prolactin, estradiol levels, estrus cycle and TSH levels in TK rats:

- In males, serum prolactin levels were reduced by approximately 50% in the lorcaserin treated males relative to control males (24 vs. 58 ng/ml in control males) at week 56. It should be noted that MD and HD male rats had higher incidence of mammary tumors than the controls.
- In females, there was no significant difference in the prolactin levels among groups. The basal prolactin levels in female rats were nearly 2 fold higher than males (115 vs. 58 ng/ml in males).
- There was no difference in serum estradiol levels among groups which was about 2 pg/ ml.
- There was also no apparent effect on estrus cycle in female rats.
- TSH levels measured in couple of males and female TK rats per group appear to be similar among groups ranging from ~ 0.2 to 1.8 ng/ml.

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Endpoint	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Mean Cycle Length (Days)	5.9	2.02	4	6.2	2.77	11	7.2	3.88	14	7.1	1.52	8
No. of Cycles (Count)	1.5	0.58	4	2.7	1.10	11	2.4	1.28	14	1.8	0.71	8

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 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Toxicokinetic Neuroendocrine Hormone Values - MALE

Endpoint	Interval of Study	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Prolactin ng/mL	Week 55	57.87	32.562	6	28.24 ^b	12.479	14	29.93 ^b	10.908	10	23.69 ^b	16.140	14

Summary of Toxicokinetic Neuroendocrine Hormone Values - FEMALE

Endpoint	Interval of Study	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Prolactin ng/mL	Week 56	114.78	79.726	5	129.91	55.678	14	106.12	67.995	13	116.62	62.920	10
Estradiol pg/mL	Week 56	2.0	0.00	5	3.7	3.10	14	2.2	0.60	13	2.0	0.00	10

N - Number of measures used to calculate mean ^bSignificantly different from control; (p<0.01)
 SD - Standard Deviation

Prolactin Positive Staining of Pituitary gland:

- There were no apparent differences in the number of prolactin positive staining cells in male rats suggesting no notable drug effect on prolactin releasing cells in pituitary. The prolactin labeling index was similar among groups in male rats.
- The number of prolactin positive staining cells appeared to be slightly higher in female treated with 30 and 100 mg/kg but the significance of that is not clear since, a) the number of animals in the control group was smaller (5 vs. 14) relative to treated group, b) the number of positive staining cells was only slightly increased with no clear dose-dependency, c) mammary gland prolactin positive staining cells were reduced at HD and similar among control, LD and MD females, e) previous studies in intact and ovariectomized rats had found no notable difference between lorcaserin treated and control females.

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 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Prolactin Positive Stained Cell Counts in the Pituitary Gland - MALE

Endpoint	Estrous Stage	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Prolactin Labeling Index (%)	All Stages	26.30	6.423	6	28.65	5.910	14	23.58	8.926	11	27.52	7.303	14

(b) (4) Study Number 900-063
 A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Prolactin Positive Stained Cell Counts in the Pituitary Gland - FEMALE

Endpoint	Estrous Stage	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Prolactin Labeling Index (%)	All Stages	60.56	7.566	5	70.42	13.442	14	79.01 ⁹	14.942	14	79.82 ⁹	11.268	10

N - Number of measures used to calculate mean ⁹Significantly different from control; (p<0.05)
 SD - Standard Deviation

Prolactin Stain of the Pituitary Glands			
Females			
Toxicokinetic			
Dose levels (mg/kg/day)	10	30	100
Number examined	14	14	10
Prolactin stain, increased	8 (57%)	7 (50%)	7 (70%)
Prolactin stain, decreased	2 (14%)	1 (7%)	0 (0%)
Prolactin stain, normal	4 (29%)	6 (43%)	3 (30%)

Prolactin Positive Staining cells of the Mammary gland (immunohistochemistry staining)

- The incidence and severity of prolactin positive staining cells were similar among control, LD and MD and 40% lower in the HD female rats. No actual data was provided.
- To quote the sponsor “*the mammary gland lobular hyperplasia with atypia, and benign and malignant mammary tumors were primarily prolactin negative. There was no correlation between incidence of mammary gland prolactin stain and the incidence of pituitary gland prolactin stain in females at any dose level*”.

Immunohistochemistry staining of brain tissues in rats with astrocytoma:

In an effort to characterize the lineage of brain astrocytoma in 20 male rats with brain tumors (astrocytoma), new sections of the brain tissue tumors were pretreated with Tris buffer solution and incubated with the primary antibodies. Three immunohistochemistry stains/antibodies used in the study were GFAP (glial fibrillary acidic protein), ED-1 (anti-CD68 clone ED-1) and MCHII (major histocompatibility complex II). Slides were incubated with a secondary antibody conjugated to horse radish peroxidase and detected via enzymatic color reaction using Ventana's diaminobenzidine detection kit (GLP, at (b) (4) facility). The sponsor had provided little or no description of the method and the immunohistochemistry stains. The background information on the immunohistochemistry stains described below was acquired from the literature by the reviewer.

Brief description of the stains:**GFAP**

GFAP is the predominant component of astrocyte intermediate filaments in the central nervous system. Any cell staining positive to GFAP would identify the cell as an astrocyte in humans. Although it is known to be a marker for astrocytes, it has also been detected in the glial cells of the enteric nervous system and some Schwann cells in the peripheral nervous system. It is involved in many cellular functioning processes, such as cell structure and movement, cell communication, and the functioning of the blood brain barrier leading to conclusion that GFAP may be involved in the long term maintenance of normal CNS myelination. Astrocytes lacking GFAP do not form the extensions normally present in neurons.

ED-1

ED-1 is an anti CD-68 antibody used to stain various white blood cells. This stain has been effectively used to characterize many macrophage and monocytes expressing CD68 in normal and neoplastic tissue using light microscopy. Since CD-68 is an intracellular glycoprotein found in plasma and cell membrane of many cell types (macrophages, monocytes, basophils, neutrophils and large lymphocytes) the stain will bind any such cell in brain tissue.

MCHII

MHC II represents external/ foreign peptides/proteins digested by the immune cells. As such they are generally found in antigen presenting cells. Antibodies against MCH II polypeptide is used to identify and to differentiate types of lymphocytes (T and B cells) and macrophages.

Results

- Out of 20 rats with brain tumors used in the test, 7/20 did not have any tumors (astrocytoma) present on the newly prepared tissue sections. The remaining 13 with tumors were all positive for ED-1 antibody (anti-CD 68) suggesting cells with macrophage/ histiocytes cell lineage and only one positive for MCHII (a HD male, 4141).
- None of the sections were positive for GFAP, marker for human astrocyte

- According to the sponsor’s description, there was no distinctive staining pattern among the different astrocytoma. They appeared solid homogenous neoplastic cell population, less cellular infiltrating pattern with prominent perivascular cuffing or necrotic areas surrounded by a palisading cell formation.
- According to the sponsor, some astrocytes did stain positive with GFAP within the tumors but these cells were part of the normal brain tissue and not neoplastic cells
- Based on strong reactivity to ED-1, neoplastic cells in the tumors appeared to be of macrophage/histiocyte lineage and not from astrocytes leading to the conclusion that “lorcaserin dose-dependably increased spontaneously” forming brain tumors.

Immunohistochemistry Evaluation of Malignant Astrocytomas in the Brain			
Antibody	ED 1	MHC II	GFAP
Control males			
Animal # 1145	No tumor on sections		
Group 3 (30 mg/kg) males			
Animal # 3113	+++	-	-
Animal # 3131	No tumor on sections		
Animal # 3160	++	-	-
Animal # 3165	+++	-	-
Group 4 (100 mg/kg) males			
Animal # 4101	++	-	-
Animal # 4110	No tumor on sections		
Animal # 4136	+	-	-
Animal # 4140	++++	-	-
Animal # 4141	++	+	-
Animal # 4162	+	-	-
Animal # 4170	No tumor on sections		
Animal # 4159	+++	-	-
Group 8 (100 mg/kg) males			
Animal # 8102 (TK)	+	-	-
Group 2 (10 mg/kg) females			
Animal # 2203	+++	-	-
Animal # 2242	++	-	-
Group 7 (30 mg/kg) females			
Animal # 7210 (TK)	No tumor on sections		
Group 4 (100 mg/kg) females			
Animal # 4238	No tumor on sections		
Group 8 (100 mg/kg) females			
Animal # 8211 (TK)	++	-	-
Animal # 8212 (TK)	No tumor on sections		
-: negative staining +: approximately less than 10% of neoplastic cells positive ++: positive neoplastic cells represent approximately between 10 and 40% of total neoplastic cell population +++: positive neoplastic cells represent approximately between 40 and 70% of total neoplastic cell population ++++: approximately more than 70 % of neoplastic cells positive TK: toxicokinetic animals			

In summary, brain tumors identified in 20 rats from the main and TK study were processed for immunohistochemistry staining (GFAP, ED1 and MHCII). Out of 20 brain tumors, only 13 had tumors present on the newly prepared brain tumor section. This itself suggests that brain tumors can be easily missed with new tissue sectionings. All of these 13 tumors stained positive with ED1, a marker of macrophage/ histiocyte cell lineage and one stained positive for MHCII, a marker for antigen presenting cells. None were positive for GFAP, a marker for human astrocyte, however various number of astrocytes were positive for GFAP within the tumors but these cells were part of the normal brain tissue and not neoplastic. Based on the findings, the sponsor concluded that the neoplastic cells in the brain tumors were of macrophage/histiocyte lineage and did not derive from astrocytes as is the case in humans. The sponsor's finding appears to be in agreement with the literature that reports a lack of GFAP staining in rat astrocytoma, and with a paper by Nagatani et al suggesting that rat astrocytoma has a monocytic or neuronal progenitor lineage.

The study has several shortcomings that cast a shadow on utility of the findings, a) at least 7/20 sections did not contain tumors/neoplastic cells, suggesting that tissues collected/used in the immunohistochemistry might have been collected peripheral to the tumor site which may include a high macrophage population, b) although some "normal" astrocytes within the tumor were GFAP positive, it is not clear if the sponsor used a positive control to show that the assay was carried out properly, c) the sponsor provided no evidence that these tumors from whatever origin were not drug-related, d) the emergence of drug-related, fatal brain neoplasms in rats is evidence of a tumorigenic mode of action which signals a tumor hazard to human subjects, regardless of the specific cellular lineage of the neoplasm. Evidence that key events of that tumorigenic mode of action are irrelevant to human biology would be a more persuasive argument that human risk is negligible.

Hematology:

- There was slight elevation of neutrophils and monocytes in both sexes at LD and MD males where sufficient live animals were available for sampling at the time of necropsy.
- The numbers of reticulocytes in both sexes were increased by as much as 2 fold relative to the controls, in response to slightly lower number of RBCs.
- These findings are consistent with the 6-month rat study.

(b) (4) Study Number 900-063
 A 2-Year Carcinogenicity Study of AP0356 Given by Oral Gavage to Rats

Summary of Hematology Values - MALE

Endpoint	Interval of Study	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Leukocytes 10 ³ /μL	Terminal	11.53	4.249	21	15.48 ^a	3.903	16	19.25 ^b	7.412	20	11.88	3.528	4
Erythrocytes 10 ⁶ /μL	Terminal	7.815	0.7242	21	6.823 ^a	1.4478	16	6.876 ^a	1.2147	20	6.745	0.3743	4
Hematocrit %	Terminal	48.00	3.697	21	43.26	9.792	16	43.95	7.417	20	43.53 ^b	1.919	4
Absolute Reticulocytes 10 ³ /μL	Terminal	188.26	70.669	21	413.96 ^b	220.847	16	396.36 ^b	188.317	20	393.18 ^b	36.030	4
Neutrophils 10 ³ /μL	Terminal	4.292	3.0663	21	6.943 ^a	2.8634	16	10.378 ^b	6.6572	20	4.683	2.0160	4

N - Number of measures used to calculate mean ^aSignificantly different from control; (p<0.05)
 SD - Standard Deviation ^bSignificantly different from control; (p<0.01)

Summary of Hematology Values - FEMALE

Endpoint	Interval of Study	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Leukocytes 10 ³ /μL	Terminal	7.63	2.843	23	11.38 ^a	4.178	12	10.14	5.704	5	NA	NA	0
Platelets 10 ³ /μL	Terminal	839.0	222.93	23	1050.4 ^a	285.52	12	1290.4 ^b	209.05	5	NA	NA	0
Absolute Reticulocytes 10 ³ /μL	Terminal	199.65	86.749	23	304.64 ^a	141.973	12	293.22	94.532	5	NA	NA	0
Neutrophils 10 ³ /μL	Terminal	2.473	1.6037	23	5.679 ^b	3.1428	12	4.134	3.7658	5	NA	NA	0
Monocytes 10 ³ /μL	Terminal	0.292	0.1619	23	0.554 ^b	0.2304	12	0.652 ^b	0.3871	5	NA	NA	0

N - Number of measures used to calculate mean ^aSignificantly different from control; (p<0.05)
 SD - Standard Deviation ^bSignificantly different from control; (p<0.01)
 NA - Not available/not applicable

Gross Pathology

Male rats

- Dose-dependent increase in subcutis masses in male rats
- Dose-related increase in incidence of discoloration of liver, liver masses
- Dose-related increase in discoloration of lungs and enlarged spleen
- Dose-related increase in incidence of palmar/plantar ulcers was noted in the feet. The greatest number of palmar ulcerations were seen in the LD where survival rate was greater than MD and HD. The lower incidence of ulcers in HD males was therefore likely due to high incidence of premature death. It is not clear why rats treated with lorcaseerin were more susceptible to foot ulceration than controls.

Treatment-Related Macroscopic Changes Terminal Necropsy*** Male				
Dose level: mg/kg/day	0	10	30	100
Number of Animals Examined	65	65	65	75
Liver				
Discoloration/foci*	3	10	9	10
Mass	0	1	3	6
Skin, Subcutis				
Mass	6	15	30	45
Lung				
Discoloration/foci**	2	2	4	18
Foot/Feet				
Ulcer, plantar/palmar	15	33	34	28
Spleen				
Enlarged	2	4	10	14
* Brown, red, tan, white, yellow, gray discoloration or foci combined.				
** Tan, white discoloration or foci combined.				
*** Scheduled and unscheduled deaths.				

Female rats

- Dose-dependent increase in incidence of subcutis masses
- Majority of the masses appeared to be mammary tumors. Although similar observation was made in male rats as well, the incidences of these masses were 4 fold higher in females. It should be noted that lorcaseerin exposure in females was about 1.5 fold greater than males. The similar incidence of subcutaneous masses between HD and MD females (n=221 vs. 225 in MD) may have been impacted by greater premature mortality in HD rats.
- Dose-related increase in the incidence and severity of lung nodules and discoloration
- Dose-related increase in incidence and severity enlarged spleen
- Drug-related increases in incidence of palmar/plantar ulcers in the feet were seen at all doses of lorcaseerin relative to the control. The greatest increase in foot ulcers was in the LD where survival rate was greater than MD and HD. The pattern was

similar to male rats suggesting that high premature mortality was affecting the total incidence of foot ulcers at 30 and 100 mg/kg.

Direct Test-Article Related Macroscopic Findings Terminal Necropsy Female				
Dose level: mg/kg/day	0	10	30	100
Number Examined	65	65	65	75
Skin, subcutis*				
Total number of masses				
-present	60	179	225	221
Total number of subcutaneous masses compatible with mammary masses				
-present*	59	174	221	217
Lung				
Nodule				
-present	1	1	5	4
-mild	1	1	2	3
-moderate	0	0	0	1
-severe	0	0	3	0
Mass				
-present	0	0	3	0
Discoloration, white				
-moderate	0	0	1	3
Discoloration, tan				
-mild	0	0	1	8
-moderate	0	0	1	3
-severe	0	0	0	4
Focus/foci, white				
-mild	0	0	2	5
-moderate	0	0	2	0
Focus/foci, tan				
-moderate	1	0	0	3
-severe	1	0	0	2
-mild	0	0	0	1
* Total incidences in respective number of animals examined.				
41 out of 65 at 0 mg/kg/day	61 out of 65 at 30 mg/kg/day			
54 out of 65 at 10 mg/kg/day	69 out of 75 at 100 mg/kg/day			

Macroscopic Findings Terminal Necropsy Female				
Dose level: mg/kg/day	0	10	30	100
Number of Females/Dose Group	65	65	65	75
Spleen				
Enlarged	2	7	12	30
-minimal	0	1	0	0
-mild	1	3	5	16
-moderate	0	3	5	13
-severe	1	0	2	1
Lymph node, axillary				
Enlarged	2	8	9	13
-minimal	0	0	1	0
-mild	1	4	7	11
-moderate	1	4	1	2
Lymph node, mandibular				
Enlarged	2	5	3	2
-mild	2	3	3	2
-moderate	0	2	0	0
Lymph node, inguinal				
Enlarged	0	3	7	5
-minimal	0	0	1	0
-mild	0	2	5	4
-moderate	0	1	1	0
-severe	0	0	0	1
Foot/feet				
Ulcer, plantar/palmar	8	22	17	11
-no grade	0	1	0	0
-minimal	1	1	1	0
-mild	4	11	4	8
-moderate	3	8	11	3
-severe	0	1	1	0

Histopathology

Comments on Peer Review:

The histopathology evaluations of male and female rats were performed by (b) (6) at (b) (4) respectively. It appears that slides in the bimonthly updates were reviewed by (b) (6) (b) (4). She is currently working for another company in (b) (4).

There appeared to be two external peer review pathologists. The first was (b) (6) of (b) (4). He peer reviewed all slides and all diagnoses from 10% of randomly selected control and HD male and female rats. All diagnosis for liver, kidney, lung, thyroid, spleen, epididymis, seminal vesicle, skin, skeletal muscle and subcutaneous masses for all male rats and mammary gland, liver, pituitary gland, lung, bone marrow, bone, adrenal gland, spleen, kidney, vagina and lymphoid tissues for all females. The heart tissue was not reviewed during the peer review. Differences in the opinions of the pathologist were resolved and agreement was reached on diagnosis.

The toxicokinetic animals were also peer reviewed by [REDACTED] (b) (4). He peer reviewed several tissues (tests, epididymides, mammary glands, pituitary gland, and brain) as well as all the neoplastic and hyperplastic from 4 randomly selected TK rats (rat #5104, 8115, 5202 and 8215). [REDACTED] (b) (6) served as senior principal study director from initiation of the study to March 25, 08. [REDACTED] (b) (6) served as senior study director with no dates provided.

According to the sponsor, the peer review results were in agreement with the interpretations of the study pathologists. It was not clear why the tabulated incidence of adenocarcinoma in the MD (43/57 vs. 35/65) and HD females (72/75 vs. 60/75) in final report were so different than the earlier reports. One would have expected a small increase or no change with availability of more necropsied rats. In contrast, the number decreased by 10 or more. The report did not explain the changes in the incidence of adenocarcinoma. Without an explanation from the pathologists and the sponsor, it can be surmised that the pathologists had experienced difficulty in discerning fibroadenoma from adenocarcinoma.

Neoplastic Findings

- Lorcaseerin treatment resulted in a number of neoplastic tumors in rats. The tumor type and incidence were different between male and female rats. Early mortality in female rats may have affected overall duration of treatment exposure. The exposure to parent drug at 10, 30 and 100 mg/kg in male rats were 5, 17 and 5x the clinical dose of 10 mg BID based on AUC. The exposure to parent drug at 19, 30 and 100 mg/kg in female rats were 7, 24 and 82x the clinical dose, based on AUC.
- Two HD female rats that were euthanized in extremis due to presence of mammary tumors (#4202 and 4212) had no record of mammary tumor in the histopath table.
- In male rats, there were a significant dose-related increase (trend) in the incidences of brain tumors (astrocytoma), malignant schwannomas, mammary fibroadenoma, combined mammary adenocarcinoma/fibroadenoma, skin squamous cell carcinoma, and thyroid follicular cell adenoma.
- In female rats, there were significant dose-related increases (trend) in the incidence of mammary adenocarcinoma, mammary fibroadenoma and lung carcinosarcoma and uterine with cervix benign glandular polyps.
- Tumors identified in males (brain tumors) and females (mammary tumors) in the main study were also seen in the TK animals. The incidence of adenocarcinoma in LD (7/14), MD(6/14) and HD (7/10) female rats at the interim TK were also higher than control TK females (0/5).
- Due to the high incidence of premature mortalities from mammary and brain tumors in rats, the sponsor submitted bimonthly reports of the incidences of mammary and brain tumors for both main and TK animals as they became available (per FDA request). Although the final report in the NDA did not contain the incidence of tumors in the TK animals, the summary of the bimonthly reports collected from earlier submissions are presented in this review.
- The incidence of tumors and p values (in parenthesis) for the trend analysis in male and female rats are provided in tables below

The incidences of tumors with statistical significance for trend and pair wise comparisons provided by the sponsor are listed in tables below.

Neoplastic tumors in male rats treated with lorcaserin ^a

(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in male rats		Lorcaserin dose, mg/kg				Dose Response
		0	10	30	100	
Brain	astrocytoma	1	0	4 ND	8 ^b p=0.0019	< 0.0001
	mixed glioma	0	0	0	1	NS
	reticularis	0	1	0	1	NS
	granular cell	0	0	1	1	NS
	oligodendroglioma	1	0	0	0	NS
	combined	2	1	5	20	
Liver	hepatocellular carcinoma	1	3	2	4	NS
	hepatocellular adenoma	1	1	2	6 p=0.030	p=0.033
	combined	2	4	4	10 p=0.0048	p=0.0012
Lung,	carcinosarcoma, sec.	0	1	1	0	NS
	fibrosarcoma, sec.	0	0	0	1	
	osteosarcoma, sec.	0	0	1	0	
	mesothelioma, sec.	1	1	0	0	
Mammary	adenocarcinoma,	0	0	2	2 NS	p=0.046
	fibroadenoma	0	1	4 NS	6 NS	p=0.0001
	combined	0	1	6 p=0.0131	8 p=0.0009	p< 0.0003
Pituitary, adenoma, pars distalis, benign		32	22 NS	22 NS	15 p=0.0003	p=0.0005
Skin, subcutis	benign fibroma	3	7 NS	11 p=0.017	17 p<0.0001	p<0.0001
	fibrosarcoma	1	0	0	0	
	combined	4	7	11	17	
Skin	squamous cell carcinoma,	0	0	4 NS	5 p=0.014	p=0.003
	papilloma	1	0	1	2	NS
	combined	1	0	5	7	
Schwannoma, all sites		0	0	2	9 p<0.0037	p<0.0001
Schwannoma, Subcutis alone			0	1	5 p=0.047	p=0.0053
Thyroid, follicular cell adenoma, benign		0	5 p=0.02	4 NS	8 p=0.0011	p=0.0035

^a Statistical analysis and p values in the table were provided by the FDA statistician, Dr. Jackson

^b One of the astrocytomas in the HD males was reclassified as infarct due to lymphocytic leukemia in an amendment to the NDA.

Neoplastic tumors in female rats treated with lorcaserin ^a
(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in female rats		Lorcaserin dose, mg/kg				Dose Response
		0	10	30	100	
Brain	astrocytoma	0	2	0	1	NS
	mixed glioma	0	0	0	1	NS
	combined	0	2	0	2	
Lung	carcinoma, secondary	0	4	9	6	NS
	carcinosarcoma, second	0	0	0	1	NS
	Combined	0	4	9	7	
Mammary	carcinosarcoma	0	0	0	1	NS
	adenocarcinoma	28	34 NS	35 p=0.02	60 p<0.0001	p < 0.0001
	fibroadenoma	20	47 p<0.0001	53 p<0.0001	45 p<0.0001	p < 0.0001
	combined	40	56 p<0.0001	61 p<0.0001	71 p<0.0001	p<0.0001
Skin,	fibrosarcoma, subcutis	0	0	0	2	p=0.067
Pituitary	adenoma, pars distalis, benign	50	46 (NS)	31 (p=0.001)	20 (p<0.0001)	p<0.0001
	carcinoma, pars distalis	0	2	0	0	NS
	combined	50	48	31	20	
Thyroid, adenoma follicular	2	2	4	3	p=0.03	
Uterus with cervix, benign glandular polyp	0	0 NS	3 NS	3 NS	p=0.003	

^a The statistical analysis and p values in the table were provided by the FDA statistician, Dr. Mathew Jackson.

NS = not significant

Brain Tumors (Astrocytoma)

Lorcaserin significantly increased brain astrocytoma in male rats in a dose-dependent manner (trend p<0.0001). There was no significant increase in brain tumors in female rats. The incidence of brain astrocytoma was significantly increased in HD males (10.67%, 55x the clinical dose of 10 mg BID based on AUC) and was numerically higher in the MD male (6.15%) vs. concurrent control (1%) and ^{(b) (4)} historical control data (0-5%). There were several cases of astrocytoma in the TK rats. Overall, out of 20 astrocytomas in rat study (main and TK, male and female rats), 19 were in the lorcaserin treated rats suggesting that astrocytoma was clearly a lorcaserin related effect and that astrocytoma can appear within a year of lorcaserin treatment in rats.

Brain astrocytoma in SD rats		Lorcaserin dose, mg/kg			
		0	10	30	100
Main study, astrocytoma	M	1/65	0/65	4/65	8/75
	F	0/65	2/65	0/65	1/75
TK study, astrocytoma	M	0/6	0/14	0/11	1/14
	F	0/5	0/14	1/14	2/10
Combined		1	2	5	12

The astrocytomas were characterized as a homogeneous neoplastic cell population with poorly defined borders and round nuclei. They were found in the fore, mid and hindbrain. According to the sponsor some of the cells around the surrounded vessels infiltrated the meninges or the surrounding preexisting neurons. Larger tumors had a palisading pattern which is considered to be secondary to necrosis. These tumors were fatal in nearly all cases (control and MD) except for some HD rats where they may have succumbed early to other tumors or causes (M#4101M, 4110, 4136, 4140, 4159 and F#4238).

In the original report (submitted with the NDA) the sponsor had identified 9 astrocytomas in HD males. However, in an amendment to the NDA, one of the previously identified astrocytoma was removed and reclassified as infarct in male #4111 due to lymphocytic leukemia. Since astrocytomas occasionally had infiltrated neurons and surrounding blood vessels, it is not clear if this reclassification was justified.

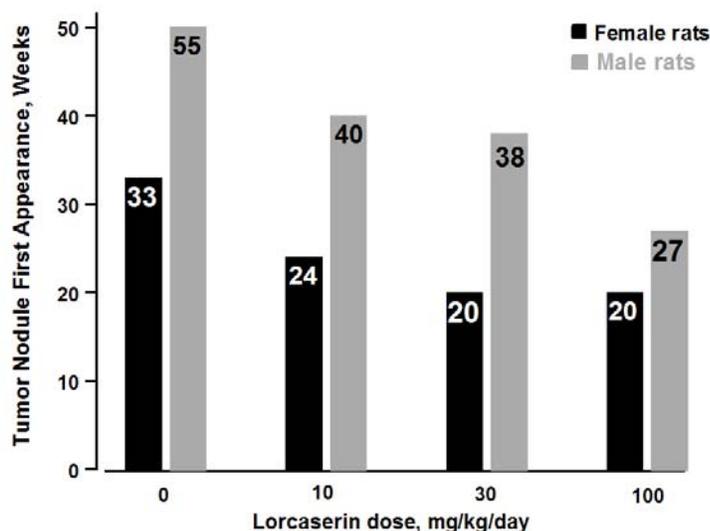
In an effort to identify the lineage of the brain astrocytes in male rats, 20 astrocytoma identified in the carci study were processed by immunohistochemistry staining (ED1, GFAP and MHCII). Out of 20 tumors, only 13 of the new slides had astrocytoma. Seven of the tumors were missed in the new slides. All the 13 slides stained positive for ED1 and none for GFAP and only one was positive for MHCII suggesting a macrophage/histiocyte lineage (ED1). The lineage of astrocytoma has been questioned for over 20 years and remains controversial. The high incidence of astrocytoma is a real concern since they were, a) generally lethal, b) 7 out of 20 tumors were missed suggesting that a slight change in sectioning could easily miss a brain tumor, c) the new tissue samples may have been closer to the periphery and closer to the surrounding vessels, d) lorcaserin significantly partitions to the brain (up to 35x the plasma). With no clinical data, it is assumed that the brain levels in humans is likely to be similar to that in monkeys (10x the plasma). The brain exposure in monkeys can vary from 3 to 23 fold, suggesting that large variability may also exist in humans, with some individuals having significantly more brain lorcaserin than others, translating to a greater clinical risk for some but not others.

In the final analysis, the numerical and statistically significant increase in astrocytoma at the mid- and high doses (17x and 55x clinical dose) was considered related to lorcaserin.. Actual safety margins based on brain concentrations of lorcaserin are unknown, because the degree of partitioning of lorcaserin to the CNS in human subjects is unknown.

Mammary tumors

In female rats, there was a dose-dependent and statistically significant increase in the incidence of fibroadenoma at all doses versus control (72%, 82%, 60% vs. 31%, in LD, MD and HD vs. C). Statistical significance for adenocarcinoma was achieved at the HD, although a numerical increase is seen at the LD and MD: (52%, 54% and 80% vs. 43%, in LD, MD and HD vs. C). The combined incidence of fibroadenoma and adenocarcinoma was significantly ($p < 0.0001$) increased at all dose levels, but combining these two tumor types is not necessary to achieve statistical significance at all doses for the increase in fibroadenoma. The mean ^{(b) (4)} historical data for mammary fibroadenoma (22 to 54%, mean=36) and adenocarcinoma (8.3 to 37%, mean=24%) appear to be lower than the control in this study.

In males, the incidence of mammary fibroadenoma was also increased in MD and HD. Although the incidence of adenocarcinoma was not significantly increased, the combined incidence of fibroadenoma and adenocarcinoma was significant in MD and HD males. Tumor nodule (all types) first appearance in males was notably later (7 to 17 weeks) than that in female rats (mammary tumors). The shortened duration of exposure to lorcaserin in female may explain why some tumors such as astrocytoma were not increased in female rats. Furthermore, since brain measurements of lorcaserin were made only in male rats, there might be gender effect on brain levels as well.



For some reason two HD females (4202 and 4212) that were euthanized due to mammary tumor burden, had not histopath record suggesting these animals ever had mammary tumors.

The exact mechanism by which lorcaserin increased the incidence of mammary tumors in rats was not well characterized by the sponsor. The mechanistic studies submitted with the NDA are described in section 10. It has been hypothesized that CNS active drugs may increase prolactin levels, leading to hyperplasia of mammary gland which can eventually lead to mammary tumors. This hypothesis was tested by the sponsor by measuring serum prolactin, estradiol and prolactin positive cells in the mammary and pituitary gland in the surviving TK animals at week 52. In male rats, the prolactin levels at all doses of lorcaserin were 48 to 59% lower than the corresponding control (57.8 ng/ml vs. 23.7 ng/ml in HD). In females, there were no notable differences in prolactin levels in control and lorcaserin treated rats (114.8 ng/ml vs. 116.62 ng/ml in HD females). Analysis of estradiol in female rats found no difference in estradiol levels among groups (2 pg/ml). The mean cycle length appeared to be slightly greater in MD and HD females (7.2 days) than control and LD females (5.9-6.2 days). When estradiol levels and estrous cycle were monitored, there was no notable effect on estradiol and the number of estrous cycle suggesting that estradiol was not involved in high incidence of mammary tumors in lorcaserin treated rats. Using immunohistochemistry, the sponsor found no apparent difference in the number of

prolactin positive cells in pituitary in male rats. In female rats, the numbers of prolactin positive cells were slightly higher in MD and HD. The percent prolactin positive cells in mammary glands were similar among control, LD and MD females but 40% lower in HD females. The reliability of this data is questionable since the numbers of control animals tested in the study were small (5 vs. 14 in the treated group). Analysis of serum prolactin in other toxicology and clinical studies found no notable change in prolactin levels in female rats. In ovariectomized rats supplemented with suprapharmacological doses of progesterone and estradiol (up to 20x the normal levels), an elevation in prolactin was seen in lorcaserin treated rats. In absence of evidence of direct elevation in prolactin levels in rats and humans, makes the ovariectomized rat model treated with pharmacological doses of estradiol/progesterone irrelevant and not resembling the animals in the study. The antipsychotic drug with high incidence of mammary tumors were shown to produce a robust and persistent increase in prolactin levels no matter what model was used.

According to the sponsor own statement, "*The mammary gland lobular hyperplasia with atypia, benign and malignant mammary tumors were primarily prolactin negative. There was no correlation between incidence of mammary gland prolactin stain and the incidence of pituitary gland prolactin stain in females at all dose levels.*" With decrease in prolactin in males or no change in lorcaserin treated females and 40% lower prolactin positive cells in mammary gland in females suggests that the lorcaserin induced mammary tumors in rats was independent of prolactin.

In the final analysis, lorcaserin significantly increased mammary fibroadenoma in females at all doses (< 7x the clinical dose of 10 mg BID on AUC basis) and at 100 mg/kg/d in males (55x the MRHD). The incidence of adenocarcinoma was increased only at 100 mg/kg/d of lorcaserin (82x the MRHD) but was numerically higher in MD than control and historical data. Oral administration of lorcaserin produced, a) no change in serum prolactin in the carci, b) no change in prolactin in the mechanistic studies in intact or ovariectomized female rats, c) increase in prolactin in ovariectomized female rats replenished with hormone in an altered model is irrelevant to the rat study d) no change in the number of prolactin positive staining cells in the pituitary or mammary gland, e) no change in the brain dopamine or serotonin levels [haloperidol → ↑dopamine; dexfenfluramine → ↑ serotonin], f) no change in estradiol, progesterone, FSH or LH levels. The main trust of the sponsor hypothesis and supportive studies aimed to explain a role for prolactin failed to show a positive link between prolactin and lorcaserin induced mammary tumors in rats.

Pituitary adenoma

The incidence of benign pituitary tumors of pars distalis (encompassing anterior pituitary) was significantly lower in the lorcaserin treated male (LD: 22/65, MD: 22/65, HD: 15/75) and female (LD: 46/65, MD: 31/65, HD: 20/75) than control male (32/65) and female rats (50/65). The incidence of benign pituitary tumors in the concurrent control males (49%) and females (77%) were within the ^{(b) (4)} historical data range for male (44 to 67%) and female SD rats (74 to 84.6%). In pairwise comparisons, the incidence was increased in males at 100 mg/kg and in females at ≥ 30 mg/kg. Pars distalis makes up the biggest section of the pituitary gland. It arises from Rathke's pouch surrounding pars nervosa from the brain. Pars distalis is under control of the brain and responsible for

release of several hormones [prolactin (ϵ -cells), GH(α -cells FSH(δ -cells), LH (δ -cells), TSH]. Prolactin released by the pars distalis of the pituitary gland is responsible for mammary gland development and milk secretion. Based on anatomical location and physiological function, a benign tumor of the pars distalis is likely to lead to excessive production these hormones, particularly prolactin leading to mammary gland hyperplasia and eventually to mammary tumor. The high incidence of pituitary tumors in the controls rats may explain the unusually higher incidence of mammary tumor in female rats (43%) and feminization of the control male rats (~50%). Most of the CNS active drugs that cause mammary tumors have been frequently associated with pituitary tumors.

Interestingly, microscopic evaluation of the pituitary gland found significantly higher incidence of pituitary hyperplasia (particularly pars distalis) with increase in lorcaserin dose ($p < 0.05$ at 30 mg/kg) suggesting that a) premature deaths had prevented full development to tumor, b) perhaps lorcaserin prevented aggressive growth of pituitary tumors from hyperplasia to adenoma which would support the decrease in prolactin in male rats.

Pituitary gland findings in male and female rats		Lorcaserin dose, mg/kg (p value)				Dose Response
		0	10	30	100	
Pituitary, adenoma, pars distalis, benign	M	32	22 (NS)	22 (NS)	15 (p=0.0003)	p=0.0005
	F	50	46 (NS)	31 (p=0.001)	20 (p<0.0001)	p<0.0001
Pituitary, hypertrophy/hyperplasia	M	16	23	21	29	
	F	14	12	20	28	

Malignant Schwannomas

There was a lorcaserin related increase in the incidence of malignant schwannomas (all sites combined) in male rats at 30 (2/65) and 100 (9/75) mg/kg. There was no schwannomas in the control or LD males (4.8x the clinical dose of 10 mg BID). The increase at the MD and HD is considered related to lorcaserin, thus providing a safety margin of 5x the clinical dose. Schwannomas across all locations (kidney, eyes thoracic and abdominal cavity, bone, skin subcutis) were characterized as small round neoplastic cells with unclear border. In at least 3 of the HD male rats their metastasis were seen in the lungs and thymus. Overall, the incidence of combined schwannomas in the HD males ($p=0.0037$) was above the ^{(b) (4)} historical control suggesting that schwannomas were drug-related.

Skin fibroma and Squamous cell carcinoma

Lorcaserin increased the incidence of benign skin fibroma in male rats in a dose-dependent manner (3/65C, 7/65 LD, 11/65 MD and 17/75 HD). Although the incidence of fibroma was statistically increased only in MD (16.9%) and HD (22.7%) male, the rate in the LD males (10.8%) was numerically higher than concurrent control (4.6%) and the ^{(b) (4)} historical control data (0 to 5%). There were only few incidences of fibroma in female rats (3LD and 1 MD). Lorcaserin also increased ($p > 0.05$) the incidence of skin squamous cell carcinoma in MD (4/65, 6.15%) and HD (5/75, 6.67%) male rats relative

to corresponding control and (b) (4) historical control data (0 to 1.7%). Only two HD females had squamous cell carcinomas. The increase in skin fibroma and squamous carcinoma at the MD and HD are considered related to lorcaserin. The squamous cell carcinomas were observed in the anogenital/inguinal, head and neck area. The skin in these animals was visibly ulcerated. The increased incidence of fibroma at 10 mg/kg (4.8x the clinical dose, based on AUC) in male rats suggest that lorcaserin may have an off target peripheral tumorigenic effect at close to the clinical dose. Skin fibroma and squamous cell carcinomas occurred primarily in male rats. Whether shorter life span had played a role in female rats is not clear. .

Lung Tumors

Secondary lung carcinomas were increased in females in all dose groups, with an incidence of 0, 4, 9, and 6 in the C, LD, MD, and HD groups, respectively. Examination of individual histopathology reports indicates that the lung metastases arose from a primary mammary malignancy in 2, 7, and 6 cases in the LD, MD, and HD groups, with no cases observed in the control group. This suggests that lorcaserin increased the aggressiveness of mammary malignancies in females at all doses.

Liver and Thyroid tumors

Lorcaserin significantly increased the trend for hepatocellular adenoma in male rats (1/65, 2/65 and 6/75 in C, LD, MD and HD, respectively) but the increase was not significant in the pair wise comparisons relative to control. Similarly, higher incidence of hepatocellular carcinoma in male rats was not significant in the trend and pairwise comparisons (1, 3, 3 and 4 in C, LD, MD and HD).

Since the incidences of both adenoma and carcinoma in HD (adenoma 8% and carcinoma 5.3%) males were greater than the (b) (4) historical data for adenoma (0-5.7%) and carcinoma (0 to 1.7%) suggests that liver tumors in males at 100 mg/kg were likely drug metabolism related. With no significant increase in pair wise comparison and high exposure multiples (> 55x the clinical dose of 10 BID on AUC basis), the potential risk to humans is deemed minimal.

The trend for incidence of thyroid follicular cell adenoma was significant in lorcaserin treated males. The incidence in the C, LD, MD and HD male rats were 0/65, 5/65, 4/65 and 8/75, respectively. Although the trend was positive only the incidence in the HD (10.6%) was statistically significant. The (b) (4) historical control for follicular cell adenoma in male rats ranged from 0 to 11.7% (x=2.2%). The incidence of follicular cell adenoma was within the historical range but it should be noted that historical range is skewed by one group at 11.7% with remaining between 0 and 3%. The trend for this tumor was not significant in female rats.

Lorcaserin induced UGT and cytochrome P450 enzymes and resulted in a greater degree of hepatocellular hypertrophy in males than in females. Thyroid follicular cell tumors often accompany hepatic tumors that arise from induction of drug-metabolizing enzymes, a likely consequence of increased triiodothyronine turnover by the liver with secondary chronic thyroid follicular cell stimulation. Induction of drug-metabolizing enzymes in the liver of human subjects is not known to lead to hepatic carcinogenesis, as typified by clinical experience with phenobarbital. With a reasonable safety margin to non-tumorigenic exposure and, more significantly, a recognized mechanism of tumorigenesis, the potential risk of hepatic and thyroid tumors to humans is considered minimal.

Non-neoplastic findings in male rats:

Microscopic evaluation of the tissues in the control and lorcaserin treated male rats found significant drug related histopath findings (hypertrophy, hyperplasia and vacuolation) in male rats. The most prominent organs affected by lorcaserin were the liver, brain, thyroid, lung, epididymides, pituitary, skeletal muscle, spinal cord, spleen and adrenal gland. Some of the changes may have been due to adaptation to drug metabolism (i.e. liver hypertrophy) and age (atrophy) however, the increased incidences of vacuolation and degeneration were likely lorcaserin related. It is not clear how the early euthanization of HD male rats (48%) relative to control (15.4%), LD (21.5%) and MD (23.1%) had affected the incidence of some these findings. Since lorcaserin is a weight loss drug and reduced BW in male rats, one would have expected the severity and incidence of the histopath findings to be more attenuated but this was not the case. The most affected target organs in males are listed below.

- Adrenal gland, cortical angiectasis/cystic degeneration
- Brain, minimal gliosis and mineralization
- Bone, marrow atrophy and granulocytic hyperplasia
- Epididymal, vacuolation
- Liver, centrilobular hypertrophy, vacuolation, focal cystic degeneration, and cellular alteration Lung, alveolar histocytosis and lipidosis or cholesterol cleft
- Pituitary gland hyperplasia of pars distalis
- Skeletal muscle atrophy and degeneration
- Spinal cord degeneration
- Spleen and mesenteric lymph node lymphoid depletion
- Skin ulceration
- Thyroid, follicular cell hyperplasia and hypertrophy, follicular cysts

The tables below show the incidence and severity of histopath findings in male rats treated with 10, 30 and 100 mg/kg of lorcaserin in the 2-year rat carcinogenicity study. The table provides the histopathology of target organs in animals that died/ euthanized during the study (DOS) and scheduled necropsy (SNC).

Summary of Microscopic Observations - MALE									
Terminal									
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
adrenal glands		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, cortical, benign, primary		0	0	0	0	0	1	1	0
angiectasis/cystic degeneration, focal cortical		8	5	14	6	16	9	28	2
- minimal		5	4	9	5	10	6	16	1
- mild		2	1	3	1	5	3	11	1
- moderate		0	0	2	0	1	0	0	0
- severe		1	0	0	0	0	0	1	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
bone marrow, femur		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
atrophy		0	0	2	0	1	1	24	0
	- minimal	0	0	1	0	0	0	2	0
	- mild	0	0	1	0	0	1	4	0
	- moderate	0	0	0	0	1	0	4	0
	- severe	0	0	0	0	0	0	14	0
hyperplasia, granulocytic		8	6	15	11	20	14	34	2
	- minimal	1	1	1	4	1	0	6	1
	- mild	5	5	10	4	12	7	14	1
	- moderate	2	0	4	3	7	7	14	0
bone marrow, sternum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
atrophy		1	0	0	0	1	2	23	0
	- minimal	0	0	0	0	0	1	3	0
	- mild	1	0	0	0	0	1	7	0
	- moderate	0	0	0	0	1	0	11	0
	- severe	0	0	0	0	0	0	2	0
hyperplasia, granulocytic		9	1	18	7	18	14	32	1
	- minimal	4	0	3	3	5	1	4	1
	- mild	4	1	12	4	8	12	24	0
	- moderate	1	0	3	0	5	1	4	0

DOS - Died or euthanized on study
 SNC - Scheduled necropsy
 () - Number observed

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
bone, femur		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hyperostosis		0	0	1	0	1	0	6	0
	- minimal	0	0	0	0	1	0	3	0
	- mild	0	0	1	0	0	0	3	0
bone, sternum		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hyperostosis		0	0	1	0	0	0	6	0
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	1	0	0	0	4	0
	- moderate	0	0	0	0	0	0	1	0

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		43	22	49	16	45	20	71	4
brain		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
gliosis		1	0	1	0	2	0	4	0
	- minimal	0	0	1	0	1	0	2	0
	- mild	0	0	0	0	1	0	1	0
	- moderate	1	0	0	0	0	0	1	0
mineralization, focal		0	0	0	1	1	0	8	1
	- minimal	0	0	0	1	0	0	7	0
	- mild	0	0	0	0	1	0	1	1
reticulosis, malignant, primary		0	0	0	1	0	0	1	0

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
epididymides		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
vacuolation		29	18	40	15	37	20	67	4
	- minimal	28	18	37	14	28	18	7	1
	- mild	1	0	3	1	9	2	34	3
	- moderate	0	0	0	0	0	0	26	0

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
liver		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hypertrophy, hepatocyte, centrilobular		0	0	0	0	2	0	46	4
	- minimal	0	0	0	0	2	0	17	1
	- mild	0	0	0	0	0	0	22	3
	- moderate	0	0	0	0	0	0	6	0
	- severe	0	0	0	0	0	0	1	0
hyperplasia, bile duct		17	14	26	8	16	16	33	3
	- minimal	11	10	10	4	5	6	20	1
	- mild	6	3	16	4	11	10	11	2
	- moderate	0	1	0	0	0	0	2	0
necrosis, hepatocytes, centrilobular		2	0	6	0	4	0	6	0
	- minimal	0	0	0	0	2	0	3	0
	- mild	0	0	2	0	0	0	1	0
	- moderate	2	0	3	0	2	0	1	0
	- severe	0	0	1	0	0	0	1	0
pigment, increased kupffer cell		1	0	0	0	5	0	21	0
	- minimal	1	0	0	0	4	0	17	0
	- mild	0	0	0	0	1	0	4	0
vacuolation, hepatocellular		2	5	23	3	24	17	48	3
	- minimal	1	3	18	3	20	10	28	2
	- mild	0	2	5	0	4	6	17	1
	- moderate	1	0	0	0	0	1	3	0

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
lung		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
carcinoma, hepatocellular, malignant, secondary		0	0	1	0	0	1	0	0
cholesterol clefts	- minimal	0	1	0	0	3	0	5	0
histiocytosis	- mild	0	0	1	0	0	0	0	0
histiocytosis, alveolar		10	5	14	2	10	9	48	4
	- minimal	6	5	11	2	10	6	18	2
	- mild	4	0	3	0	0	3	23	2
	- moderate	0	0	0	0	0	0	7	0
lipidosis, alveolar		0	0	0	0	0	0	6	1
	- minimal	0	0	0	0	0	0	1	0
	- mild	0	0	0	0	0	0	4	1
	- severe	0	0	0	0	0	0	1	0

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
mammary gland		(41)	(22)	(49)	(16)	(44)	(20)	(70)	(4)
fibroadenoma, benign, primary		0	0	1	0	3	1	5	1

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
pancreas		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
depletion, secretory		0	0	7	0	2	0	9	0
	- minimal	0	0	2	0	1	0	4	0
	- mild	0	0	5	0	1	0	5	0

Pituitary hyperplasia of pars distalis was increased in a dose dependent manner in lorcaserin treated male rats while the incidence of pituitary adenoma of pars distalis was decreased. It appears that rats treated with lorcaserin had lower incidence of adenoma but higher incidence of hyperplasia. Since the survival was significantly reduced by lorcaserin, the reviewer concludes that the time to pituitary tumor was reduced by premature deaths resulting in high incidence of pituitary hyperplasia and low incidence of adenoma in male rats.

The analysis of TSH in TK animals at 1 year found no significant rise in TSH when pituitary hyperplasia was in place. Since prolactin levels were also not increased suggests that pituitary was not releasing more hormones in the lorcaserin treated rats.

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
pituitary gland		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
adenoma, pars distalis, benign, primary		21	11	15	7	15	7	14	1
hyperplasia, pars distalis		10	6	16	7	14	7	26	3
	- minimal	3	1	4	1	3	3	9	1
	- mild	4	3	6	3	6	1	9	1
	- moderate	2	2	5	1	4	1	5	1
	- severe	1	0	1	2	1	2	3	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Skeletal muscle atrophy and degeneration was increased with dose. It is not clear if the severity or incidence had been altered due to early deaths and lower BW.

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
skeletal muscle, biceps femoris		(40)	(22)	(47)	(16)	(45)	(20)	(71)	(4)
atrophy		5	2	6	6	8	8	18	2
	- minimal	3	1	6	5	4	7	9	2
	- mild	2	1	0	1	4	1	9	0
degeneration, myofiber		1	0	6	0	4	2	19	0
	- minimal	1	0	5	0	4	2	14	0
	- mild	0	0	1	0	0	0	4	0
	- moderate	0	0	0	0	0	0	1	0

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
skin		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
atrophy		1	0	5	0	5	0	9	0
	- minimal	1	0	3	0	5	0	7	0
	- mild	0	0	2	0	0	0	2	0
carcinoma, squamous cell, malignant, primary		0	0	0	0	3	1	5	0
hyperkeratosis		1	1	2	1	4	0	5	0
	- minimal	0	1	2	1	4	0	3	0
	- mild	1	0	0	0	0	0	2	0
skin, subcutis		(7)	(0)	(13)	(1)	(6)	(9)	(22)	(3)
fibroma, benign, primary		3	0	7	0	4	7	14	3

Spinal cord degeneration in the lumbar region appeared to be higher in the HD male rats but overall trend was weaker since the incidence of degeneration was also relatively high in the control groups.

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
spinal cord, cervical		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration, axonal/myelin		1	3	2	3	7	5	5	2
	- minimal	1	1	2	2	7	5	5	2
	- mild	0	2	0	1	0	0	0	0
spinal cord, lumbar		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
cyst	- mild	0	0	0	0	1	0	0	0
degeneration, axonal/myelin		11	16	17	12	21	18	42	4
	- minimal	10	14	17	10	18	16	35	3
	- mild	0	2	0	2	3	2	7	1
	- severe	1	0	0	0	0	0	0	0
spinal cord, thoracic		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
degeneration, axonal/myelin		5	8	5	2	8	6	12	3
	- minimal	5	6	5	2	7	6	12	3
	- mild	0	2	0	0	1	0	0	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
thyroid gland		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
cyst, follicular		1	0	1	0	3	1	9	2
	- minimal	1	0	1	0	2	1	6	0
	- mild	0	0	0	0	1	0	1	2
	- moderate	0	0	0	0	0	0	2	0
hyperplasia, c-cell, diffuse		2	7	4	9	8	9	14	2
	- minimal	2	6	4	7	5	5	10	1
	- mild	0	1	0	2	3	4	4	1
hyperplasia, c-cell, focal		6	7	8	2	6	7	6	1
	- minimal	2	5	4	1	2	2	3	1
	- mild	2	1	3	1	4	4	1	0
	- moderate	1	1	1	0	0	0	1	0
	- severe	1	0	0	0	0	1	1	0
hyperplasia, follicular cell		0	0	5	1	7	7	13	0
	- minimal	0	0	0	1	1	2	2	0
	- mild	0	0	1	0	1	2	2	0
	- moderate	0	0	2	0	3	3	6	0
	- severe	0	0	2	0	2	0	3	0

DOS - Died or euthanized on study
 SNC - Scheduled necropsy
 () - Number observed

As a serotonin agonist, lorcaserin has the theoretical potential to cause valvulopathy. Therefore the Division had requested detailed examination of the heart tissue in rats (multiple sections of myocardium, endocardium, chordae tendineae and all four valves). The severity and incidence of cardiac findings (fibrosis of endocardium, endocardial hyperplasia, hypertrophy of tendineae) did not appear to be dose-dependent. Again, it is not clear if premature mortality had affected the cardiac histopathology (lack of dose-response).

Summary of Microscopic Observations - MALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
chordae tendineae, left		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hypertrophy		0	0	4	0	3	2	0	0
	- minimal	0	0	3	0	3	2	0	0
	- mild	0	0	1	0	0	0	0	0
chordae tendineae, right		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
hypertrophy		0	0	2	0	3	2	1	0
	- minimal	0	0	1	0	3	2	1	0
	- mild	0	0	1	0	0	0	0	0
endocardium		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
fibrosis	- minimal	0	0	0	1	0	0	0	0
hyperplasia, endocardial	- moderate	0	0	1	1	0	0	0	0
heart		(43)	(22)	(49)	(16)	(45)	(20)	(71)	(4)
cardiomyopathy		27	16	38	15	29	17	58	4
	- minimal	13	9	17	10	16	10	29	2
	- mild	13	7	20	5	13	7	28	2
	- moderate	1	0	1	0	0	0	0	0
	- severe	0	0	0	0	0	0	1	0
degeneration, myofiber	- mild	0	0	0	1	0	0	0	0
dilatation, ventricular/atrial	- moderate	1	0	0	0	0	0	0	0
endocarditis, valvular vegetative	- moderate	0	0	0	0	1	0	0	0
hypertrophy/hyperplasia, mesothelial cell	- minimal	1	0	0	0	2	0	0	0
valve, aortic		(35)	(22)	(42)	(13)	(42)	(17)	(61)	(2)
endocardiosis, valvular	- minimal	0	0	1	0	2	1	1	0
hypertrophy	- minimal	0	0	1	0	1	0	2	0
valve, left atrioventricular		(38)	(20)	(47)	(14)	(40)	(19)	(68)	(4)
endocardiosis, valvular	- minimal	0	0	1	1	2	0	3	0
hypertrophy	- minimal	1	0	0	0	3	0	2	0
valve, pulmonic		(41)	(21)	(39)	(9)	(33)	(16)	(57)	(3)
hypertrophy	- minimal	1	0	0	0	1	1	0	0
valve, right atrioventricular		(42)	(22)	(48)	(16)	(45)	(20)	(71)	(4)
endocardiosis, valvular	- minimal	0	0	0	0	0	2	0	0
hypertrophy	- minimal	1	0	1	0	0	0	0	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

Non-neoplastic findings in female rats:

Notable microscopic finding (hypertrophy, hyperplasia and vacuolation) in female rats treated with 10, 30 and 100 mg/kg of lorcaserin are shown in tables below. As noted before it is not clear how the premature mortality in the females affected the dose-related relationship. Lorcaserin affected many tissues, chief among them were:

- Liver, centrilobular hypertrophy, cellular alterations and necrosis
- Bone, femur and sternum hyperostosis
- Bone marrow (femur and sternum), atrophy and granulocytic hyperplasia
- Pituitary, hyperplasia/hypertrophy
- Lung, alveolar lipidosis and histiocytosis
- Tibiofemoral cartilage joint degeneration/necrosis
- Lymph nodes, hyperplasia
- Extramedullary hematopoiesis in adrenal glands, liver, spleen
- Vaginal atrophy

Although there was a trend to lower weight in HD female, there was no significant change in BW of lorcaserin treated female rats suggesting that BW was not a factor.

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
adrenal glands		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hematopoiesis, extramedullary		2	0	4	0	8	0	13	0
	- minimal	2	0	4	0	4	0	5	0
	- mild	0	0	0	0	3	0	8	0
	- moderate	0	0	0	0	1	0	0	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		42	23	53	12	60	5	75	0
bone marrow, femur		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
atrophy		2	0	1	0	2	0	15	0
	- minimal	1	0	0	0	0	0	1	0
	- mild	1	0	1	0	1	0	5	0
	- moderate	0	0	0	0	0	0	5	0
	- severe	0	0	0	0	1	0	4	0
hyperplasia, granulocytic		10	4	18	3	31	0	41	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	3	4	4	3	10	0	9	0
	- moderate	7	0	12	0	19	0	32	0
	- severe	0	0	1	0	2	0	0	0
hyperplasia, mixed		9	0	11	3	9	1	14	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	5	0	6	3	7	1	8	0
	- moderate	3	0	5	0	2	0	6	0
bone marrow, sternum		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
atrophy		1	0	0	0	1	0	11	0
	- minimal	0	0	0	0	0	0	4	0
	- mild	1	0	0	0	0	0	5	0
	- moderate	0	0	0	0	1	0	1	0
	- severe	0	0	0	0	0	0	1	0
hyperplasia, granulocytic		10	2	17	1	29	0	42	0
	- mild	7	2	14	1	23	0	34	0
	- moderate	3	0	3	0	6	0	8	0
hyperplasia, mixed		9	2	10	5	11	1	13	0
	- minimal	1	0	0	0	0	0	0	0
	- mild	8	2	9	5	11	1	13	0
	- moderate	0	0	1	0	0	0	0	0
bone, femur		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hyperostosis		2	2	3	0	2	0	15	0
	- minimal	1	2	2	0	0	0	6	0
	- mild	1	0	1	0	2	0	9	0
bone, sternum		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hyperostosis		1	1	3	0	3	0	10	0
	- minimal	1	1	2	0	2	0	1	0
	- mild	0	0	1	0	1	0	9	0

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
joint, tibiofemoral		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
degeneration/necrosis, cartilage		2	0	5	0	3	0	10	0
	- minimal	1	0	4	0	0	0	4	0
	- mild	1	0	1	0	3	0	6	0

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
liver		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
focus of cellular alteration, basophilic		3	3	9	1	17	4	19	0
	- minimal	0	1	4	1	9	1	11	0
	- mild	3	0	4	0	8	3	7	0
	- moderate	0	2	1	0	0	0	1	0
focus of cellular alteration, eosinophilic		0	0	7	4	17	2	39	0
	- minimal	0	0	2	0	7	1	16	0
	- mild	0	0	3	4	7	0	15	0
	- moderate	0	0	2	0	3	1	8	0
hematopoiesis, extramedullary		13	2	17	2	27	0	54	0
	- minimal	12	2	16	2	19	0	37	0
	- mild	1	0	1	0	8	0	16	0
	- moderate	0	0	0	0	0	0	1	0
hyperplasia, bile duct		18	8	18	3	10	1	7	0
	- minimal	14	5	10	2	5	0	7	0
	- mild	4	3	8	1	5	1	0	0
hypertrophy, hepatocyte, centrilobular		0	1	0	0	2	0	25	0
	- minimal	0	0	0	0	2	0	16	0
	- mild	0	1	0	0	0	0	8	0
	- moderate	0	0	0	0	0	0	1	0
necrosis		2	0	5	0	12	0	16	0
	- minimal	0	0	1	0	6	0	4	0
	- mild	2	0	2	0	5	0	10	0
	- moderate	0	0	2	0	1	0	2	0
pigment, increased kupffer cell		5	2	8	2	8	0	23	0
	- minimal	4	1	5	2	5	0	18	0
	- mild	1	1	3	0	3	0	5	0

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
lung		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
histiocytosis, alveolar		7	6	15	5	28	3	47	0
	- minimal	6	6	11	4	17	3	22	0
	- mild	1	0	4	1	10	0	21	0
	- moderate	0	0	0	0	1	0	4	0
lipidosis, alveolar		0	0	0	0	3	0	24	0
	- mild	0	0	0	0	2	0	14	0
	- moderate	0	0	0	0	1	0	6	0

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
lymph node, axillary		(15)	(6)	(33)	(8)	(43)	(4)	(50)	(0)
hyperplasia, lymphocyte/plasmacyte		9	1	14	0	27	0	40	0
- minimal		1	0	1	0	1	0	3	0
- mild		2	1	4	0	10	0	12	0
- moderate		3	0	7	0	14	0	21	0
- severe		3	0	2	0	2	0	4	0
lymph node, inguinal		(9)	(4)	(14)	(1)	(17)	(3)	(19)	(0)
hyperplasia, lymphocyte/plasmacyte		1	0	4	0	8	0	8	0
- minimal		1	0	0	0	1	0	1	0
- mild		0	0	3	0	2	0	4	0
- moderate		0	0	1	0	5	0	2	0
- severe		0	0	0	0	0	0	1	0
lymph node, mandibular		(41)	(23)	(53)	(12)	(59)	(5)	(75)	(0)
hyperplasia, lymphocyte/plasmacyte		12	1	21	6	33	0	45	0
- minimal		2	0	3	1	4	0	6	0
- mild		4	1	14	3	22	0	27	0
- moderate		5	0	4	2	7	0	12	0
- severe		1	0	0	0	0	0	0	0

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
mammary gland		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hyperplasia, lobular		12	12	19	4	18	5	27	0
- minimal		1	5	2	1	0	3	2	0
- mild		9	6	15	3	14	0	19	0
- moderate		2	1	2	0	4	2	6	0
hyperplasia, lobular with atypia		14	4	17	1	26	0	22	0
- mild		5	3	8	0	8	0	10	0
- moderate		9	1	9	1	18	0	12	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
pituitary gland		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenoma, pars distalis, benign, primary		31	19	36	10	28	3	20	0
carcinoma, pars distalis, malignant, primary		0	0	2	0	0	0	0	0
hypertrophy/hyperplasia		10	4	14	2	27	2	46	0
- minimal		3	3	2	1	4	1	15	0
- mild		5	1	11	1	19	1	28	0
- moderate		2	0	1	0	4	0	3	0

Summary of Microscopic Observations - FEMALE

		Terminal								
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		
Tissue	Observation	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
skeletal muscle, biceps femoris			(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
	degeneration, myofiber		1	0	1	0	3	0	5	0
	- minimal		1	0	1	0	2	0	2	0
	- mild		0	0	0	0	1	0	3	0
	degeneration/regeneration, myofiber		0	0	0	0	0	1	2	0
	- minimal		0	0	0	0	0	1	1	0
	- mild		0	0	0	0	0	0	1	0

Summary of Microscopic Observations - FEMALE

		Terminal								
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		
Tissue	Observation	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
spleen			(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
	macrophages, pigmented		34	20	47	10	54	4	72	0
	- minimal		3	0	4	0	3	0	3	0
	- mild		18	18	27	9	33	3	42	0
	- moderate		12	2	15	1	17	1	26	0
thymus gland			(39)	(21)	(49)	(10)	(54)	(5)	(70)	(0)
	depletion, lymphoid		36	20	45	9	53	4	68	0
	- minimal		1	0	1	0	2	0	0	0
	- mild		4	6	7	2	3	0	8	0
	- moderate		24	13	23	5	35	4	33	0
	- severe		7	1	14	2	13	0	27	0
	macrophages, pigmented		21	12	23	7	38	2	62	0
	- minimal		15	11	15	7	27	2	18	0
	- mild		6	1	8	0	11	0	44	0

Summary of Microscopic Observations - FEMALE

		Terminal								
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		
Tissue	Observation	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
joint, tibiofemoral			(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
	degeneration/necrosis, cartilage		2	0	5	0	3	0	10	0
	- minima		1	0	4	0	0	0	4	0
	- mild		1	0	1	0	3	0	6	0

Summary of Microscopic Observations - FEMALE

		Terminal								
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		
Tissue	Observation	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
vagina			(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
	atrophy		15	3	18	4	22	0	37	0
	- mild		15	2	18	4	19	0	32	0
	- moderate		0	1	0	0	3	0	5	0

DOS - Died or euthanized on study

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() - Number observed

As noted earlier the detailed examination of the heart tissue (multiple sections of myocardium, endocardium, chordae tendineae and all four valves) was made in the carci study animals. Although there were one or two female rats in the LD and MD groups with hypertrophy of chordae tendineae, aortic valve, left ventricular valve and pulmonic valves, the overall severity and incidence did not appear to be dose-dependent. Whether high incidence of premature mortality in female rats had affected the incidence of cardiac histopathology developments is not clear.

Summary of Microscopic Observations - FEMALE

		Terminal							
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
chordae tendineae, left		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hypertrophy	- minimal	0	1	2	0	0	0	1	0
chordae tendineae, right		(41)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
hypertrophy		0	0	2	0	1	0	0	0
	- minimal	0	0	2	0	0	0	0	0
	- mild	0	0	0	0	1	0	0	0
endocardium		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
inflammation, chronic-active	- moderate	0	0	1	0	0	0	0	0
lymphoma, malignant, multic		0	0	1	0	0	0	0	0
heart		(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
cardiomyopathy		25	18	27	9	34	4	36	0
	- minimal	13	13	19	7	23	3	22	0
	- mild	12	5	8	2	10	1	14	0
	- moderate	0	0	0	0	1	0	0	0
valve, aortic		(38)	(22)	(45)	(12)	(49)	(3)	(67)	(0)
endocardiosis, valvular	- minimal	0	0	1	0	0	0	0	0
hypertrophy	- minimal	0	0	0	0	1	0	0	0
valve, left atrioventricular		(41)	(22)	(51)	(12)	(54)	(5)	(68)	(0)
cyst		0	0	1	0	1	0	0	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	0	0	0	0	1	0	0	0
endocardiosis, valvular	- minimal	0	0	1	0	0	0	2	0
valve, left atrioventricular		(41)	(22)	(51)	(12)	(54)	(5)	(68)	(0)
hypertrophy	- minimal	0	0	0	0	1	0	1	0
valve, pulmonic		(34)	(13)	(48)	(9)	(47)	(4)	(56)	(0)
hypertrophy	- minimal	0	0	1	0	0	0	0	0
valve, right atrioventricular		(41)	(23)	(52)	(12)	(58)	(5)	(73)	(0)
cyst	- minimal	0	1	0	0	1	0	0	0
endocardiosis, valvular	- moderate	0	0	1	0	0	0	0	0

DOS - Died or euthanized on study

SNC - Scheduled necropsy

() - Number observed

Bimonthly Mammary tumor updates during the rat carci study:

The incidence of tumors in rats had been under greater scrutiny

(b) (6)

there were at least 11 mammary tumors in lorcaseerin treated females 42 into the study. These tumors were seen in all lorcaseerin treated groups thus there was no safety margin. At the time, histopath analysis indicating adenocarcinoma was available on only 2 females and the speculation was that the mammary tumors were due to excess prolactin.

(b) (6)

The reviewer communicated the information to Drs. Bourcier and Colman.

(b) (6)

the Division received a 15-day safety report from the sponsor on May 31, 07, approximately 3 month after the initial incidence stating the unusual increase in the number of mammary tumors in lorcaseerin treated female rats.

In the initial 15-day safety report, the 2 year rat study was at week 55 and the phase 3 two year clinical study was week 80. Up on arrival of the safety report, the Division contacted the sponsor asking for bimonthly updates as more rat tumor data became available. The incidence of mammary and astrocytoma in rats at week 55 is shown in table below:

Incidence of mammary tumors at WK 55, Sept 5, 2007**Summary Statistics Relevant to Survival and Mammary Gland Alterations in Female Rats given APD356 in an Ongoing 2-Year Carcinogenicity Study**

	Dose APD356			
	vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Females initiated	65	65	65	75
Females on study Week 63	62	59	53	42
Unscheduled deaths	3	6	12	33
Females with masses Week 62	8	12	21	29
Weeks (mean±sd) treatment prior to observation of masses	35	47±9	46±8	45±6
Histopathology at Week 55 ¹				
Available for histopathology	1	4	7	15
Mammary AdCA ²	0	2	5	13
Lung, secondary AdCA	0	1	3	2
Fibroadenoma	0	1	0	2
Galactocele	0	3	3	9
Lobular hyperplasia	0	2	5	12

¹ Values are numbers of females with specified pathology² AdCA: adenocarcinoma**Female Microscopic Mammary Gland Findings – Interim (TK) Study**

Dose (mg/kg/day)	0	10	30	100
Rats per Dose (N)	5	14	14	10
Number of females affected				
Lobular Hyperplasia	3	14	12	7
Lobular Hyperplasia with Atypia	0	4	7	3
Adenoma	0	0	0	4
Fibroadenoma	3	5	8	5
Fibroadenoma with Atypia	0	3	6	3
Adenocarcinoma	0	7	6	7

All TK rats were examined in the Sept 5th report.

Incidence of mammary tumor, Sept 5th, 2007 update (#0061)**Summary Statistics Relevant to Survival and Mammary Gland Changes in Female Rats given APD356 in an Ongoing 2-Year Carcinogenicity Study**

	Dose APD356			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Females initiated	65	65	65	75
Females on study Week 76	53	50	42	15
Unscheduled deaths	12	15	23	60
Females with masses Week 76	13	26	24	15
Weeks of treatment prior to observation of masses	35	47	46	45
Histopathology at Week 68 ¹				
Histopathology available	5	6	18	46
Mammary AdCA ²	2	6	16	45
Lung, secondary AdCA	0	1	3	2
Fibroadenoma	1	1	5	20
Galactocele	0	3	4	10
Lobular hyperplasia	1	2	5	23

¹ Values are numbers of females with specified pathology² AdCA: adenocarcinoma**Incidence of mammary tumors at WK 68, 1st update (Nov 9, 2007)****Mammary Gland Histopathology in Female Rats given APD356 in an Ongoing 2-Year Carcinogenicity Study**

	Dose APD356			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Histopathology available	10	11	22	55
Mammary AdCA ¹	4	10	19	54
Lung, secondary AdCA	0	1	3	2
Fibroadenoma	1	1	9	23
Galactocele	2	3	4	9
Lobular hyperplasia	2	2	8	23

¹ AdCA: adenocarcinoma

Incidence of mammary tumors at WK88, 2nd update (Jan 09, 08)**Mammary Gland Histopathology in Female Rats given APD356 in an Ongoing 2-Year Carcinogenicity Study**

	Dose APD356			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Histopathology available	28	38	45	74
Mammary AdCA ¹	16	27	36	72
Lung, secondary AdCA	0	2	6	5
Fibroadenoma	4	16	24	35
Galactocele	6	7	2	1
Lobular hyperplasia	7	13	11	27

^a AdCA: adenocarcinoma**Incidence of mammary tumors at WK 96, 3th update (March 10, 08)****Mammary Gland Histopathology in Female Rats in an Ongoing 2-Year Carcinogenicity Study**

	Dose APD356			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Histopathology available	39	50	57	75
Mammary AdCA ^a	20	34	43	72
Lung, secondary AdCA	0	2	7	5
Fibroadenoma	10	27	36	36
Galactocele	8	10	3	1
Lobular hyperplasia	9	16	15	27

^a AdCA: adenocarcinoma**Incidence of mammary tumors, End of study for females, 6th update (May 16, 2008)****Mammary Gland Histopathology in Female Rats**

	Dose APD356			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Histopathology available	65	65	65	75
Mammary AdCA ^a	30	35	35	63
Lung, secondary AdCA	0	4	9	7
Mammary carcinosarcoma	0	0	0	3
Fibroadenoma	20	47	60	53
Galactocele	18	14	7	1
Lobular hyperplasia	24	23	23	27

^a AdCA: adenocarcinoma

Incidence of mammary tumors, End of study for females, 7th update

Mammary Gland Histopathology in Female Rats

	Dose APD356			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Histopathology available	65	65	65	75
Mammary AdCA ^a	29	35	36	62
Lung, secondary carcinoma	0	4	9	6
Mammary carcinosarcoma	0	0	0	1
Fibroadenoma	20	48	56	51
Galactocele	18	14	7	1
Lobular hyperplasia	24	23	23	27

^a AdCA: adenocarcinoma

Incidence of mammary tumors, End of study for females and males, 8th update (Sept 19, 08)

Mammary Gland Neoplasms in Male and Female Rats

	Dose APD356			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Number of females examined	65	65	65	75
Adenocarcinoma	28	34	35	61
Carcinosarcoma	0	0	0	1
Fibroadenoma	20	48	54	45
Number of males examined	65	65	65	75
Adenocarcinoma	0	0	2	2
Fibroadenoma	0	1	4	6

Incidence of mammary (b) (4) female rats submitted with the NDA (Oct 2009)

Study Number 900-063
A 2-Year Carcinogenicity Study of APD356 Given by Oral Gavage to Rats

Summary of Microscopic Observations - FEMALE

Tissue Observation	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined	42	23	53	12	60	5	75	0
mammary gland	(42)	(23)	(53)	(12)	(60)	(5)	(75)	(0)
adenocarcinoma, malignant, primary	18	10	28	6	34	1	60	0
area normal, no mammary tissue present	0	0	0	0	0	0	1	0
carcinosarcoma, malignant, primary	0	0	0	0	0	0	1	0
fibroadenoma, benign, primary	14	6	36	11	48	5	45	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy
() - Number observed

The tables below show the chronological changes in the numbers of mammary adenocarcinoma and fibroadenoma in female rats. The first report was at week 55. At week 96, the sponsor had analyzed all the female rats at 100 mg/kg at which point the incidence of adenocarcinoma was 72/75 (highlighted yellow). As more animals were necropsied, the numbers of adenocarcinoma increased up to WK 96. Post week 96, the numbers of adenocarcinoma started decreasing in spite of the increase in the numbers of necropsied rats. The sponsor had provided no explanation for the changes, other than indicating that the diagnoses had changed upon peer review.

Changes in diagnosis of adenocarcinoma and fibroadenoma over time
(from Wk 55 until the final NDA submission)

Mammary Adenocarcinoma Incidence over time in Female Rats (main study)				
Data Update (Week)	Control	10 mg/kg	30 mg/kg	100 mg/kg
Week 55 update	0 / 1	2 / 4	5 / 7	13 / 15
Week 68 update	2 / 5	6 / 6	16 / 18	45 / 46
Week 88 update	16 / 28	27 / 38	36 / 45	72 / 74
Week 96 update	20 / 39	34 / 50	43 / 57	72 / 75
Week 104 update	30 / 65	35 / 65	35 / 65	63 / 75
Final update	29 / 65	35 / 65	36 / 65	62 / 75
Final NDA	28 / 65	34 / 65	35 / 65	60 / 75

Mammary Fibroadenoma Incidence over time in Female Rats (main study)				
Data Update (Week)	Control	10 mg/kg	30 mg/kg	100 mg/kg
Week 55 update	0 / 1	1 / 4	3 / 7	2 / 15
Week 68 update	1 / 10	1 / 11	5 / 18	20 / 46
Week 88 update	4 / 28	16 / 38	24 / 45	35 / 74
Week 96 update	10 / 39	27 / 50	36 / 57	36 / 75
Week 104 update	20 / 65	47 / 65	60 / 65	53 / 75
Final update	20 / 65	48 / 65	56 / 65	51 / 75
Final NDA	20 / 65	47 / 65	53 / 65	45 / 75

Bimonthly Astrocyte tumor updates during rat carcinogenicity study:

The incidences of brain astrocytoma in male rats beginning at week 55 in the 2-year lorcaserin carcinogenicity study. With the exception of couple of cases, astrocytomas were lethal. In the NDA amendment, one of the astrocytoma in HD males was reclassified as infarct. There were total of 20 astrocytoma in the entire rat carci study (male and female, main and TK groups).

Out of 20 astrocytomas, 19 were found in the lorcaserin treated rats. If one normalizes for the number of animals examined in the treated groups, the incidence of astrocytoma is 4.1% for lorcaserin treatment and 0.7% for control, or approximately a 6-fold higher rate in the lorcaserin-treated groups. This argues against the notion that the astrocytomas were a spontaneous tumor.

Incidence of astrocytoma in the main and the interim TK animals (WK 52)
in the 2-year rat carcinogenicity study

Lorcaserin dose, mg/kg		0 n=65	10 n=65	30 n=65	100 n=75
Main study, astrocytoma	M	1	0	4	8
	F	0	2	0	1
TK study, astrocytoma	M	0	0	0	1
	F	0	0	1	2
Total astrocytoma (20)		1	2	5	12

Incidence of Astrocytoma in rats at WEEK 55**Incidence of Benign Astrocytoma**

Dose (mg/kg/day)	0	10	30	100
Interim (TK) Study²				
M	0/6	0/14	0/11	1/14
F	0/5	0/14	1/14	2/10
M+F	0/11	0/28	1/25	3/24
%Incidence M+F	0%	0%	4.0%	12.5%
Unscheduled Deaths in Main Study²				
M	0/6	0/3	1/7	0/6
F	0/1	0/4	0/7	3/15
M+F	0/7	0/7	1/14	3/21
%Incidence M+F	0%	0%	7.1%	0%
Combined Assessments at Week 55²				
M	0/12	0/17	1/18	1/20
F	0/6	0/18	1/21	2/25
M+F	0/18	0/35	2/39	3/45
%Incidence M+F	0%	0%	5.1%	6.6%
Exposure (AUC) ratio rat:human¹				
Male		2.5	7.6	34
Female		5	15	60

¹ Estimated APD356 exposure relative to estimated human exposure at 10 BID (0.032 h⁻¹g/mL)

² % - # animals affected with astrocytoma/total number of animals with available histopathology per assessment or combined.

Incidence of Astrocytoma in rats at WEEK 88

Incidence of Astrocytoma in the Rat Carcinogenicity Study

Dose (mg/kg/day)	0	10	30	100
Interim (TK) Study^a				
M	0/6	0/14	0/11	1/14
F	0/5	0/14	1/14	2/10
M+F	0/11	0/28	1/25	3/24
%Incidence M+F	0%	0%	4.0%	12.5%
Unscheduled Deaths in Main Study^a				
M	0/22	0/26	2/28	5/57
F	0/28	2/38	0/45	1/74
M+F	0/50	2/64	2/73	6/131
%Incidence M+F	0%	3.1%	2.7%	4.6%
Combined Assessments^a				
M	0/28	0/40	2/39	6/71
F	0/33	2/52	1/59	3/84
M+F	0/61	2/92	3/98	9/155
%Incidence M+F	0%	2.2%	3.1%	5.8%
Exposure (AUC) ratio rat:human^b				
Male		2.5	7.6	34
Female		5	15	60

^a % Incidence: # animals with astrocytoma/total number of animals with available histopathology.

^b Estimated APD356 exposure relative to human exposure at 10 mg BID (0.932 hr*µg/mL)

Incidence of astrocytoma in rats, final update

Incidence of Astrocytoma in the Rat Carcinogenicity Study

Dose (mg/kg/day)	0	10	30	100
Interim (TK) Study^a				
Male	0/6	0/14	0/11	1/14
Female	0/5	0/14	1/14	2/10
Main Study^a				
Male	1/65	0/65	4/65	9/75
%Incidence Male	1.5%	0%	6.1%	12%
Female	0/75	2/65	0/65	1/75
%Incidence Female	0%	3.1%	0%	1.3%
Exposure (AUC) ratio rat:human^b				
Male		4.6	16.4	54.3
Female		6.7	23.4	81.4

^a % Incidence: # animals with astrocytoma/total number of animals with available histopathology.

^b Estimated APD356 exposure relative to human exposure at 10 mg BID (1.03 hr*µg/mL), the maximum recommended dose.

The incidence of astrocytoma in was reduced by 1 to 8 in HD males in an amendment to the NDA. The astrocytoma in this case was reclassified as infarct.

Toxicokinetics

Blood samples were collected from non-fasted animals at week 52 via orbital sinus under CO₂ anesthesia. After collection of blood samples for TK analysis, the TK rats were kept on the assigned doses for an additional 3 to 4 weeks for the purpose of prolactin analysis and pituitary/mammary immunohistochemistry. Plasma samples were analyzed for the parent drug (lorcaserin, APD356), M1 (sulfamate metabolite, AR244208) and M5(AR306388) by (b) (4).

- The AUC exposure for the parent drug increased in a dose-linear manner.
- The AUC for lorcaserin in female rats was about .5x the AUC in male rats.
- Both metabolites were found in male and female rats however, the exposure to M1 metabolite was about 11x greater than lorcaserin exposure while the exposure to M5 metabolite was a fraction (4 to 27%) of plasma lorcaserin AUC.
- The exposure to lorcaserin in male rats was 5x, 17x and 55x the clinical dose of 10 mg BID based on AUC
- The exposure in female rats was 7x, 24x and 82x the clinical dose based on AUC.

Exposure margins:

Species	Dose, mg/kg	Lorcaserin, AUC, µg.h/ml	APD244208 (M1), AUC, µg.h/ml	Exposure margins based on AUC (Animal/Human)	
				Lorcaserin	M1
104-week Mouse Carci Study	5	M:4.78, F:6.86	M:170, F: 193	M:4.7, F:6.7	M:36, F:41
	25	M:16.9 F:24.1	M:319, F: 412	M:16.6, F:24	M:68, F:88
	50	M:55.9 F:83.8	M:633, F:1050	M:55, F: 82	M:136, F:225
Clinical Dose: 10 mg BID		1.02	4.66		

Toxicokinetic Parameters for APD356 Following Oral Administration at 10, 30, and 100 mg/kg/day for 52 Weeks in Male and Female Sprague-Dawley Rats.

Parameter ^a	Male			Female		
	10	30	100	10	30	100
Dose (mg/kg, APD356)	10	30	100	10	30	100
t _{1/2} (hr)	3.70	4.13	8.49	3.02	3.94	10.6
t _{max} (hr)	1.00	1.00	0.500	1.00	2.00	1.00
C _{max} (µg/mL)	0.665	1.98	4.04	1.05	2.35	5.84
t _{last} (hr)	24.0	24.0	24.0	24.0	24.0	24.0
AUC _{last} (hr•µg/mL)	4.78	16.9	55.9	6.86	24.1	83.8
AUC _{0-inf} (hr•µg/mL)	4.82	17.1	64.9	6.89	24.4	107
%AUC _{extrap}	0.992	1.23	13.9	0.430	1.55	21.8
AUMC _{last} (hr•hr•µg/mL)	29.3	118	475	39.1	156	748
MRT _{last} (hr)	6.14	6.98	8.50	5.70	6.49	8.93

^a Values were determined from the plasma concentration versus time profiles

Toxicokinetic Parameters for AR244208 Following Oral Administration at 10, 30, and 100 mg/kg/day for 52 Weeks in Male and Female Sprague-Dawley Rats.

Parameter ^a	Male			Female		
	10	30	100	10	30	100
Dose (mg/kg, APD356)	10	30	100	10	30	100
t _{1/2} (hr)	5.11	6.59	14.6	4.75	6.36	14.3
t _{max} (hr)	1.00	1.00	0.500	1.00	4.00	2.00
C _{max} (µg/mL)	15.4	23.9	36.0	16.3	31.7	63.7
t _{last} (hr)	24.0	24.0	24.0	24.0	24.0	24.0
AUC _{last} (hr•µg/mL)	170	319	633	193	412	1050
AUC _{0-inf} (hr•µg/mL)	175	338	927	200	443	1480
%AUC _{extrap}	2.83	5.84	31.7	3.24	7.04	29.2
AUMC _{last} (hr•hr•µg/mL)	1280	2620	6260	1390	3340	10200
MRT _{last} (hr)	7.50	8.21	9.89	7.18	8.11	9.74

^a Values were determined from the plasma concentration versus time profiles

Toxicokinetic Parameters for AR306388 Following Oral Administration at 10, 30, and 100 mg/kg/day for 52 Weeks in Male and Female Sprague-Dawley Rats.

Parameter ^a	Male			Female		
	10	30	100	10	30	100
Dose (mg/kg, APD356)	10	30	100	10	30	100
t _{1/2} (hr)	3.10	3.95	7.49	5.16	4.45	8.64
t _{max} (hr)	1.00	0.500	0.500	0.500	1.00	2.00
C _{max} (µg/mL)	0.178	0.559	2.03	0.0538	0.337	1.20
t _{last} (hr)	12.0	24.0	24.0	12.0	12.0	24.0
AUC _{last} (hr•µg/mL)	0.550	3.07	14.9	0.273	1.62	11.1
AUC _{0-inf} (hr•µg/mL)	0.602	3.10	16.5	0.349	1.96	12.8
%AUC _{extrap}	8.62	1.18	9.50	21.8	17.2	13.3
AUMC _{last} (hr•hr•µg/mL)	1.89	18.2	117	1.21	7.12	90.1
MRT _{last} (hr)	3.43	5.92	7.82	4.42	4.39	8.13

^a Values were determined from the plasma concentration versus time profiles

Comparison of Plasma Exposure of Lorcaserin and Circulating Metabolites Detected in Humans and Animal Species at NOEL Doses

Gender	Mice ^a				Rats ^b				Monkeys ^c				Humans ^d	
	50 mg/kg/day for 52 weeks				10 mg/kg/day for 52 weeks				2 mg/kg/day for 52 weeks				10 mg <i>b.i.d.</i> at steady-state (Simulated)	
	C _{max} (µg/mL)		AUC _{last} (µg•h/mL)		C _{max} (µg/mL)		AUC _{last} (µg•h/mL)		C _{max} (µg/mL)		AUC _{last} (µg•h/mL)		C _{max} (µg/mL)	AUC _{last} (µg•h/mL)
	M	F	M	F	M	F	M	F	M	F	M	F	Mixed	Mixed
Lorcaserin	1.51	0.890	7.50	3.72	0.665	1.05	4.78	6.86	0.141	0.136	1.01	0.598	0.061	1.02
M1	42.7	49.1	308	345	15.4	16.3	170	193	5.01	4.58	56.4	39.3	0.213	4.66
M5	1.00	0.824	3.42	3.23	0.178	0.0538	0.550	0.273	0.635	0.771	1.82	1.76	0.0723	0.765

^a CD-1 mice, NOEL dose derived from TX05004.³¹

^b S-D rats, NOEL dose derived from TX03015.²⁵

^c Cynomolgus monkeys, NOEL dose derived from TX04038.³²

^d Simulated human exposures based on 10 mg *q.d.* plasma concentration-time profiles (PDR-09-151, Rev. 01).³³

Note: Calculations were carried out using the non-rounded values; however, reported values were rounded to three significant figures.

Stability and Homogeneity

The drug concentrations in the gavage and stock solutions were measured during WK 1, 13, 26, 39, 52, 65, 78, 91 and 104. The concentrations were within the specific range ($\pm 10\%$) of the target concentrations.

- The samples from all dose levels were within 90-110% acceptance range
- 98.6 to 102.3% label claim for homogeneity samples
- 98.4 to 101.7% label claim for Weeks 1, 13, and 26 concentration samples
- 95.4 to 101.7% label claim for stability samples, and
- APD356 was not detected in the vehicle control samples for Weeks 1, 13, and 26.
- 96.0 to 101.5% label claim for Weeks 39 and 52 concentration samples
- 97.5 to 100.4% label claim for Weeks 65, 78, and 91 concentration samples
- 100.2 to 102.6% label claim for Week 104 concentration samples
- APD356 was not detected in the vehicle control samples for Weeks 39, 52, 65, 78, 91, and 104.

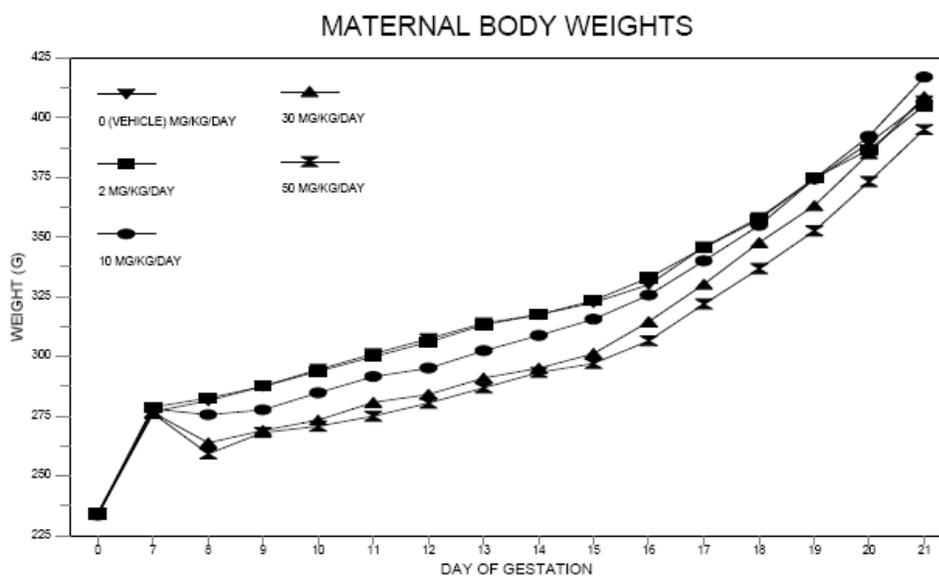
9 Reproductive and Developmental Toxicology

The sponsor had carried out several dose ranging studies in rats and rabbits. These studies will be summarized here for reference.

Study Title: Oral gavage dosage-range development toxicity study of APD356 in rats.

Methods: The study was carried out at (b) (4) (GLP protocol #6401-001P, Sponsor#TX03024). Pregnant CrI:CD®(SD)IGS BR VAF/Plus® rats (5 groups, 9/group) were treated with 2, 10, 30 and 50 mg/kg of lorcaserin (lot number AR 222154-A-04-001) from gestation day 7 through day 17 (DG 7-17). Lorcaserin was prepared in deionized water which also served as vehicle (1 ml/kg). The BW and food intake as well as the TK data were also determined. Dams were sacrificed after C-section on DG 21 and the number and distribution of corpora lutea, implantation sites and uterine content were determined. Additional evaluations included gross necropsy of the thoracic, abdominal and pelvic viscera as well as fetal weight, gross external alterations and fetal sex. TK animals were sacrificed on the last day of the treatment, DG 17 or 18. Blood samples were also collected from each fetus in the litter in the TK groups.

Results: There were drug-related deaths. Lorcaserin appeared to increase salivation at all doses. Body weight and BW gains were reduced in all lorcaserin treated groups. The BW gain was 96.6%, 94.2%, 87.5% and 74.3% of the control at 2, 10, 30 and 50 mg/kg, respectively. A decrease of 25% in BW gain suggest that the top dose of 50 mg/kg is reaching the MTD in dams. The decrease in BW was correlated with a decrease in food intake (92.6%, 87.0% and 81.5% of the control at 10, 30 and 50 mg/kg, respectively). Animals regained weight following termination of treatment.



Lorcaserin doses up to 50 mg/kg had no effect on any of the reproductive parameter evaluated in the study. There were no lorcaserin related fetal gross alterations occurred in rats.

PROTOCOL 6401-001P: ORAL (GAVAGE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF APD356 IN RATS (SPONSOR'S STUDY NUMBER: TX03024)

CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0 (VEHICLE)	II 2	III 10	IV 30	V 50
RATS TESTED	N	8	8	8	8	8
PREGNANT	N(%)	8(100.0)	8(100.0)	7(87.5)	8(100.0)	8(100.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	8	8	7	8	8
CORPORA LUTEA	MEAN±S.D.	16.9 ± 4.5	16.2 ± 2.7	16.0 ± 2.2	17.6 ± 1.3	15.1 ± 1.1
IMPLANTATIONS	MEAN±S.D.	13.8 ± 3.7	14.0 ± 2.4	14.7 ± 1.4	15.8 ± 1.3	14.5 ± 1.3
LITTER SIZES	MEAN±S.D.	13.4 ± 3.5	12.2 ± 4.1	14.4 ± 1.3	15.1 ± 1.4	14.2 ± 1.6
LIVE FETUSES	N	107	98	101	121	114
	MEAN±S.D.	13.4 ± 3.5	12.2 ± 4.1	14.4 ± 1.3	15.1 ± 1.4	14.2 ± 1.6
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.7	1.8 ± 2.0	0.3 ± 0.8	0.6 ± 0.7	0.2 ± 0.5
EARLY RESORPTIONS	N	2	14	2	4	2
	MEAN±S.D.	0.2 ± 0.7	1.8 ± 2.0	0.3 ± 0.8	0.5 ± 0.8	0.2 ± 0.5
LATE RESORPTIONS	N	1	0	0	1	0
	MEAN±S.D.	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	2(25.0)	5(62.5)	1(14.3)	4(50.0)	2(25.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	8(100.0)	8(100.0)	7(100.0)	8(100.0)	8(100.0)

a. Dosage occurred on days 7 through 17 of gestation.

PROTOCOL 6401-001P: ORAL (GAVAGE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF APD356 IN RATS (SPONSOR'S STUDY NUMBER: TX03024)

LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0 (VEHICLE)	II 2	III 10	IV 30	V 50
LITTERS WITH ONE OR MORE LIVE FETUSES	N	8	8	7	8	8
IMPLANTATIONS	MEAN±S.D.	13.8 ± 3.7	14.0 ± 2.4	14.7 ± 1.4	15.8 ± 1.3	14.5 ± 1.3
LIVE FETUSES	N	107	98	101	121	114
	MEAN±S.D.	13.4 ± 3.5	12.2 ± 4.1	14.4 ± 1.3	15.1 ± 1.4	14.2 ± 1.6
LIVE MALE FETUSES	N	51	43	53	63	55
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	46.4 ± 13.5	45.8 ± 18.0	52.7 ± 12.4	51.5 ± 13.5	47.8 ± 14.6
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.32 ± 0.24	5.59 ± 0.34	5.58 ± 0.20	5.14 ± 0.24	5.25 ± 0.35
MALE FETUSES	MEAN±S.D.	5.41 ± 0.28	5.74 ± 0.25	5.72 ± 0.16	5.28 ± 0.32	5.36 ± 0.39
FEMALE FETUSES	MEAN±S.D.	5.21 ± 0.25	5.52 ± 0.42	5.42 ± 0.20	5.03 ± 0.18	5.20 ± 0.42
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	2.3 ± 4.5	15.1 ± 22.2	1.8 ± 4.7	4.0 ± 4.7	1.8 ± 3.4

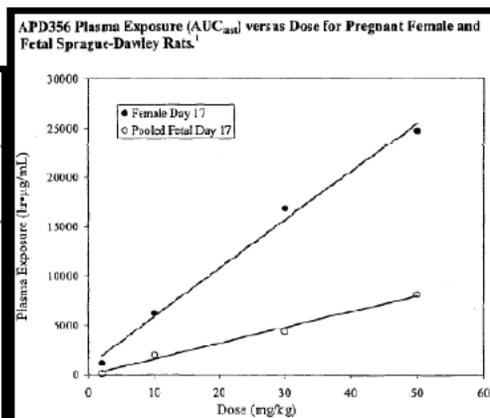
a. Dosage occurred on days 7 through 17 of gestation.

TK analysis found a dose-related increase in lorcaserin exposure in pregnant rats. The $t_{1/2}$ in pregnant rats increased with increase in dose from 3.5 hrs to 20.3 hr after single dose of 2 to 50 mg/kg of lorcaserin. The mean resident time increased from 5.5 to 9.8 hrs with increase in dose from 2 to 50 mg/kg. **Lorcaserin AUC in fetus was approximately 1/3 of the AUC in pregnant rats suggesting moderate drug transfer to fetal tissue.**

Pharmacokinetic Parameters for APD356 after a Single Oral Dose (Day 7 of Gestation) to Pregnant Female Rats.									
Dose (mg/kg/day)	λ_z (hr ⁻¹)	$t_{1/2}$ (hr)	AUC _{last} (hr•µg/mL)	AUC _{INF} (hr•µg/mL)	%AUC _{extrap}	t_{max} (hr)	C _{max} (µg/mL)	AUMC _{last} (hr•hr•µg/mL)	MRT _{last} (hr)
2	0.201	3.5	1.257	1.267	0.8	0.5	0.180	6.955	5.5
10	0.069	10.0	5.576	6.833	18.4	0.5	0.613	45.529	8.2
30	0.028	24.8	13.238	27.246	51.4	2.0	0.957	130.348	9.8
50	0.034	20.3	19.294	35.720	46.0	0.5	1.683	188.964	9.8

Pharmacokinetic Parameters for APD356 after 11 Days Oral Dosing (Day 17 of Gestation) of Pregnant Female Rats.									
Dose (mg/kg/day)	λ_z (hr ⁻¹)	$t_{1/2}$ (hr)	AUC _{last} (hr•µg/mL)	AUC _{INF} (hr•µg/mL)	%AUC _{extrap}	t_{max} (hr)	C _{max} (µg/mL)	AUMC _{last} (hr•hr•µg/mL)	MRT _{last} (hr)
2	0.192	3.6	1.162	1.172	0.9	1.0	0.163	5.585	4.8
10	0.202	3.4	6.213	6.257	0.7	0.5	0.667	35.646	5.7
30	0.114	6.1	16.833	17.878	5.9	1.0	1.820	112.213	6.7
50	0.098	7.1	24.773	27.169	8.8	1.0	2.400	173.144	7.0

Fetal Rat Pharmacokinetic Parameters for APD356 after 11 Days Oral Dosing (Day 17 of Gestation) of Pregnant Female Rats.						
Dose Group	Dose (mg/kg/day)	AUC _{last} (hr•µg/mL)	t_{max} (hr)	C _{max} (µg/mL)	AUMC _{last} (hr•hr•µg/mL)	MRT _{last} (hr)
2	2	0.154	4.0	0.028	0.784	5.1
3	10	1.974	4.0	0.220	12.656	6.4
4	30	4.360	8.0	0.306	35.760	8.2
5	50	8.156	8.0	0.552	67.984	8.3



Study Title: Oral Dosage-Range Development Toxicity Study of APD356 in Rabbit.

Methods: The study was also carried out at (b) (4) (GLP protocol #6401-002P, Sponsor#TX03025). This was a two part study.

In part 1, non-pregnant New Zealand White [Hra:(NZW)SPF] rabbits (4 groups, 3/group) were treated with 3 10, 30 and 100 mg/kg of lorcaserin (lot number AR 222154-A-04-001) for 5 days (Study day DS1 to 5). As stated for other studies, lorcaserin was prepared in deionized water and also served as control vehicle (5 ml/kg). Rabbits were sacrificed on DS6 for gross necropsy of thoracic, abdominal and pelvic vices along with changes in BW and food intake.

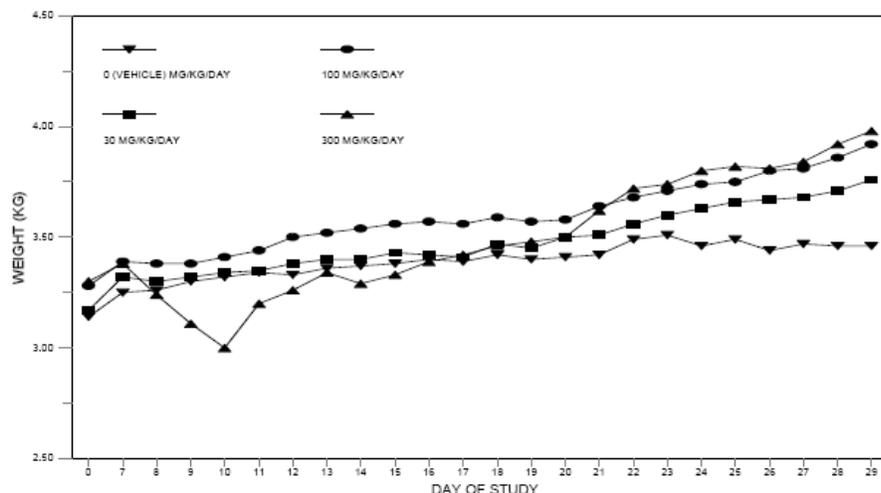
All non-pregnant rabbits survived lorcaserin treatment up to 100 mg/kg. There were no adverse findings or toxicity at doses up to 100 mg/kg. There was no change in BW or food intake.

In Part 2, higher doses of lorcaserin were administered since there was no toxicity at 100 mg/kg in part 1. Two rabbits were given 1000 mg/kg but due to toxicity, a lower dose of 300 mg/kg lorcaserin was given for 4 days.

Clinical signs of toxicity at 1000 mg/kg included opisthotonus, tonic extensor convulsion, vocalization, dark mucosa areas and trismus. Based on this data pregnant rabbits were treated with 30, 100 and 300 mg/kg of lorcaserin from DG 7 through DG19. Rabbits were sacrificed on DG 29 by C-section and standard maternal and fetal data was collected (i.e. number and distribution of corpora lutea, implantation sites and uterine contents, gross examination of the thoracic, abdominal and pelvic viscera, fetal weight and sex as well as gross external alterations). Blood samples were also collected from each fetus for TK analysis.

Results Part 2

- Of all the treated pregnant rabbits, there were two dead rabbits at 300 mg/kg one due to drug, the other due to intubation error. Adverse clinical signs included ptosis, tachypnea at ≥ 100 mg/kg and scant feces at 300 mg/kg. Red area around fundic mucosa was seen in one dead rabbits at 300 mg/kg.
- Lorcaserin reduced BW and BW gain at 30, 100 and 300 mg/kg. Food intake was reduced at ≥ 100 mg/kg. Fetal BW was also reduced at 300 mg/kg.
- There were no adverse findings in reproductive parameters at doses up to 300 mg/kg. There were no fetal gross alterations.



PROTOCOL 6401-002P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF APD356 IN RABBITS (SPONSOR'S STUDY NUMBER: TX03025)					
CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - MATED FEMALE RABBITS - PART B					

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	30	100	300

RABBITS TESTED	N	5	5	5	5
PREGNANT	N(%)	4 (80.0)	5 (100.0)	4 (80.0)	5 (100.0)
FOUND DEAD	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (60.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION					
	N	4	5	4	2
CORPORA LUTEA	MEAN±S.D.	7.5 ± 1.0	8.6 ± 1.3	10.2 ± 0.5	11.0 ± 1.4
IMPLANTATIONS	MEAN±S.D.	6.2 ± 1.2	8.4 ± 1.5	10.0 ± 0.9	11.0 ± 1.4
LITTER SIZES	MEAN±S.D.	6.2 ± 1.2	8.2 ± 1.1	8.8 ± 1.5	10.5 ± 0.7
LIVE FETUSES	N	25	41	35	21
	MEAN±S.D.	6.2 ± 1.2	8.2 ± 1.1	8.8 ± 1.5	10.5 ± 0.7
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.0 ± 0.0	0.2 ± 0.4	1.2 ± 1.2	0.5 ± 0.7
EARLY RESORPTIONS	N	0	0	2	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.6	0.0 ± 0.0
LATE RESORPTIONS	N	0	1	3	1
	MEAN±S.D.	0.0 ± 0.0	0.2 ± 0.4	0.8 ± 1.0	0.5 ± 0.7
DOES WITH ANY RESORPTIONS	N(%)	0 (0.0)	1 (20.0)	3 (75.0)	1 (50.0)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DOES WITH VIABLE FETUSES	N(%)	4 (100.0)	5 (100.0)	4 (100.0)	2 (100.0)
PLACENTAE APPEARED NORMAL	N(%)	4 (100.0)	5 (100.0)	4 (100.0)	2 (100.0)

a. Dosage occurred on days 7 through 19 of gestation.

PROTOCOL 6401-002P: ORAL (STOMACH TUBE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF APD356 IN RABBITS (SPONSOR'S STUDY NUMBER: TX03025)					
LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - MATED FEMALE RABBITS - PART B					

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	30	100	300

LITTERS WITH ONE OR MORE LIVE FETUSES	N	4	5	4	2
IMPLANTATIONS	MEAN±S.D.	6.2 ± 1.2	8.4 ± 1.5	10.0 ± 0.9	11.0 ± 1.4
LIVE FETUSES	N	25	41	35	21
	MEAN±S.D.	6.2 ± 1.2	8.2 ± 1.1	8.8 ± 1.5	10.5 ± 0.7
LIVE MALE FETUSES	N	15	24	17	6
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	58.8 ± 11.8	58.9 ± 16.3	48.8 ± 15.3	27.7 ± 25.0
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	46.14 ± 7.96	45.44 ± 3.27	44.62 ± 7.91	40.50 ± 0.44
MALE FETUSES	MEAN±S.D.	47.66 ± 8.58	46.32 ± 3.38	44.69 ± 7.89	48.24 ± 7.10
FEMALE FETUSES	MEAN±S.D.	44.17 ± 7.77	44.74 ± 3.84	44.86 ± 8.17	38.55 ± 1.26
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	0.0 ± 0.0	1.8 ± 4.1	12.6 ± 12.6	4.2 ± 5.9
NO FETAL ALTERATIONS WERE IDENTIFIED AT GROSS EXTERNAL EXAMINATION					

a. Dosage occurred on days 7 through 19 of gestation.

TK analysis

- There was a dose-proportional increase in lorcaserin exposure in pregnant rabbits.
- The $t_{1/2}$ in pregnant rabbits increased with increase in dose from 2.2 hrs at 30 mg/kg to 4.4hrs at 300 mg/kg.
- Exposure on DG7 (day 1) was similar DG19 at doses up to 100 mg/kg. At 300 mg/kg there was a 3.6 and 4.6 fold higher exposure on DG19 than DG7.
- Fetal blood levels were **below limits** of quantification on GD19. The relative lorcaserin bioavailability was very poor in rabbits, less than mice. Therefore poor fetal lorcaserin exposure is due to low parental drug related.

Day	Dose (mg/kg/day)	C_{max} ($\mu\text{g/mL}$)	t_{max} (hr)	AUC_{last} ($\text{hr}\cdot\mu\text{g/mL}$)	AUC_{INF} ($\text{hr}\cdot\mu\text{g/mL}$)
Female					
1	30	0.073 ± 0.008	0.5 ± 0.0	0.259 ± 0.023	0.279 ± 0.023
	100	0.205 ± 0.082	2.1 ± 1.3	0.956 ± 0.359	1.011 ± 0.367
	300	0.555 ± 0.182	1.6 ± 0.5	4.216 ± 0.832	4.315 ± 0.827
19	30	0.076 ± 0.021	0.5 ± 0.0	0.270 ± 0.065	0.290 ± 0.070
	100	0.243 ± 0.127	1.0 ± 0.8	1.454 ± 0.779	1.605 ± 0.724
	300 (n=2)	2.535 ± 0.983	0.5 ± 0.0	15.313 ± 0.988	15.376 ± 0.929

Pharmacokinetic Parameters for APD356 after a Single Oral Dose (Day 7 of Gestation) of 100 mg/kg/day to Pregnant Female Rabbits (Group III).										
Animal Number	λ_e (hr^{-1})	$t_{1/2}$ (hr)	AUC_{last} ($\text{hr}\cdot\mu\text{g/mL}$)	AUC_{INF} ($\text{hr}\cdot\mu\text{g/mL}$)	% AUC_{extrap}	t_{max} (hr)	C_{max} ($\mu\text{g/mL}$)	$AUMC_{last}$ ($\text{hr}\cdot\text{hr}\cdot\mu\text{g/mL}$)	MRT_{last} (hr)	
1591	0.232	3.0	0.874	1.055	17.2	2.0	0.164	2.734	3.1	
1592	0.305	2.3	1.348	1.498	10.1	2.0	0.273	4.064	3.0	
1593 ¹	--	--	1.306	--	--	4.0	0.311	4.833	3.7	
1594	0.252	2.7	0.537	0.640	16.1	2.0	0.125	1.579	2.9	
1595	0.247	2.8	0.717	0.851	15.7	0.5	0.153	2.092	2.9	
Average	0.259	2.7	0.956	1.011	14.8	2.1	0.205	3.060	3.1	
Std	0.032	0.3	0.359	0.367	3.2	1.3	0.082	1.359	0.3	
¹ The terminal phase of the plasma concentration versus time curve could not be estimated for this animal.										
Pharmacokinetic Parameters for APD356 after 13-Days of Oral Dosing (Day 19 of Gestation) of 100 mg/kg/day to Pregnant Female Rabbits (Group III).										
Animal Number	λ_e (hr^{-1})	$t_{1/2}$ (hr)	AUC_{last} ($\text{hr}\cdot\mu\text{g/mL}$)	AUC_{INF} ($\text{hr}\cdot\mu\text{g/mL}$)	% AUC_{extrap}	t_{max} (hr)	C_{max} ($\mu\text{g/mL}$)	$AUMC_{last}$ ($\text{hr}\cdot\text{hr}\cdot\mu\text{g/mL}$)	MRT_{last} (hr)	
1591	0.232	3.0	1.179	1.183	0.4	1.0	0.192	4.971	4.2	
1592	0.201	3.4	1.347	1.357	0.7	0.5	0.221	6.188	4.6	
1593	0.141	4.9	1.183	1.728	31.6	1.0	0.215	4.048	3.4	
1594	0.282	2.5	2.794	2.798	0.1	0.5	0.460	11.758	4.2	
1595	0.207	3.4	0.769	0.958	19.7	2.0	0.126	2.529	3.3	
Average	0.213	3.4	1.454	1.605	10.5	1.0	0.243	5.899	3.9	
Std	0.051	0.9	0.779	0.724	14.4	0.8	0.127	3.538	0.6	

Pharmacokinetic Parameters for APD356 after a Single Oral Dose (Day 7 of Gestation) of 300 mg/kg/day to Pregnant Female Rabbits (Group IV).									
Animal Number	λ_z (hr ⁻¹)	t _{1/2} (hr)	AUC _{last} (hr•µg/mL)	AUC _{INF} (hr•µg/mL)	%AUC _{extrap}	t _{max} (hr)	C _{max} (µg/mL)	AUMC _{last} (hr•hr•µg/mL)	MRT _{last} (hr)
1596	0.199	3.5	3.641	3.671	12.2	1.0	0.591	18.860	5.2
1597	0.203	3.4	5.555	5.589	0.6	2.0	0.590	31.737	5.7
1598	0.114	6.1	3.730	3.931	5.1	2.0	0.305	26.042	7.0
1599	0.185	3.8	3.645	3.683	1.0	1.0	0.806	17.730	4.9
1600	0.129	5.4	4.508	4.702	4.1	2.0	0.484	27.444	6.1
Average	0.166	4.4	4.216	4.315	4.6	1.6	0.555	24.372	5.8
Std	0.041	1.2	0.832	0.827	4.7	0.5	0.182	5.952	0.8
Pharmacokinetic Parameters for APD356 after 13-Days of Oral Dosing (Day 19 of Gestation) of 300 mg/kg/day to Pregnant Female Rabbits (Group IV).									
Animal Number	λ_z (hr ⁻¹)	t _{1/2} (hr)	AUC _{last} (hr•µg/mL)	AUC _{INF} (hr•µg/mL)	%AUC _{extrap}	t _{max} (hr)	C _{max} (µg/mL)	AUMC _{last} (hr•hr•µg/mL)	MRT _{last} (hr)
1596	0.201	3.4	14.614	14.718	0.7	0.5	3.230	77.120	5.3
1597 ¹	--	--	--	--	--	--	--	--	--
1598 ¹	--	--	--	--	--	--	--	--	--
1599 ¹	--	--	--	--	--	--	--	--	--
1600	0.277	2.5	16.011	16.033	0.1	0.5	1.840	34.810	5.3
Average	0.239	3.0	15.313	15.376	0.4	0.5	2.535	30.965	5.3
Std	0.054	0.7	0.988	0.929	0.4	0.0	0.983	5.438	0.0

¹These animals died before Day 19.

9.1 Fertility and Early Embryonic Development

Study title: Oral Male and Female Fertility and Early Embryonic Development to Implantation in Rats

Study no.: TX04057 (05-4283)
 Study report location: Arena electronic NDA
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Jan 28, 2005
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Lot# 04L018, Purity of 96.45%

Key Study Findings

- Slight but lower trend in BW was noted at 15 and 50 mg/kg in male rats (6-7%)
- Consistent with other studies, there was no change in BW of female rats
- A transient decrease in food intake was noted during the first few days
- Fertility parameters i.e. mating/fertility Index, corpora lutea, preimplantation Loss was not affected by lorcaserin doses up to 50 mg/kg
- There was no change in testes, epididymis, prostate, seminal vesicles and coagulating glands in male rats (sperm count and motility were not evaluated)
- Pregnancy rate was 95 to 100% with no change in estrous cycle
- NOAEL for embryonic development and fertility was 50 mg/kg

Methods

Doses: 5, 15 and 50 mg/kg
 Frequency of dosing: Daily
 Dose volume: 5 ml/kg
 Route of administration: oral
 Formulation/Vehicle: Distilled water
 Species/Strain: Albino SD rat strain, CrI:CD@ IGS BR, (b) (4)
 Number/Sex/Group: 20/sex/dose
 Satellite groups: Male TK rats
 Study design: Males: 9 WK treatment (4 Wks before and 5 Wks after mating)
 Females: 4-5 Wks (2 Wks before mating then until GD 7 with terminated on GD14)

Deviation from study protocol: No notable deviation of consequence

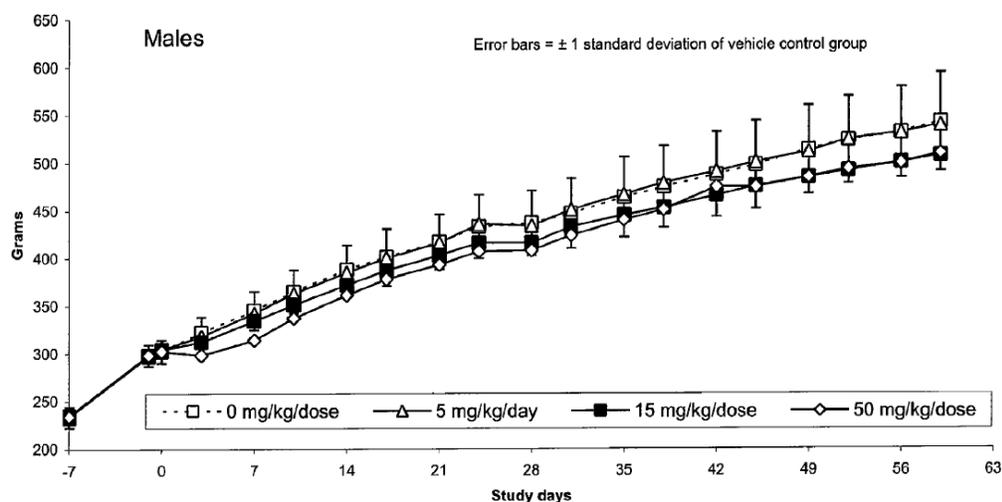
Stability and Homogeneity: Lorcaserin solutions were stable for at least 7 days and the concentrations in the solutions varied less than 4% of target concentrations which is well within the $\pm 10\%$ criterion.

Observations and Results

Mortality: There were no drug-related deaths in the study. A high dose male rat that was sacrificed on Day 12 had nasal bone fracture due to trauma.

Clinical Signs: There were minimal signs of toxicity.

Body Weight: Slight decrease in BW gain was noted in HD males (50 mg/kg) during the first week (9%). The final BW gain at week 9 of treatment in the HD males was about 6-7% less than control. The slight decrease in BW gain in the MD (15 mg/kg) within the first 3 days was similar to control at the end of the treatment. There was no change in BW in the LD (5 mg/kg) group. There was no clear drug related effect on BW gain in female rats. Female rats with initial decrease in BW gain in the first 3 days of the treatment recovered rapidly.



Feed Consumption: There was a transient decrease (up to 25%) in the food intake in the first few days corresponding to the decrease in BW in the HD male and female rats. The effect on food intake at the end of the study was insignificant.

Toxicokinetics: TK analysis was performed in male but not female rats. Blood samples were collected via orbital sinus at 1, 2, 4, 8 and 24 hrs post dose after anesthesia with CO₂/O₂ mixture. Lorcaserin exposure in male rats increased in a dose-proportional manner. The C_{max} and AUC in male rats at NOAEL dose of 50 mg/kg were 2.511 µg/ml and 29.287 µg.h/ml, respectively.

Toxicokinetic Summary after Oral Administration of APD356 to Male Sprague-Dawley Rats on Day 63.

Dose (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr•µg/mL)	AUC _{0-inf} (hr•µg/mL)
5	0.345	1.0	2.685	2.719
15	1.037	1.0	9.911	10.020
50	2.511	2.0	29.287	30.502

Necropsy

Fertility Parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.) were not affected by lorcaserin doses up to 50 mg/kg. Sperm motility and numbers were not determined. The pregnancy rate was 95 to 100%. There was no drug-related effect on estrous cycle, mating performance, fertility or the maintenance of normal pregnancy up to mid gestation. There were no drug related changes in BW, testes, epididymis, prostate, seminal vesicles and coagulating glands in male rats. The NOAEL dose for fertility in males was 50 mg/kg. The NOAEL for early embryonic development was also 50 mg/kg.

APD356 HEMIHYDRATE: ORAL MALE AND FEMALE FERTILITY
AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION IN RATS

SUMMARY OF SURVIVAL AND PREGNANCY					
DOSE GROUP		1	2	3	4
DOSE LEVEL (MG/KG/DAY)		0	5	15	50
No. of males at start	N	20	20	20	20
Premating					
- Died/sacrificed	N	0	0	0	0
Postmating					
- Died/sacrificed	N	0	0	0	0
No. of females cohabitated	N	20	20	20	20
- Without evidence of mating	N	4	2	1	2
Pregnant	N	4	2	1	2
Nonpregnant	N	0	0	0	0
Pregnant	N	20	20	19	19
- Died/sacrificed	N	0	0	0	0
- Aborted died/sacrificed	N	0	0	0	0
Nonpregnant	N	0	0	1	1
- Died/sacrificed	N	0	0	0	0
Total females died/sacrificed	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Examined at scheduled c-section	N	16	18	19	18
- Nonpregnant	N	0	0	1	1
- With total implant loss	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
- With viable fetuses	N	16	18	18	17
	%	100.0	100.0	94.7	94.4

No statistically significant differences

APD356 HEMIHYDRATE: ORAL MALE AND FEMALE FERTILITY
AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION IN RATS

SUMMARY OF CESAREAN SECTION DATA					
DOSE GROUP		1	2	3	4
DOSE LEVEL (MG/KG/DAY)		0	5	15	50
Pregnant (at scheduled sacrifice)	N	20	20	19	19
Dams with no Viable Fetuses	N	0	0	0	0
Dams with Viable Fetuses	N	20	20	19	19
Corpora Lutea	TOTAL	322	330	324	313
No. per animal	MEAN	16.1	16.5	17.1	16.5
	S.D.	2.69	2.31	1.93	1.78
Implantation Sites	TOTAL	293	302	292	286
No. per animal	MEAN	14.6	15.1	15.4	15.1
	S.D.	2.58	3.13	1.50	1.54
Preimplantation Loss	TOTAL	29	28	32	27
No. per animal	MEAN	1.5	1.4	1.7	1.4
	S.D.	1.57	2.04	1.97	1.26
% per animal	MEAN%	9.1	8.8	9.1	8.3
	S.D.	9.02	14.36	9.85	7.17
Live Fetuses	TOTAL	284	284	280	274
No. per animal	MEAN	14.2	14.2	14.7	14.4
	S.D.	2.59	3.35	1.52	1.84

No statistically significant differences

9.2 Embryonic Fetal Development

Study title: Lorcaserin: Oral Developmental Toxicity (segment II) Study in Sprague Dawley Rats

Study no:	TX03038	(b) (4)
Study report location:		(b) (4)
Conducting laboratory and location:		(b) (4)
Date of study initiation:	Dec 5, 03 (GD 0), ended on Dec 28, 03	
GLP compliance:	Yes	
QA statement:	Yes	
Drug, lot #, and % purity:	Lot#AR222154-A-04-001, purity 100%	

Key Study Findings

- Administration of 2, 10 and 50 mg/kg of lorcaserin from DG 7 to 17 did not result in mortality in dams. The prominent clinical signal was salivation.
- Maternal BW was slightly reduced at 10 and 50 mg/kg, corresponding to the decrease in food intake. There were no statistically significant differences in BW at GD 21. Although the decrease in BW was not considered a toxicological signal since a decrease in BW can affect reproductive parameters, a dose that decreases BW is considered a maternally toxic dose. The pregnancy rate in Control, 2, 10 and 100 mg/kg were 100%, 96%, 100% and 96%, respectively.
- Mean gravid and uterine weight were not affected by the treatment.
- There was a significant difference in fetal sex ratio at 10 and 50 mg/kg. The difference was not due to lorcaserin but due to slightly skewed proportion of male to female ratio in the control. The ratio of male to female in the treated animals was close ($\pm 1.6\%$) relative controls ($\pm 6.7\%$). The difference in sex ratio was of little clinical consequence since the imbalance was primarily in the control group. Furthermore, the historical control with a range of 0.72 to 1.16% was closer to that in the MD and HD rats.
- There were no statistically significant or toxicologically meaningful differences in C-section parameters among groups.
- There were no drug-related changes in fetal external, visceral or skeletal malformations or developmental variations between lorcaserin-treated groups and control.
- Minor variations such as an incidence of omphalocele in one fetus at 10 mg/kg and anal atresia with shortened torso and filamentous tail in one fetus in the HD groups were likely incidental.
- The sponsor selected 2 mg/kg as NOAEL due to small decrease in BW at 10 and 50 mg/kg. Since lorcaserin is a weight loss drug, the slight decrease in BW is likely a pharmacological response rather than a toxicological response.
- The maternal NOAEL was 10 mg/kg based on weight loss, with fetal NOAEL being the highest dose of lorcaserin tested in the study (50 mg/kg).
- Exposure at NOAEL of 10 and 50 mg/kg were 8x and 48x the clinical dose of 10 mg BID, based on AUC.

Methods

Doses: 2, 10 and 50 mg/kg
 Frequency of dosing: Once a day
 Dose volume: 1 ml/kg
 Route of administration: oral
 Formulation/Vehicle: Deionized water
 Species/Strain: CrI:CD[®](CD)IGS BR
 Number/Sex/Group: 25/sex/group
 Satellite groups: TK rats
 Deviation from study protocol: Small deviations with minimal consequence
 Study Design: Gestation Day (GD7-17) with C-section on GD21

Group	No. of Females	Dosage Material	Dosage Level (mg/kg/day)	Dosage Conc. (mg/mL)	Dosage Volume (mL/kg/day)
1	25	RODI water	0	0	1
2	25	APD356	2	2	1
3	25	APD356	10	10	1
4	25	APD356	50	50	1

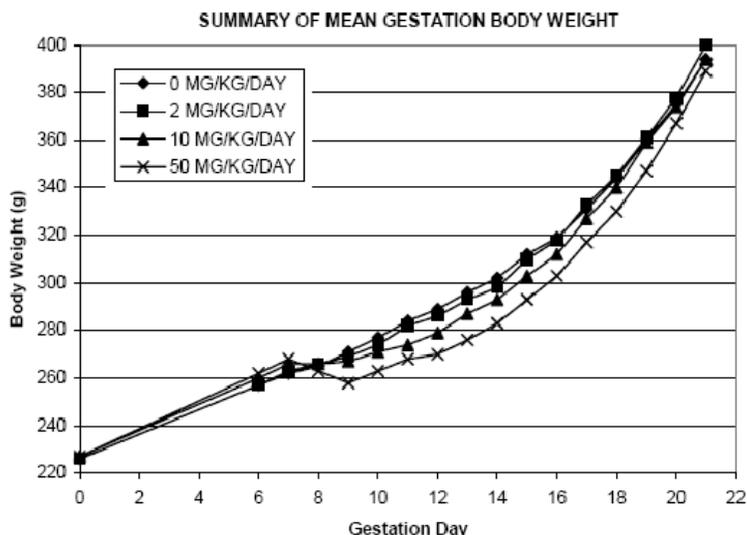
Note: Animals were dosed once daily by oral gavage from gestation days 7-17. A TK phase was performed on nine satellite TK animals from each group on the first and last days of dosing (gestation days 7 and 17).

Observations and Results

Mortality: There were no drug-related deaths in the study

Clinical Signs: Salivation appeared to be the most prominent clinical signs. Some rats had dark material around nose or mouth at 50 mg/kg.

Body Weight: Maternal BW was slightly reduced at 10 and more at 50 mg/kg, corresponding to slight decrease in food intake. There were no statistically significant differences in BW at GD 21. Mean gravid and uterine weight were not affected by the treatment



AN ORAL SEGMENT II STUDY IN RATS
SUMMARY OF GESTATION BODY WEIGHT DATA (GRAMS)

GROUP:		1	2	3	4
LEVEL (MG/KG/DAY):		0	2	10	50
DAY 12	MEAN	289 d	286	279*	270**
	S. D.	14.6	14.1	10.6	15.4
	N	25	24	25	24
DAY 13	MEAN	296 d	293	287	276**
	S. D.	14.2	14.2	11.5	15.7
	N	25	24	25	24
DAY 14	MEAN	302 d	298	293	283**
	S. D.	15.8	14.2	12.1	15.5
	N	25	24	25	24
DAY 15	MEAN	312 d	310	303	293**
	S. D.	16.0	15.3	12.5	16.7
	N	25	24	25	24
DAY 16	MEAN	319 d	318	312	303**
	S. D.	16.9	16.5	12.6	17.7
	N	25	24	25	24
DAY 17	MEAN	331 d	333	327	317**
	S. D.	18.4	15.4	15.3	17.3
	N	25	24	25	24
DAY 18	MEAN	344 d	345	340	330*
	S. D.	19.2	18.4	17.4	19.7
	N	25	24	25	24

Statistical key: d=ANOVA/DUNNETT-TEST * = P<0.05 ** = P<0.01

Toxicokinetics: Blood samples were collected from pregnant rats on GD 7 and 17. The exposure at 50 mg/kg was approximately 44x the clinical dose of 10 mg BID, based on AUC.

Day	Dosage	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)	AUC _{INF} (hr·µg/mL)
	Level (mg/kg/day)				
7	2	0.170	2.0	0.872	1.050
	10	0.600	0.5	6.874	6.936
	50	2.050	2.0	26.712	31.388
17	2	0.206	1.0	1.342	1.346
	10	0.890	1.0	7.949	7.988
	50	3.897	0.5	44.470	48.737

Stability and Homogeneity: Lorcaseerin solution was stable and concentrations were within ±10 of the target concentrations.

Necropsy: There were no notable gross findings except for a single subcutaneous mass found in the abdominal region in one female HD female rat.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- The pregnancy rate in C, 2, 10 and 100 mg/kg were 100%, 96%, 100% and 96%, respectively.
- Although there was a significant difference in the fetal sex ratio at 10 and 50 mg/kg, relative to control, the ratio of male to female in the treated animals was close (±1.6%) relative controls (±6.7%). The difference in sex ratio was of little clinical consequence since the ratio in MD and HD was closer to normal range than the control.
- There were no statistically significant or toxicologically meaningful differences in C-section parameters among groups.

AN ORAL SEGMENT II STUDY IN RATS

SUMMARY OF CESAREAN SECTION DATA

GROUP: LEVEL (MG/KG/DAY):		1 0	2 2	3 10	4 50
Pregnant	N	25	24	25	24
Dams with no Viable Fetuses	N	0	0	0	0
Dams with Viable Fetuses	N	25	24	25	24
Corpora Lutea	TOTAL	358	344	365	337
No. per animal	MEAN	14.3 d	14.3	14.6	14.0
	S. D.	1.89	1.86	1.98	1.81
Implantation Sites	TOTAL	317	330	336	319
No. per animal	MEAN	12.7 d	13.8	13.4	13.3
	S. D.	2.19	1.70	2.71	1.52
Preimplantation Loss	TOTAL	41	14	29	18
No. per animal	MEAN	1.6 d	0.6	1.2	0.8
	S. D.	2.48	1.10	1.99	1.03
% per animal	MEAN%	10.7 k	3.7	8.0	4.9
	S. D.	15.28	6.98	13.70	6.71
Live Fetuses	TOTAL	304	316	320	306
No. per animal	MEAN	12.2 d	13.2	12.8	12.8
	S. D.	2.61	1.69	2.89	1.48
Males	TOTAL	171	162	156	147
	MEAN%	56.7 k	51.8	48.6*	48.4*
	S. D.	13.28	14.85	12.47	12.98
Females	TOTAL	133	154	164	158
	MEAN%	43.3 k	48.2	51.4*	51.6*
	S. D.	13.28	14.85	12.47	12.98

Statistical key: d=ANOVA/DUNNETT-TEST k=KRUSKAL-WALLIS * = P<0.05

Offspring (Malformations, Variations, etc.)

- There were no drug-related changes in fetal external, visceral or skeletal malformations or developmental variations between lorcaserin-treated groups and control.
- Minor variations such as an incidence of omphalocele in one fetus at 10 mg/kg and anal atresia with shortened torso and filamentous tail in one fetus in the HD groups were incidental.
- The sponsor selected 2 mg/kg as NOAEL due to small decrease in BW at 10 and 50 mg/kg. Since the changes in the BW at 10 mg/kg was minimal, the reviewer selected 10 mg/kg as maternal NOAEL dose (8x the MRHD).

AN ORAL SEGMENT II STUDY IN RATS

SUMMARY OF CESAREAN SECTION DATA

GROUP:		1	2	3	4
LEVEL (MG/KG/DAY):		0	2	10	50
Postimplantation Loss	TOTAL	13	14	16	13
	No. per animal	MEAN	0.5 d	0.6	0.6
		S.D.	0.92	0.72	1.04
% implants per animal	MEAN%	4.8 k	4.1	5.2	3.9
		S.D.	8.14	4.83	8.78
					5.42
Dead Fetuses	TOTAL	0	0	0	0
	No. per animal	MEAN	0.0 k	0.0	0.0
		S.D.	0.00	0.00	0.00
% of implants per animal	MEAN%	0.0 k	0.0	0.0	0.0
		S.D.	0.00	0.00	0.00
					0.00
Resorptions: Early	TOTAL	13	14	16	13
	No. per animal	MEAN	0.5 k	0.6	0.6
		S.D.	0.92	0.72	1.04
% of implants per animal	MEAN%	4.8 k	4.1	5.2	3.9
		S.D.	8.14	4.83	8.78
					5.42
Resorptions: Late	TOTAL	0	0	0	0
	No. per animal	MEAN	0.0 k	0.0	0.0
		S.D.	0.00	0.00	0.00
% of implants per animal	MEAN%	0.0 k	0.0	0.0	0.0
		S.D.	0.00	0.00	0.00
					0.00

Statistical key: d=ANOVA/DUNNETT-TEST k=KRUSKAL-WALLIS

AN ORAL SEGMENT II STUDY IN RATS

SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

GROUP:		1	2	3	4
LEVEL (MG/KG/DAY):		0	2	10	50
Litters Examined Externally		25	24	25	24
Fetuses Examined		304	316	320	306
MICROPHthalmia/ANOPhtthalmia					
Fetal Incidence		2	0	0	0
Litter Incidence		2 f	0	0	0
OMPHALOCELE					
Fetal Incidence		0	0	1	0
Litter Incidence		0 f	0	1	0
ANAL ATRESIA					
Fetal Incidence		0	0	0	1
Litter Incidence		0 f	0	0	1
SHORTENED TORSO					
Fetal Incidence		0	0	0	1
Litter Incidence		0 f	0	0	1
FILAMENTOUS TAIL					
Fetal Incidence		0	0	0	1
Litter Incidence		0 f	0	0	1
Litters Examined Viscerally		25	24	25	24
Fetuses Examined		153	158	159	152
INTERVENTRICULAR SEPTAL DEFECT					
Fetal Incidence		1	0	0	0
Litter Incidence		1 f	0	0	0
Litters Examined Skeletally		25	24	25	24
Fetuses Examined		151	158	161	154
JUGAL AND SQUAMOSAL FUSED					
Fetal Incidence		0	1	0	0
Litter Incidence		0 f	1	0	0
CERVICAL VERTEBRAE FUSED					
Fetal Incidence		0	1	0	0
Litter Incidence		0 f	1	0	0
COSTAL CARTILAGE ANOMALY					
Fetal Incidence		2	0	0	0
Litter Incidence		1 f	0	0	0

Statistical key: f=CHI-SQUARE/FISHERS EXACT TEST

AN ORAL SEGMENT II STUDY IN RATS
SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

GROUP: LEVEL (MG/KG/DAY):	1 0	2 2	3 10	4 50
TOTAL MALFORMATIONS				
NUMBER WITH EXTERNAL MALFORMATIONS				
Fetal Incidence	2	0	1	1
Litter Incidence	2 f	0	1	1
NUMBER WITH VISCERAL MALFORMATIONS				
Fetal Incidence	1	0	0	0
Litter Incidence	1 f	0	0	0
NUMBER WITH SKELETAL MALFORMATIONS				
Fetal Incidence	2	1	0	0
Litter Incidence	1 f	1	0	0
TOTAL NUMBER WITH MALFORMATIONS				
Fetal Incidence	4	1	1	1
Litter Incidence	3 f	1	1	1

Statistical key: f=CHI-SQUARE/FISHERS EXACT TEST

AN ORAL SEGMENT II STUDY IN RATS
SUMMARY OF FETAL OBSERVATIONS - VARIATIONS

GROUP: LEVEL (MG/KG/DAY):	1 0	2 2	3 10	4 50
Litters Examined Externally	25	24	25	24
Fetuses Examined	304	316	320	306
Number with findings	0	0	0	0
Litters Examined Viscerally	25	24	25	24
Fetuses Examined	153	158	159	152
MAJOR BLOOD VESSEL VARIATION				
Fetal Incidence	1	0	0	0
Litter Incidence	1 f	0	0	0
URETER(S) DISTENDED				
Fetal Incidence	0	0	0	1
Litter Incidence	0 f	0	0	1
Litters Examined Skeletally	25	24	25	24
Fetuses Examined	151	158	161	154
27 PRESACRAL VEREBRAE				
Fetal Incidence	0	0	1	0
Litter Incidence	0 f	0	1	0
ACCESSORY SKULL BONE(S)				
Fetal Incidence	0	1	0	2
Litter Incidence	0 f	1	0	2
ALISHENOID VARIANT				
Fetal Incidence	3	7	6	13
Litter Incidence	3 f	5	4	4
ZYGOMATIC ARCH FUSION				
Fetal Incidence	0	0	0	1
Litter Incidence	0 f	0	0	1
JUGAL(S) REDUCED OSSIFICATION				
Fetal Incidence	0	0	1	0
Litter Incidence	0 f	0	1	0
SUPRAOCCIPITAL REDUCED OSSIFICATION				
Fetal Incidence	0	1	0	0
Litter Incidence	0 f	1	0	0
7TH CERVICAL RIB(S)				
Fetal Incidence	0	1	2	2
Litter Incidence	0 f	1	2	1
CERVICAL ARCH(ES) REDUCED OSSIFICATION				
Fetal Incidence	0	1	0	0
Litter Incidence	0 f	1	0	0
STERNEBRA(E) MALALIGNED				
Fetal Incidence	5	3	3	2
Litter Incidence	3 f	3	2	1
7TH STERNEBRA				
Fetal Incidence	1	0	0	0
Litter Incidence	1 f	0	0	0
RIB(S) 14TH RUDIMENTARY				
Fetal Incidence	9	11	11	7
Litter Incidence	7 f	7	6	4
REDUCED OSSIFICATION OF THE 13TH RIB(S)				
Fetal Incidence	1	2	2	0
Litter Incidence	1 f	1	2	0

Statistical key: f=CHI-SQUARE/FISHERS EXACT TEST

Study title: Lorcaserin: Oral Developmental Toxicity (segment II) Study in New Zealand White Rabbits

Study no: TX03039 (b) (4)
Study report location: (b) (4)
Conducting laboratory and location: (b) (4)
Date of study initiation: Dec 16, 03, Ended on Jan 16, 04
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Lot#AR222154-A-04-001, purity 100%

Key Study Findings

- The death of 1 female at 200 mg/kg on Day 8 was considered a dosing error.
- Clinical signs in the dead and other high dose rabbits and to lesser degree in MD included few/soft stool, rapid breathing and arched back
- One HD and a LD aborted on Day 23 and 26, respectively while one MD female delivered prematurely on GD 28. The incidence of spontaneous abortion in LD and HD was within the historical control (0 to 6.9%).
- Significant decreases in BW and food intake were observed at 200 mg/kg.
- The pregnancy rate was 94.7% in control and 100% in the treated rabbits
- Gross necropsy findings were limited to one LD, MD and HD dam. These animals had either empty implantation sites or early resorption in the case of MD and LD females.
- Overall, there were no statistically significant differences in C-section parameters (corpora lutea, implantation sites, pre-implantation loss, live fetuses, post-implantation loss, dead fetuses, early resorption, late resorption and mean fetal body weight) between lorcaserin treated dams and control
- There were no statistically significant differences in the incidence of total fetal external, visceral or skeletal malformations or developmental variations among groups; however, the incidence of several variations i.e. heart and greater vessel anomaly in 2 HD fetuses were greater than historical background. The remaining variations in the HD appeared to be within the historical control range.
- The relative bioavailability of lorcaserin in rabbits appears to be very poor compared to rats, monkeys and humans but somewhat close to that in mice.
- There appeared to be large variation in the lorcaserin exposure in LD and MD vs. HD dams. The nonlinear 43x increase in exposure from 60 to 200 mg/kg on Day 19 is unusual, a pattern that was also seen in the dose ranging study. The dramatic increase in AUC at 200 mg/kg was likely due to greater drug absorption following inappetence /empty GI and /or possibly due to decreased metabolism.
- Based on significant decrease in BW, the NOAEL for maternal toxicity was 60 mg/kg was 0.6x the clinical dose of 10 mg BID, based on AUC.
- The NOAEL for fetal toxicity was 60 mg/kg (<1x the MRHD) due to nonsignificant but slightly higher incidence fetal variations such as heart and greater vessel anomaly at high dose.

Methods

Doses: 20, 60 and 200 mg/kg
 Frequency of dosing: Daily from GD 7 to 19
 Dose volume: 5 ml/kg
 Route of administration: oral
 Formulation/Vehicle: deionized water
 Species/Strain: New Zealand White Rabbits
 Number/Sex/Group: 20/group
 Satellite groups: Yes (6/group)
 Study design: GD 7 through GD19 with sacrifice on GD 29
 Deviation from study protocol: Minor

Group	Number of Females	Dosage Material	Dosage Level (mg/kg/day)	Dosage Conc. (mg/mL)	Dosage Volume (mL/kg/day)
1	20	RODI Water	0	0	5
2	20	APD356	20	4	5
3	20	APD356	60	12	5
4	20	APD356	200	40	5

This test article will be considered 100% pure for dosage calculations. Animals will be dosed once daily from gestation days 7-19.

Observations and Results**Mortality**

- There was a dead female (Day 8) which appeared to be due to intubation error.

Clinical Signs

- Clinical signs consisted of few/soft stool, rapid breathing and arched back in the HD rabbits. These signs were also noted in the female that died on GD8.
- Some of the clinical signs were seen in MD but not in the LD females
- One HD and a LD aborted on Day 23 and 26 while one MD female delivered prematurely on GD 28

Body Weight

- Significant decreases in BW and BW gain were seen in the females at 200 mg/kg. The weight lost during treatment phase was recovered during post treatment phase (GD 19-29).
- There was no significant difference in BW, BW gain and gravid uterine weight between lorcaseerin treated and control group at the end of the study.

AN ORAL SEGMENT II STUDY IN RABBITS

SUMMARY OF GESTATION BODY WEIGHT CHANGES (GRAMS)

GROUP:		1	2	3	4
LEVEL (MG/KG/DAY):		0	20	60	200
DAYS 7 TO 19	MEAN	183 d	209	178	-43**
	S.D.	99.5	109.8	118.3	165.7
	N	19	20	20	19
DAYS 19 TO 29	MEAN	167 d	154	222	371**
	S.D.	107.8	148.3	139.4	110.1
	N	18	19	19	18

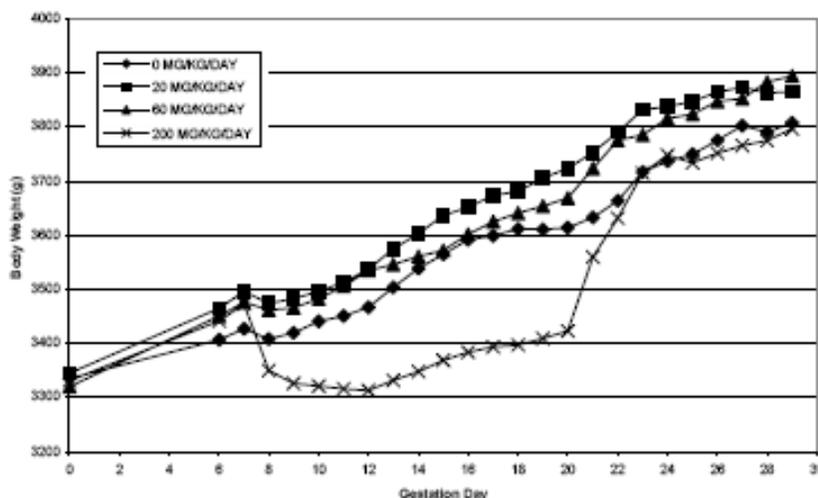
Statistical key: d=ANOVA/DUNNETT-TEST ** = P<0.01

AN ORAL SEGMENT II STUDY IN RABBITS
SUMMARY OF GESTATION BODY WEIGHT DATA (GRAMS)

GROUP : LEVEL (MG/KG/DAY) :		1 0	2 20	3 60	4 200
DAY 7	MEAN	3427 d	3496	3476	3473
	S.D.	332.3	317.5	312.1	265.7
	N	19	20	20	20
DAY 19	MEAN	3611 d	3706	3654	3409
	S.D.	375.4	303.6	316.7	250.2
	N	19	20	20	19
DAY 25	MEAN	3750 d	3848	3824	3734
	S.D.	363.9	272.6	337.4	271.4
	N	18	20	20	18

Statistical key: d-ANOVA/DUNNETT-TEST

SUMMARY OF MEAN GESTATION BODY WEIGHT



Feed Consumption

- The food intake was significantly decreased in the HD with corresponding decrease in BW (seen above). The decrease in food intake reversed after cessation of lorcaserin treatment.

Toxicokinetics

- The exposure at 20 and 60 mg/kg on Day 7 were similar to GD 19.
- AUC exposure at 200 mg/kg on Day 19 was 6.6 fold less than Day 7.
- Lorcaserin exposure appeared to be dose-proportional from 20 to 60 mg/kg but not from 60 to 200 mg/kg, specifically on Day 19 (GD 19). The exposure on Day 19 at 200 mg/kg was 43x higher than AUC at 60 mg/kg.
- Since there were no changes in exposure at 20 and 60 mg/kg on Day 7 vs. Day 19, the high exposure on Day 19 suggests changes to absorption (empty GI) or decreased metabolism.
- Based on AUC, lorcaserin bioavailability is very poor in rabbits relative to rats, monkeys and humans but closer to mice.
- In the dose ranging study, lorcaserin exposure at 300 mg/kg on Day 1 and 19 was 4.2 and 15.3 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively, which is significantly less than AUC exposure at 200 mg/kg on Day 19. The overall trend suggests changes in absorption and possible metabolism. The fetal exposure is likely to be minimal due to poor maternal exposure. This also suggests that maternal incidences may not be drug related but due to maternal weight loss.

Day	Dosage Level (mg/kg/day)	C _{max} (µg/mL)	t _{max} (hr)	AUC _{last} (hr·µg/mL)	AUC _{INF} (hr·µg/mL)
7	20	0.056	0.5	0.179	0.189
	60	0.153	0.5	0.549	0.554
	200	0.315	1.0	2.921	2.934
19	20	0.068	0.5	0.155	0.162
	60	0.193	0.5	0.443	0.552
	200	1.639	2.0	19.295	19.304

Exposure margin

Species	Dose, mg/kg	lorcaserin AUC, µg.h/ml	Exposure margins (rabbit AUC /Human AUC)
Oral Embryo-fetal development in rabbits, NOAEL=60 mg/kg	20	0.155	0.15
	60	0.443	0.43
	200	19.295	19
Clinical Dose: lorcaserin , 10 mg BID		1.02	

Stability and Homogeneity: The drug solutions were stable for at least 7 days. The target concentrations were 103 and 105% of the target concentrations which is within the ±10% specification.

Necropsy

- One HD (#R0537) and a LD (#R0490) aborted on Day 23 and 26, respectively while one MD (#R0513) female delivered prematurely on GD 28
- Internal gross necropsy of one female (#R0537) at 200 mg/kg that aborted on Day 23 included one placenta in the vagina. She had 13 empty implantation sites. The aborted material consisted of 2 early resorption and 6 placentas.
- Internal gross necropsy of one MD female (#R0513) that delivered prematurely on GD 28 had vaginal and pale. This female also had 13 empty implantation sites. The aborted material consisted of 7 placentas, 12 late resorption with one partially cannibalized late resorption. One viable fetus developed normally.
- Internal gross necropsy of one LD female (#R0490) that aborted on GD 26 had also abnormal material in the uterus and red fluid in vagina. This female had 2 viable fetuses that developed normally. She had one placenta, two early resorptions and four late resorptions. The aborted material consisted of one late resorption.
- Internal gross examination of one HD female (#R0538) that was found dead on GD 8 due to apparent intubation error had reddish brown fluid in the abdominal cavity with autolysis in the abdominal tissue. She had distended cecum and colon and dark areas in the heart with pale kidneys, dark red trachea and reddened linear mucosa in the urinary bladder. She had seven implantation sites. The death of this female was due to intubation error.
- One control female was euthanized moribund on GD 22 due to significant clinical observation that included worsening wobbly gait, few feces and mobility. The cause of death was not determined. She had 10 viable fetuses normally developed externally.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- There was no notable difference in C-section parameters among groups regarding corpora lutea, implantation sites, pre-implantation loss, live fetuses, post-implantation loss, dead fetuses, early resorption, late resorption and mean fetal body weight
- The pregnancy rate was 94.7% in control and 100% in the treated rabbits

AN ORAL SEGMENT II STUDY IN RABBITS

SUMMARY OF SURVIVAL AND PREGNANCY

GROUP:		1	2	3	4
LEVEL(MG/KG/DAY):		0	20	60	200
NO. OF FEMALES	N	20	20	20	20
PREGNANT	N	19	20	20	20
- DIED/EUTHANIZED	N	1	0	0	1
- ABORTED DIED/EUTHANIZED	N	0	1	0	1
- EUTHANIZED/PREMATURE DELIVERY	N	0	0	1	0
NONPREGNANT	N	1	0	0	0
- DIED/EUTHANIZED	N	0	0	0	0
TOTAL FEMALES DIED/EUTHANIZED	N	1 f	1	1	2
	%	5.0	5.0	5.0	10.0
EXAMINED AT SCHEDULED C-SECTION	N	19	19	19	18
- NONPREGNANT	N	1	0	0	0
- WITH TOTAL IMPLANT LOSS	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
- WITH VIABLE FETUSES	N	18 f	19	19	18
	%	94.7	100.0	100.0	100.0

STATISTICAL KEY: f-CHI-SQUARE/FISHERS EXACT TEST

AN ORAL SEGMENT II STUDY IN RABBITS

SUMMARY OF CESAREAN SECTION DATA

GROUP:		1	2	3	4
LEVEL(MG/KG/DAY):		0	20	60	200
Pregnant	N	18	19	19	18
Dams with no Viable Fetuses	N	0	0	0	0
Dams with Viable Fetuses	N	18	19	19	18
Corpora Lutea	TOTAL	181	206	202	177
No. per animal	MEAN	10.1 d	10.8	10.6	9.8
	S.D.	1.86	1.21	2.03	1.76
Implantation Sites	TOTAL	170	183	184	170
No. per animal	MEAN	9.4 d	9.6	9.7	9.4
	S.D.	1.72	1.89	2.00	1.69
Preimplantation Loss	TOTAL	11	23	18	7
No. per animal	MEAN	0.6 d	1.2	0.9	0.4
	S.D.	0.85	1.72	1.03	0.61
% per animal	MEAN%	5.8 k	10.9	8.9	3.8
	S.D.	7.72	15.59	10.03	5.64
Live Fetuses	TOTAL	161	171	175	162
No. per animal	MEAN	8.9 d	9.0	9.2	9.0
	S.D.	1.95	2.13	2.15	1.50
Males	TOTAL	79	82	89	78
	MEAN%	48.3 k	46.7	51.0	48.4
	S.D.	13.43	19.09	17.05	17.92
Females	TOTAL	82	89	86	84
	MEAN%	51.7 k	53.3	49.0	51.6
	S.D.	13.43	19.09	17.05	17.92

Statistical key: d-ANOVA/DUNNETT-TEST k-KRUSKAL-WALLIS

AN ORAL SEGMENT II STUDY IN RABBITS

SUMMARY OF CESAREAN SECTION DATA

GROUP:		1	2	3	4
LEVEL(MG/KG/DAY):		0	20	60	200
Postimplantation Loss No. per animal	TOTAL	9	12	9	8
	MEAN	0.5 d	0.6	0.5	0.4
	S.D.	1.20	0.90	0.77	0.98
% implants per animal	MEAN%	5.1 k	7.2	5.0	4.2
	S.D.	12.10	11.44	8.51	8.18
Dead Fetuses No. per animal	TOTAL	0	0	0	0
	MEAN	0.0 k	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00
% of implants per animal	MEAN%	0.0 k	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00
Resorptions: Early No. per animal	TOTAL	7	6	5	4
	MEAN	0.4 k	0.3	0.3	0.2
	S.D.	0.98	0.58	0.45	0.43
% of implants per animal	MEAN%	4.1 k	3.0	2.8	2.3
	S.D.	9.96	5.70	5.00	4.64
Resorptions: Late No. per animal	TOTAL	2	6	4	4
	MEAN	0.1 k	0.3	0.2	0.2
	S.D.	0.32	0.67	0.54	0.73
% of implants per animal	MEAN%	1.0 k	4.2	2.2	1.8
	S.D.	2.98	10.15	5.75	5.80
Fetal Body Weight (g)	MEAN	41.7 d	41.4	42.0	42.3
	S.D.	4.26	5.12	4.67	4.73
	N	18	19	19	18
Male Fetuses	MEAN	42.7 d	42.6	42.4	43.5
	S.D.	3.67	5.26	4.94	4.85
Female Fetuses	MEAN	41.3 d	40.8	40.9	41.4
	S.D.	5.26	5.11	5.31	4.82

Statistical key: d=ANOVA/DUNNETT-TEST

Offspring (malformations, variations, etc.)

- There was statistically significant in incidence of total fetal external, visceral or skeletal malformations or developmental variations between lorcaseerin treated animals and control. The incidence of heart and or great vessel anomaly was slightly higher than the background. Since serotonin agonists are known to affect the valves, the significance of this observation is uncertain. It is very likely the small variations at 200 mg/kg were due to significant weight.
- Since fetal exposure was likely below detection limits, any fetal variation was likely due to poor food intake and maternal BW gain than lorcaseerin.

Fetal Finding(s)	Type	Group 4		Historical Control	
		Incidence	Percent	Incidence	Percent
Short Tail	EM	1/18	5.6	Not in current HC	
Heart and/or Great Vessel Anomaly	VM	2/18	11.1	1/17	5.9
Cervical Vertebral Anomaly	SM	1/18	5.6	2/17	11.8
Ovary Cyst	VV	1/18	5.6	1/20	5.0
27 Presacral Vertebrae	SV	14/18	77.8	14/17	82.4
28 Presacral Vertebrae	SV	1/18	5.6	Not in current HC	
Hyoid Arch(es) Bent	SV	4/18	22.2	9/15	60.0
7 th Sternebrae	SV	2/18	11.1	Not in current HC	
Rib(s) 13 th Full	SV	18/18	100	19/19	100
Rib(s) 14 th Full	SV	1/18	5.6	Not in current HC	
Talus Unossified	SV	1/18	5.6	2/17	11.8

Note: EM = External Malformation, VM = Visceral Malformation, SM = Skeletal Malformation, VV = Visceral Variation and SV = Skeletal Variation.

Notable fetal observations are shown in table below. Since most findings occurred at the high dose of 200 mg/kg (19x the clinical dose) were likely due to significant weight loss.

SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

GROUP: LEVEL (MG/KG/DAY):	1 0	2 20	3 60	4 200
Litters Examined Externally	18	19	19	18
Fetuses Examined	161	171	175	162
OMPHALOCELE				
Fetal Incidence	0	1	1	0
Litter Incidence	0 f	1	1	0
SPINA BIFIDA				
Fetal Incidence	0	0	1	0
Litter Incidence	0 f	0	1	0
CLUB FOOT				
Fetal Incidence	0	1	0	0
Litter Incidence	0 f	1	0	0
HINDLIMB ROTATION				
Fetal Incidence	0	0	1	0
Litter Incidence	0 f	0	1	0
SHORT TAIL				
Fetal Incidence	0	0	0	1
Litter Incidence	0 f	0	0	1
Litters Examined Viscerally	18	19	19	18
Fetuses Examined	161	171	175	162
HEART AND/OR GREAT VESSEL ANOMALY				
Fetal Incidence	0	0	0	2
Litter Incidence	0 f	0	0	2
INFERIOR VENA CAVA ANOMALY				
Fetal Incidence	0	0	1	0
Litter Incidence	0 f	0	1	0
DIAPHRAGM - HYPOPLASTIC				
Fetal Incidence	1	0	0	0
Litter Incidence	1 f	0	0	0
UNASCENDED KIDNEY(S)				
Fetal Incidence	0	0	1	0
Litter Incidence	0 f	0	1	0
LUNG LOBE(S) AGENESIS				
Fetal Incidence	0	2	2	0
Litter Incidence	0 f	1	2	0
Litters Examined Skeletally	18	19	19	18
Fetuses Examined	161	171	175	162
INTERPARIETAL BIPARTITE				
Fetal Incidence	0	0	1	3
Litter Incidence	0 f	0	1	1
NASALS FUSED				
Fetal Incidence	1	0	0	0
Litter Incidence	1 f	0	0	0
PARIETALS FUSED				
Fetal Incidence	0	0	1	0
Litter Incidence	0 f	0	1	0
CERVICAL VERTEBRAL ANOMALY				
Fetal Incidence	0	0	0	1
Litter Incidence	0 f	0	0	1
VERTEBRAL ANOMALY WITH/WITHOUT RIB ANOMALY				
Fetal Incidence	1	0	0	1
Litter Incidence	1 f	0	0	1
EXTRA SITE OF OSSIFICATION ANTERIOR TO STERNEBRA #1				
Fetal Incidence	2	0	2	1
Litter Incidence	2 f	0	2	1
TOTAL MALFORMATIONS				
NUMBER WITH EXTERNAL MALFORMATIONS				
Fetal Incidence	0	2	2	1
Litter Incidence	0 f	2	2	1
NUMBER WITH VISCERAL MALFORMATIONS				
Fetal Incidence	1	2	4	2
Litter Incidence	1 f	1	3	2
NUMBER WITH SKELETAL MALFORMATIONS				
Fetal Incidence	4	0	5	5
Litter Incidence	4 f	0	4	2
TOTAL NUMBER WITH MALFORMATIONS				
Fetal Incidence	5	4	9	8
Litter Incidence	5 f	3	7	4

Statistical key: f=CHI-SQUARE/FISHERS EXACT TEST

9.3 Prenatal and Postnatal Development

Study title: Lorcaserin: Oral Pre- and Postnatal Developmental Toxicity Study in Rats

Study no: TX06025 ((b) (4))
Study report location: Arena Pharmaceuticals, San Diego, CA
Conducting laboratory and location: (b) (4)
Date of study initiation: June 19, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: #06A009, purity of 97.5%

Key Study Findings

- All dams survived to the end of the study
- Salivation was noted in few MD (15 mg/kg) but a lot of HD (50 mg/kg) dams
- Lorcaserin dose-dependently and significantly reduced both BW gain and food intake at 5, 15 and 50 mg/kg during Gestation and lactation.
- Pregnancy rate was similar among dams
- Pup weight was reduced at ≥ 5 mg/kg but were significant at 50 mg/kg
- The percentage of live pups was reduced and the number of pups found dead was increased by lorcaserin dose of 50 mg/kg resulting in reduced viability index (87.3% vs. 98% in control).
- The gestation index was similar among groups while lactation index was reduced at 15 and 50 mg/kg.
- Necropsy of the dams found minimal changes in dams such as fluid cyst in right kidney of 1 MD and papillary process with cyst in live of 1 HD dam.
- F1 male to female ratio was unaffected by the lorcaserin treatment.
- Although the terminal BW of F1 generation males and females were similar among groups, there was a significant reduction in postweaning BW of F1 males at 50 mg/kg and in females at all doses, corresponding to decline in BW of dams.
- Food intake was reduced in both F1 generation (male and females) correlating with the decline in BW.
- There were no notable differences in the behavioral evaluation using passive avoidance paradigm and water maze test among groups.
- There was no significant difference in mating or fertility parameters in the F1 generation. The pregnancy rate in the control, LD, MD and HD F1 generation was 96%, 84%, 96% and 88%, respectively.
- The pup generation from mating of F1 generation did not resulted in significant gross alterations distinguishable among groups. Litter parameters were not affected by the lorcaserin treatment.
- Due to weight loss at all doses of lorcaserin, the maternal NOAEL was considered to be less than 5 mg/kg while the reproductive NOAEL in dams was considered to be 5 mg/kg due to the reduction in lactation index at 15 mg/kg and increased in percentage of stillborn pups, the number of dead or cannibalized pups at 50 mg/kg.

Methods

Doses: 0, 5, 15 and 50 mg/kg (C, LD, MD & HD)
 Frequency of dosing: daily
 Dose volume: 5 ml/kg
 Route of administration: oral gavage
 Formulation/Vehicle: deionized water
 Species/Strain: Crl:CD(SD) female rats
 Number/Sex/Group: 25/group
 Satellite groups: No
 Study design: Seg III: DG9 though DL 20 or GD24 (no litter)
 Deviation from study protocol: Minimal

Female rats (F0) were treated with lorcaserin from gestation day (DG 7) through lactation day 20 (DL 20). Dams that did not deliver a litter were treated until DG 24 before sacrifice. F1 generation did not receive drug directly except for any drug during maternal gestation or via milk during nursing. Milk samples were collected following intravenous oxytocin injection for evaluation of lorcaserin (but not evaluated/submitted).

F0 Generation Rats

Dosage Group	Number of Rats	Dosage ^a (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Assigned F0 Generation Rat Numbers
I	25	0	0	5	18301 - 18325
II	25	5	1	5	18326 - 18350
III	25	15	3	5	18351 - 18375
IV	25	50	10	5	18376 - 18387, 4444 ^b , 18389-18400

a. A correction factor of 1.025 was used to adjust the dosage concentration for the hemihydrate form.

b. Rat #18388 was found dead on DG 8 due to an intubation error and was replaced with rat #4444.

F1 generation were evaluated for sexual maturation (beginning on postpartum day 28 in females and postpartum day 39 in males) and behavioral changes (passive avoidance test, water maze test, reproductive capacity). The reproductive capacity of F1 generation was tested at approximately 90 days of age in both males and females. After completion of the data collection, rats were sacrificed by carbon dioxide asphyxiation.

F1 Generation Rats

Maternal Dosage Group	Number of Rats Per Sex	Assigned F1 Generation Rat Number	
		Male Rats	Female Rats
I	25	1701 - 1709, 9000 ^a , 1711 - 1725	1801 - 1825
II	25	1726 - 1750	1826 - 1850
III	25	1751 - 1775	1851 - 1875
IV	25	1776 - 1800	1876 - 1900

a. Rat #1710 was excluded from study on day 21 postpartum due to adverse clinical observations and was replaced with rat #9000.

Observations and Results

F0 Dams

Survival: There were no drug-related deaths during dosing

Clinical signs: Salivation and chromorrhinorrhea were noted at 15 and 50 mg/kg.

Other findings did not appear to be dose-dependent thus not considered drug-related.

Body weight:

- Body weight gain was significantly reduced in a dose-dependent manner at 5, 15 and 50 mg/kg (87%, 82% and 72% of control value).
- The absolute BW at DG 20 (390 g, 377 g, 374g and 361g) and DL 21 (332 g, 327 g, 322 g and 310g) in the control, LD, MD and HD, respectively.
- Significant decrease in BW was noted at 15 and 50 mg/kg. The decrease in BW in the HD is similar to fetal developmental study at 50 mg/kg.
- The decrease in BW and BW gain continued after parturition during the lactation phase.

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	22	25	23
INCLUDED IN ANALYSES	N	25	21b	25	23
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+38.6 ± 7.8	+38.8 ± 8.0	+41.8 ± 7.5	+36.8 ± 7.7
DAYS 7 - 10	MEAN±S.D.	+11.6 ± 4.8	+2.2 ± 5.2**	-3.7 ± 7.2**	-9.0 ± 7.3**
DAYS 10 - 12	MEAN±S.D.	+11.2 ± 4.3	+10.0 ± 3.8	+8.6 ± 5.0	+3.6 ± 5.3**
DAYS 12 - 15	MEAN±S.D.	+20.4 ± 5.9	+19.9 ± 5.3	+20.3 ± 7.2	+18.3 ± 7.8
DAYS 15 - 18	MEAN±S.D.	+36.2 ± 8.5	+32.7 ± 9.6	+34.5 ± 6.5	+37.7 ± 7.5
DAYS 18 - 20	MEAN±S.D.	+28.5 ± 8.7	+28.6 ± 7.0	+29.2 ± 7.1	+27.6 ± 6.4
DAYS 7 - 20	MEAN±S.D.	+107.9 ± 23.9	+93.5 ± 20.1*	+88.8 ± 17.7**	+78.3 ± 11.1**
DAYS 0 - 20	MEAN±S.D.	+146.5 ± 26.2	+132.3 ± 21.4*	+130.6 ± 20.8*	+115.0 ± 13.2**

DAYS = DAYS OF GESTATION

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Excludes values for dam 18332; only one early resorption was present in utero on day 25 of gestation.

* Significantly different from the control group value (p≤0.05).

** Significantly different from the control group value (p≤0.01).

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MATERNAL BODY WEIGHTS - LACTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	22	25	23
DELIVERED A LITTER	N	25	21	25	23
INCLUDED IN ANALYSES	N	25	21	25	21b
MATERNAL BODY WEIGHT (G)					
DAY 14	MEAN±S.D.	337.9 ± 17.9	332.4 ± 16.8	329.0 ± 17.9	312.5 ± 18.7**
DAY 15	MEAN±S.D.	336.5 ± 19.5	333.1 ± 13.0	329.0 ± 18.7	316.7 ± 20.8**
DAY 16	MEAN±S.D.	338.7 ± 18.3	335.8 ± 12.6	329.4 ± 22.2	314.6 ± 20.2**
DAY 17	MEAN±S.D.	337.6 ± 18.5	334.4 ± 13.6	325.5 ± 19.8*	312.8 ± 18.3**
DAY 18	MEAN±S.D.	336.7 ± 20.3	330.3 ± 15.0	327.8 ± 21.0	311.5 ± 19.6**
DAY 19	MEAN±S.D.	334.5 ± 16.3	328.5 ± 14.7	323.6 ± 19.3*	311.3 ± 16.9**
DAY 20	MEAN±S.D.	330.6 ± 17.1	324.5 ± 12.7	319.3 ± 20.0*	308.3 ± 20.0**
DAY 21	MEAN±S.D.	331.6 ± 19.5	326.7 ± 14.4	322.0 ± 18.1	309.6 ± 18.8**

DAY = DAY OF LACTATION

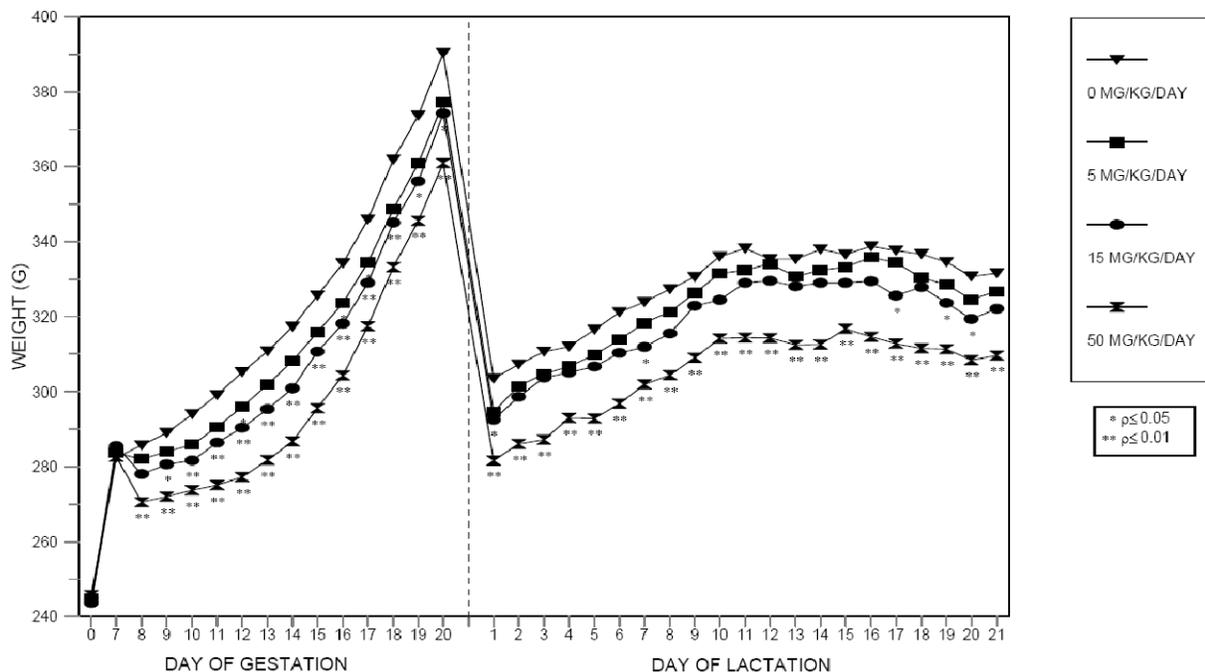
a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Excludes values for dams that were sacrificed due to no surviving pups.

* Significantly different from the control group value (p≤0.05).

** Significantly different from the control group value (p≤0.01).

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS



Feed consumption:

- The food intake was affected in the same manner as BW changes. Food intake was significantly reduced during gestation phase at 5, 15 and 50 mg/kg (91%, 86% and 75% of the control value). By far the highest dose, 50 mg/kg had the greatest impact on food intake suppression.

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MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0	II 5	III 15	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	22	25	23
INCLUDED IN ANALYSES	N	25	21 ^b	25	23
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 0 - 7	MEAN±S.D.	22.9 ± 1.8	22.6 ± 1.9	23.5 ± 2.4 [24] ^c	22.6 ± 2.0 [22] ^c
DAYS 7 - 10	MEAN±S.D.	24.3 ± 1.7	21.1 ± 3.4**	17.7 ± 2.9**	13.5 ± 3.9**
DAYS 10 - 12	MEAN±S.D.	25.7 ± 2.3	23.3 ± 3.5**	21.4 ± 2.7** [24] ^c	16.6 ± 2.1** [22] ^c
DAYS 12 - 15	MEAN±S.D.	25.9 ± 1.9	23.1 ± 2.0**	22.6 ± 2.3**	19.8 ± 2.8**
DAYS 15 - 18	MEAN±S.D.	24.6 ± 3.0	23.5 ± 2.4	22.7 ± 2.2*	21.9 ± 2.8**
DAYS 18 - 20	MEAN±S.D.	24.4 ± 2.3	22.7 ± 2.5*	23.3 ± 2.2	21.7 ± 2.2**
DAYS 7 - 20	MEAN±S.D.	25.0 ± 1.6	22.7 ± 2.0**	21.4 ± 1.6**	18.7 ± 1.6**
DAYS 0 - 20	MEAN±S.D.	24.2 ± 1.4	22.7 ± 1.8**	22.1 ± 1.7**	20.0 ± 1.4**

DAYS = DAYS OF GESTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Excludes values for dam 18332; only one early resorption was present *in utero* on day 25 of gestation.

c. Excludes values that could not be calculated as well as those associated with spillage.

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

** Significantly different from the control group value (p<0.01).

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - LACTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0	II 5	III 15	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	22	25	23
DELIVERED A LITTER	N	25	21	25	23
INCLUDED IN ANALYSES	N	25	21	25	21 ^b
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 1 - 4	MEAN±S.D.	33.3 ± 8.9 [24] ^c	31.3 ± 6.5 [20] ^c	30.1 ± 5.4	28.5 ± 4.4
DAYS 4 - 7	MEAN±S.D.	43.1 ± 8.7	38.6 ± 9.6 [20] ^c	39.0 ± 8.4	38.1 ± 5.6
DAYS 7 - 10	MEAN±S.D.	51.1 ± 9.7	47.7 ± 10.9	45.5 ± 9.3	46.3 ± 6.3
DAYS 10 - 14 ^d	MEAN±S.D.	59.2 ± 10.2 [24] ^c	53.7 ± 10.3*	52.5 ± 7.7*	51.2 ± 6.4**
DAYS 1 - 14 ^d	MEAN±S.D.	47.5 ± 7.9 [24] ^c	44.0 ± 7.9	42.6 ± 6.5*	41.8 ± 4.8**

DAYS = DAYS OF LACTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Excludes values for dams that were sacrificed due to no surviving pups.

c. Excludes values that were associated with spillage.

d. Because it is presumed that the pups begin to consume maternal feed after day 14 of lactation, maternal feed consumption values were not tabulated on days 14 to 21 of lactation.

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

** Significantly different from the control group value (p<0.01).

Uterine content:

- All pregnant dams delivered litters with exception of one LD which was found to be pregnant at terminal sacrifice on DG 25.
- The number of pregnant dams were 25 (100%), 22 (88%), 25 (100%) and 23 (92%) females at 0, 5, 15 and 50 mg/kg, respectively.
- The gestation index (live birth/pregnant) was 100%, 95%, 100% and 100% at 0, 5, 15 and 50 mg/kg.
- The number of dams with stillborn were 1, 1, 1 and 4 in the control, LD, MD and HD, respectively

- Only two dams lost all their pups (post partum Day 1 and 2) and they occurred at 50 mg/kg. There were no such findings in the control, LD or MD dams. It is not clear if this was due to significant weight loss or not.

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NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS					
DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0	II 5	III 15	IV 50
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25
PREGNANT	N	25(100.0)	22(88.0)	25(100.0)	23(92.0)
DELIVERED LITTERS	N(%)	25(100.0)	21(95.4)b	25(100.0)	23(100.0)
DURATION OF GESTATION c	MEAN±S.D.	22.6 ± 0.5	22.6 ± 0.7	22.6 ± 0.5	22.5 ± 0.5
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	376 15.0 ± 4.4	303 14.4 ± 3.1	393 15.7 ± 3.0	358 15.6 ± 1.7
DAMS WITH STILLBORN PUPS	N(%)	1(4.0)	1(4.0)	1(4.0)	4(17.4)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX d	% N/N	100.0 25/ 25	95.4 21/ 22	100.0 25/ 25	100.0 23/ 23
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	2(8.7)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

- a. Dosage occurred on day 7 of gestation through day 20 of lactation.
 b. Excludes values for dam 18332; only one early resorption was present in utero on day 25 of gestation.
 c. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the time the first pup was delivered.
 d. Number of rats with live offspring/number of pregnant rats.

Necropsy observation:

- Necropsy observation included fluid filled cyst on the papillary process of the liver in 1 HD dam.
- Two HD dams that were sacrificed on Day 1 or 2 of lactation due to having no surviving pups. Parts of the pups were found in the stomach suggesting cannibalization of the pups.

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NECROPSY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS					
DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0	II 5	III 15	IV 50
RATS EXAMINED b	N	25	25	25	25
MORTALITY	N	0	0	0	0
APPEARED NORMAL	N	24	25	24	22
LIVER: PAPILLARY PROCESS, CLEAR FLUID FILLED CYST	N	0	0	0	1
STOMACH: PUP TISSUE PRESENT	N	0	0	0	2
KIDNEYS: RIGHT AND LEFT, PELVIS, SLIGHT AND MODERATE DILATION	N	1	0	0	0
RIGHT, CLEAR FLUID FILLED CYST	N	0	0	1	0

- a. Dosage occurred on day 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
 b. Refer to the individual clinical observations table (Table A15) for external observations confirmed at necropsy.

Toxicokinetics: Plasma drug levels were not determined. According to the protocol, milk samples were collected but no data on analysis was provided to show drug presence in the milk.

Stability and homogeneity: Lorcaserin was stable in the solutions. The drug concentrations in the solutions were within the specified range of $\pm 10\%$.

F1 Generation

Survival:

- Overall, the number of pups delivered among groups was similar but the number of surviving pups per litter was lower in the HD group relative to control.
- 1 male in the MD and 2 in HD were sacrificed or found dead during postweaning Days 2 and 3 due to poor health caused either poor maternal care or presence of lorcaserin in the milk. The clinical signs in these pups included one or all of the clinical signs: cold to touch, dehydration, reduced motor activity, reduced BW.
- Percent male to female ratio was similar among groups (no treatment effect)
- The percentage of live born pups delivered was significantly reduced in HD group
- Dams with still borne pups totaled 1, 1, 1 and 7 in control, LD, MD and HD, respectively.
- As noted before, 2 dams in the HD group lost all their pups on postpartum day 1 and 2.
- The viability index was significantly but slightly lower in the HD dams (98%, 98%, 98% & 87% in C, LD, MD & HD, respectively).
- The lactation index was also significantly but slightly lower in the MD and HD dams (99%, 98%, 96% & 96% in C, LD, MD & HD, respectively).

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LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0	II 5	III 15	IV 50	
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS		N	25	21	25	23
SURVIVING PUPS/LITTER b						
DAY 1c	MEAN \pm S.D.	13.9 \pm 3.9	13.4 \pm 3.9	13.5 \pm 3.5	13.3 \pm 2.9	
DAY 4	MEAN \pm S.D.	13.6 \pm 3.8	13.1 \pm 3.8	13.2 \pm 3.6	11.6 \pm 4.1**	
DAY 7	MEAN \pm S.D.	13.6 \pm 3.8	13.1 \pm 3.7	13.2 \pm 3.6	11.6 \pm 4.0**	
DAY 14	MEAN \pm S.D.	13.6 \pm 3.8	13.0 \pm 3.7	13.0 \pm 3.5	11.4 \pm 3.9**	
DAY 21	MEAN \pm S.D.	13.6 \pm 3.8	12.8 \pm 3.6	12.7 \pm 3.7	11.2 \pm 4.0**	
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED						
DAY 1c	MEAN \pm S.D.	46.2 \pm 15.4	57.0 \pm 18.2	51.4 \pm 16.2	49.5 \pm 18.2	
DAY 4	MEAN \pm S.D.	46.6 \pm 15.6	57.1 \pm 18.4	50.8 \pm 16.1	52.3 \pm 16.8 [21]d	
DAY 7	MEAN \pm S.D.	46.6 \pm 15.6	57.2 \pm 18.3	51.0 \pm 16.0	52.5 \pm 16.7 [21]d	
DAY 14	MEAN \pm S.D.	46.4 \pm 15.6	56.7 \pm 18.5	50.7 \pm 16.1	52.9 \pm 16.1 [21]d	
DAY 21	MEAN \pm S.D.	46.6 \pm 15.6	56.9 \pm 18.5	49.9 \pm 17.9	53.4 \pm 17.1 [21]d	

DAY = DAY POSTPARTUM

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Average number of live pups per litter, including litters with no surviving pups.

c. Includes liveborn pups and pups that died before weighing on day 1 postpartum.

d. Excludes values for litters that had no surviving pups.

** Significantly different from the control group value ($p \leq 0.01$).

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LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS						
DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) a		0	5	15	50	
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS		N	25	21	25	23
PUPS DELIVERED (TOTAL)		N	350	282	339	323
		MEAN±S.D.	14.0 ± 3.9	13.4 ± 3.9	13.6 ± 3.5	14.0 ± 2.2
LIVEBORN		MEAN±S.D.	13.9 ± 3.9	13.4 ± 3.9	13.5 ± 3.5	13.3 ± 2.9
		N(%)	348 (99.4)	281 (99.6)	338 (99.7)	307 (95.0)**
STILLBORN		MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.3 ± 0.9
		N(%)	1 (0.3)	1 (0.4)	1 (0.3)	7 (2.2)**
UNKNOWN VITAL STATUS		N	1	0	0	9
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED						
DAY		N/N(%)	4/348 (1.1)	0/281 (0.0)	4/338 (1.2)	15/307 (4.9)**
DAYS	2- 4	N/N(%)	3/344 (0.9)	5/281 (1.8)	3/334 (0.9)	24/292 (8.2)**
DAYS	5- 7	N/N(%)	0/341 (0.0)	1/276 (0.4)	2/331 (0.6)	1/268 (0.4)
DAYS	8-14	N/N(%)	1/341 (0.3)	3/275 (1.1)	5/329 (1.5)	4/267 (1.5)
DAYS	15-21	N/N(%)	1/340 (0.3)	3/272 (1.1)	7/324 (2.2)	5/263 (1.9)
VIABILITY INDEX b		%	98.0	98.2	97.9	87.3
		N/N	341/348	276/281	331/338	268/307**
LACTATION INDEX c		%	99.4	97.5	95.8	96.3
		N/N	339/341	269/276	317/331**	258/268**

DAY(S) = DAY(S) POSTPARTUM

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.

c. Number of live pups on day 21 postpartum/number of live pups on day 4 postpartum.

** Significantly different from the control group value (p≤0.01).

Clinical signs:

- The Clinical signs were minor and did not appear to be drug-related. Some of the observations included cold to the touch (among all groups) and purple or black tail tip appearance in 3 pups in HD.
- Tip of tail was missing in 1 pup in LD and HD

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CLINICAL OBSERVATIONS FROM BIRTH TO DAY 21 POSTPARTUM - SUMMARY - F1 GENERATION PUPS					
MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY) a		0	5	15	50
LITTERS EXAMINED (N)		25	21	25	23
TRANSIENT CLINICAL OBSERVATIONS: b		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS			
EMACIATION	N/N	0/0	1/1	0/0	0/0
PTOSIS	N/N	0/0	1/1	0/0	0/0
LOST RIGHTING REFLEX	N/N	0/0	3/1	0/0	0/0
LOWER MIDLINE, CYST	N/N	6/1	0/0	0/0	0/0
PERSISTENT CLINICAL OBSERVATIONS: b		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS			
TIP OF TAIL MISSING	N/N	0/0	12/1	0/0	15/1
PORTION OF SNOUT MISSING	N/N	0/0	0/0	0/0	1/1

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Tabulation restricted to adverse observations; all other pups appeared normal.

Body weight:

- The BW of pups tended to follow the BW trend in dams discussed earlier. High dose male pups had lower BW than controls up to Day 50 with terminal BW being similar to the control group (516 g vs. 542 g in controls). The BW changes in the F1 HD males tend to be significantly less than control on several time intervals up to day 36 but not later.

- The BW of female pups (F1) in LD, MD and HD rats were significantly less than controls up to Day 50, corresponding to lower maternal BW. The terminal BW in treated females was similar to control. Interestingly LD and MD were affected longer than HD. BW during cohabitation was similar.

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LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	21	25	23
PUP WEIGHT/LITTER (GRAMS)					
MALE PUPS					
DAY 1	MEAN±S.D.	6.7 ± 0.7 [24]b	6.4 ± 0.6	6.4 ± 0.7	5.6 ± 0.6** [22]b
DAY 4	MEAN±S.D.	9.4 ± 1.2 [24]b	9.0 ± 1.4	8.9 ± 1.1	7.9 ± 1.0** [21]c
DAY 7	MEAN±S.D.	12.6 ± 2.0 [24]b	12.2 ± 2.2	12.2 ± 2.0	10.9 ± 1.5** [21]c
DAY 14	MEAN±S.D.	22.5 ± 3.6 [24]b	21.5 ± 4.0	20.6 ± 5.2	19.9 ± 3.4 [21]c
DAY 21	MEAN±S.D.	35.2 ± 6.4 [24]b	32.7 ± 6.8	32.0 ± 8.9	29.9 ± 5.4 [21]c
FEMALE PUPS					
DAY 1	MEAN±S.D.	6.4 ± 0.7	5.9 ± 0.3** [19]d	6.0 ± 0.4* [24]d	5.2 ± 0.6** [21]c
DAY 4	MEAN±S.D.	9.1 ± 1.3	8.1 ± 0.6** [19]d	8.3 ± 0.9* [24]d	7.4 ± 0.9** [21]c
DAY 7	MEAN±S.D.	12.4 ± 2.3	11.0 ± 1.5* [19]d	11.4 ± 1.6 [24]d	10.4 ± 1.4** [21]c
DAY 14	MEAN±S.D.	22.5 ± 4.4	19.6 ± 3.6* [19]d	19.2 ± 4.7* [24]d	19.0 ± 3.1** [21]c
DAY 21	MEAN±S.D.	35.1 ± 7.2	30.1 ± 5.4* [19]d	29.6 ± 7.5** [24]d	28.7 ± 4.5** [21]c

DAY = DAY POSTPARTUM

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Litters 18309 and 18390 no male pups.

c. Excludes values for litters that had no surviving pups.

d. Litters 18331, 18334 and 18353 had no female pups.

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

** Significantly different from the control group value (p<0.01).

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BODY WEIGHTS - PRECOHABITATION - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0	5	15	50
RATS TESTED	N	25	25	25	25
BODY WEIGHT (G)					
DAY 1	MEAN±S.D.	40.7 ± 6.6	34.3 ± 5.6**	33.3 ± 7.2**	32.9 ± 5.8**
DAY 8	MEAN±S.D.	74.4 ± 9.3	63.8 ± 7.7**	63.0 ± 10.5**	62.9 ± 9.8**
DAY 15	MEAN±S.D.	120.8 ± 11.0	106.0 ± 9.6**	103.2 ± 13.7**	103.8 ± 13.5**
DAY 22	MEAN±S.D.	158.4 ± 11.1	143.5 ± 11.6**	139.6 ± 14.2**	141.6 ± 14.9**
DAY 29	MEAN±S.D.	186.1 ± 13.8	167.1 ± 16.3**	165.6 ± 14.4**	168.2 ± 15.4**
DAY 36	MEAN±S.D.	209.2 ± 16.4	191.8 ± 16.6**	187.2 ± 14.7**	191.2 ± 17.7**
DAY 43	MEAN±S.D.	229.0 ± 19.4	213.7 ± 17.3**	205.9 ± 15.5**	210.9 ± 18.5**
DAY 50	MEAN±S.D.	246.0 ± 21.3	229.7 ± 21.8*	226.0 ± 19.7**	229.3 ± 21.6**
DAY 57	MEAN±S.D.	258.1 ± 21.2	240.7 ± 24.2*	234.4 ± 20.7**	242.9 ± 23.7*
DAY 64	MEAN±S.D.	268.2 ± 23.0	250.9 ± 25.3*	243.2 ± 20.5**	254.4 ± 26.8
DAY 71a	MEAN±S.D.	276.6 ± 24.5	259.0 ± 27.0*	250.1 ± 20.3**	262.0 ± 29.7
PRECOHABITATION b	MEAN±S.D.	277.8 ± 26.0	260.2 ± 26.5*	253.6 ± 19.7**	263.3 ± 28.3

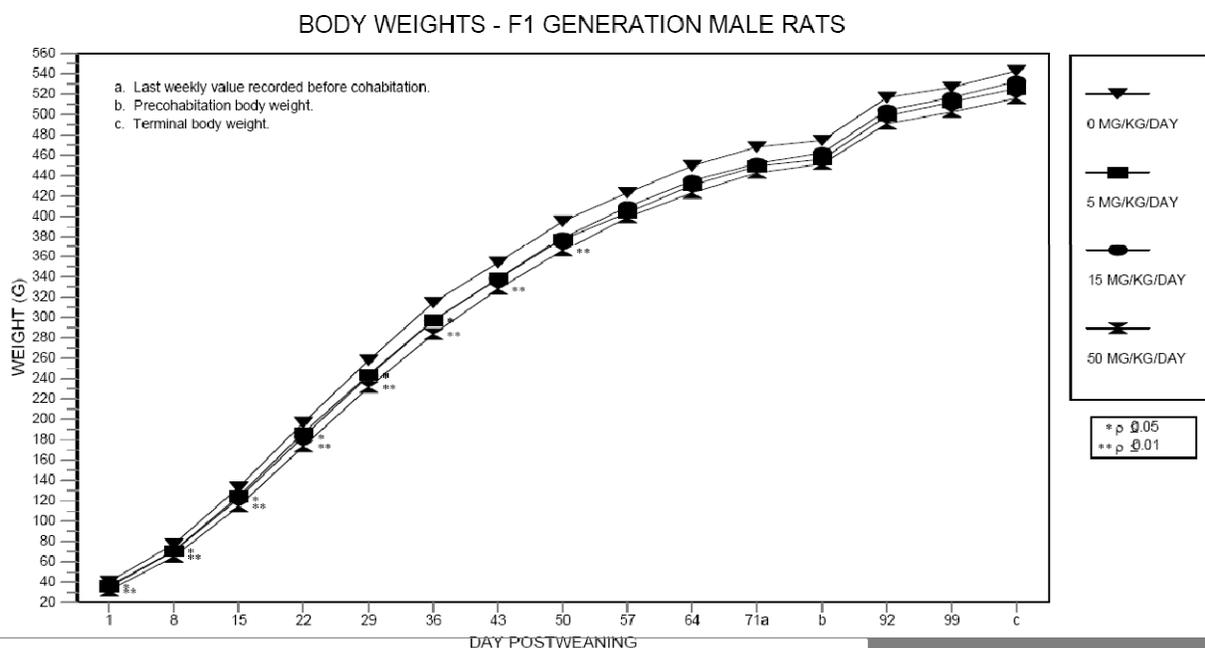
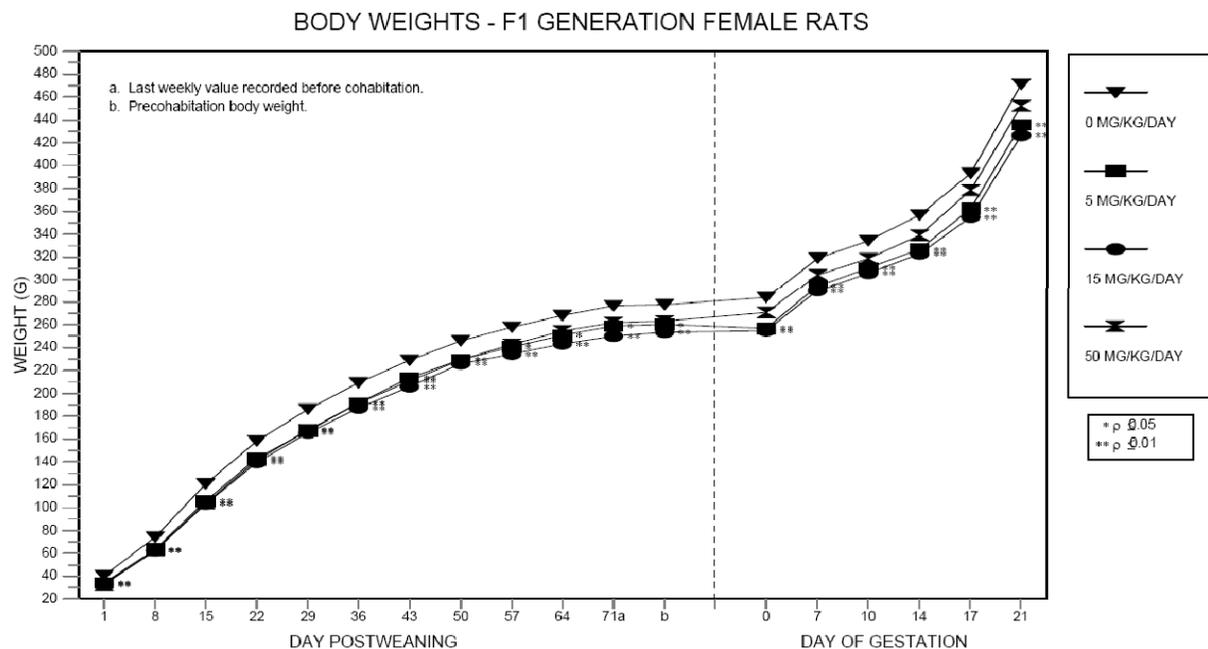
DAY = DAY POSTWEANING

a. Because body weight values were recorded at weekly intervals, based on each rat's day postweaning, day 71 postweaning was the last day in which the youngest rats had a body weight value recorded before cohabitation.

b. Precohabitation body weights were recorded on the day cohabitation began for the F1 generation rats; at that time these rats were 93 to 98 days of age.

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

** Significantly different from the control group value (p<0.01).



Feed consumption:

- The absolute food intake in F1 males was reduced in LD, MD and HD initially. The effect of MD and HD lasted longer (day 29 of postweaning in MD and day 71 in HD).
- The absolute food intake was also reduced in F1 females at LD, MD and HD. Again the effect lasted longer at higher doses.
- The food intake during gestation was unaffected.

Necropsy observation:

- Examination of the stomach of the 2, 1, 2 and 12 pups in control, LD, MD and HD groups found no milk in stomach, respectively.
- One pup in the HD group on DL1 had black heart and intestine.
- All pups except for 1 LD pup (herniated diaphragm) appeared normal at the scheduled sacrifice on lactation day 21.

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY) ^a		I 0	II 5	III 15	IV 50
LITTERS EVALUATED	N	25	21	25	23
TOTAL PUPS STILLBORN OR FOUND DEAD ^{b,c}	N	6	4	8	27
STILLBORN	N	1	1	1	7
FOUND DEAD	N	5	3	7	20
NO MILK IN STOMACH ^d	N(%)	2(40.0)	1(33.3)	2(28.6)	12(60.0)
HEART AND INTESTINES: BLACK	N(%)	0(0.0)	0(0.0)	0(0.0)	1(3.7)
PUPS SACRIFICED AND NECROPSIED ON DAY 21 POSTPARTUM ^c					
LITTERS EVALUATED	N	24 ^e	20 ^e	25	21
PUPS EVALUATED	N	275 ^e	208 ^e	267	207 ^e
APPEARED NORMAL					
LITTER INCIDENCE	N(%)	24(100.0)	19(95.0)	25(100.0)	21(100.0)
PUP INCIDENCE	N(%)	275(100.0)	207(99.5)	267(100.0)	207(100.0)
DIAPHRAGMATIC HERNIA					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
PUP INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)

a. Dosage occurred on day 7 of gestation through day 20 of lactation.

b. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded full evaluation.

c. Refer to the individual pup clinical observations table (Table A25) for external clinical observations confirmed at necropsy.

d. Analysis restricted to pups found dead and necropsied.

e. Excludes values for pups that did not have necropsy observations recorded.

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0	II 5	III 15	IV 50
RATS TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	25	24 ^a	23 ^a
TERMINAL BODY WEIGHT	MEAN±S.D.	542.8 ± 41.8	525.4 ± 63.8	532.6 ± 51.5	516.1 ± 46.9
EPIDIDYMIDES PAIRED	MEAN±S.D.	1.57 ± 0.13	1.44 ± 0.16**	1.58 ± 0.11	1.52 ± 0.17
	[] ^b	[24]b	[23]b	[22]b	[22]b
EPIDIDYMIDES PAIRED (%)	MEAN±S.D.	0.290 ± 0.030	0.278 ± 0.045	0.299 ± 0.031	0.296 ± 0.033
	[] ^b	[24]b	[23]b	[22]b	[22]b
TESTES PAIRED	MEAN±S.D.	3.62 ± 0.26	3.38 ± 0.40	3.52 ± 0.25	3.45 ± 0.27
	[] ^b	[24]b	[23]b	[22]b	[22]b
TESTES PAIRED (%)	MEAN±S.D.	0.673 ± 0.074	0.648 ± 0.094	0.665 ± 0.072	0.677 ± 0.080
	[] ^b	[24]b	[23]b	[22]b	[22]b

ALL WEIGHTS WERE RECORDED IN GRAMS (G).

RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

[] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.

b. Excludes values for rats that had abnormal organs (weight affected).

** Significantly different from the control group value (p≤0.01).

Neurological assessment:

- The passive avoidance test found no statistically significant differences among groups in learning, short-term retention, long-term retention or response inhibition in the F1 males and female. The significance of reduced latency in MD and HD males in trial 1 is not clear since other tests did not show any change.

- In water maze test, there was no statistically significant watermaze performance regarding learning, short-term retention or response inhibition. However, the number of error per trial in the MD groups was significantly increased. Again the significance of this finding is also not clear as it occurred in session 1 but not 2.

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

PASSIVE AVOIDANCE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0	5	15	50
<u>MALE RATS</u>					
SESSION 1a	N	24	21	24b	21
TRIALS TO CRITERION	MEAN±S.D.	4.4 ± 1.0	4.0 ± 0.9	4.1 ± 1.0	4.1 ± 1.2
LATENCY TRIAL 1c	MEAN±S.D.	6.2 ± 5.8	6.2 ± 4.6	4.9 ± 3.4	11.6 ± 13.3
LATENCY TRIAL 2c	MEAN±S.D.	31.0 ± 23.8	36.5 ± 21.8	36.0 ± 20.5	38.0 ± 21.3
FAILED TO LEARN d	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	24	21	24b	20e
TRIALS TO CRITERION	MEAN±S.D.	2.7 ± 0.6	2.9 ± 0.8	3.0 ± 0.4	3.2 ± 0.6
LATENCY TRIAL 1c	MEAN±S.D.	35.0 ± 23.2	37.5 ± 21.4	21.4 ± 17.1*	19.5 ± 20.2*
<u>FEMALE RATS</u>					
SESSION 1a	N	25	19	24	21
TRIALS TO CRITERION	MEAN±S.D.	4.2 ± 0.7	4.4 ± 0.9	4.1 ± 1.2	4.2 ± 0.7
LATENCY TRIAL 1c	MEAN±S.D.	4.8 ± 3.8	4.8 ± 4.2	9.5 ± 11.6	5.4 ± 3.4
LATENCY TRIAL 2c	MEAN±S.D.	27.6 ± 21.0	26.6 ± 20.8	32.2 ± 23.0	35.1 ± 22.9
FAILED TO LEARN d	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	25	19	23f	21
TRIALS TO CRITERION	MEAN±S.D.	3.0 ± 0.7	3.1 ± 0.6	3.1 ± 0.7	3.1 ± 0.4
LATENCY TRIAL 1c	MEAN±S.D.	29.6 ± 21.4	16.7 ± 14.9	22.0 ± 20.6	20.1 ± 17.1

- Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
 - Excludes values for rat 1760, which was sacrificed on day 2 postweaning due to adverse clinical observations.
 - The latency was recorded in seconds.
 - Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
 - Excludes values for rat 1782, which was found dead on day 3 postweaning.
 - Excludes values for rat 1852, which was tested five days early.
- * Significantly different from the control group value (p≤0.05).

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

WATERMAZE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0	5	15	50
<u>MALE RATS:</u>					
SESSION 1a	N	24	21	24b	20b
TRIALS TO CRITERION	MEAN±S.D.	7.8 ± 2.1	8.8 ± 2.5	9.1 ± 2.4	8.6 ± 2.9
ERRORS PER TRIAL	MEAN±S.D.	0.34 ± 0.22	0.39 ± 0.16	0.35 ± 0.15	0.36 ± 0.21
LATENCY TRIAL 2c	MEAN±S.D.	16.0 ± 9.3	16.0 ± 12.2	14.0 ± 8.6	14.4 ± 11.1
FAILED TO LEARN d	N(%)	0(0.0)	1(4.8)	0(0.0)	0(0.0)
SESSION 2a	N	24	20	24b	20b
TRIALS TO CRITERION	MEAN±S.D.	6.7 ± 2.2	6.4 ± 2.7	6.6 ± 2.7	5.8 ± 1.1
ERRORS PER TRIAL	MEAN±S.D.	0.16 ± 0.18	0.06 ± 0.09	0.10 ± 0.14	0.10 ± 0.13
LATENCY TRIAL 1c	MEAN±S.D.	11.8 ± 9.6	8.0 ± 2.9	10.5 ± 7.6	10.1 ± 5.6
<u>FEMALE RATS:</u>					
SESSION 1a	N	25	19	24	21
TRIALS TO CRITERION	MEAN±S.D.	8.2 ± 2.3	7.7 ± 2.0	8.8 ± 2.5	8.7 ± 2.6
ERRORS PER TRIAL	MEAN±S.D.	0.33 ± 0.12	0.31 ± 0.15	0.43 ± 0.16*	0.34 ± 0.13
LATENCY TRIAL 2c	MEAN±S.D.	16.3 ± 9.8	16.3 ± 10.9	18.0 ± 14.5	16.0 ± 8.1
FAILED TO LEARN d	N(%)	0(0.0)	0(0.0)	1(4.2)	1(4.8)
SESSION 2a	N	25	19	23	20
TRIALS TO CRITERION	MEAN±S.D.	7.1 ± 2.6	6.8 ± 2.1	7.1 ± 2.7	6.6 ± 2.6
ERRORS PER TRIAL	MEAN±S.D.	0.21 ± 0.26	0.20 ± 0.20	0.13 ± 0.13	0.15 ± 0.18
LATENCY TRIAL 1c	MEAN±S.D.	13.1 ± 9.1	16.6 ± 9.1	11.1 ± 5.1	14.4 ± 9.3

- Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
 - Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.
 - The latency was recorded in seconds.
 - Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
- * Significantly different from the control group value (p≤0.05).

Reproduction (F1 generation):

- Sexual maturation was unaffected by the lorcaserin treatment. There were no apparent differences among groups in the fertility index, number of rats mated, and the number of confirmed pregnancies.
- Pregnancy rate was 96%, 84%, 96% and 88% in F1 generation.
- The vaginal patency was delayed in the HD F1 females than control ($p < 0.05$). The delay may have been due to smaller size in the HD pups.
- The reproductive parameters listed in table below were not affected by the lorcaserin treatment. The significantly lower corpora lutea in the LD rats was likely a coincidental finding.

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TXJ6025)

CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0	II 5	III 15	IV 50
RATS TESTED		N 25	25	25	25
PREGNANT DELIVERED AND SACRIFICED		N(%) 24 (96.0) N(%) 1 (4.2)	21 (84.0) 1 (4.0)	24 (96.0) 0 (0.0)	22 (88.0) 0 (0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION		N 23a	20	24	22a
CORPORA LUTEA		MEAN±S.D. 17.5 ± 2.3	15.7 ± 1.6*	16.2 ± 1.6	16.7 ± 1.8
IMPLANTATIONS		MEAN±S.D. 16.6 ± 2.6	15.2 ± 1.7	15.2 ± 3.4	16.2 ± 1.7
LITTER SIZES		MEAN±S.D. 15.9 ± 2.9	14.4 ± 1.9	13.5 ± 4.3	15.7 ± 2.2
LIVE FETUSES		N 365 MEAN±S.D. 15.9 ± 2.0	287 14.4 ± 1.9	325 13.5 ± 4.3	345 15.7 ± 2.2
DEAD FETUSES		N 1 MEAN±S.D. 0.0 ± 0.2	0 0.0 ± 0.0	0 0.0 ± 0.0	0 0.0 ± 0.0
RESORPTIONS		MEAN±S.D. 0.6 ± 1.3	0.8 ± 1.1	1.6 ± 2.6	0.5 ± 1.2
EARLY RESORPTIONS		N 15 MEAN±S.D. 0.6 ± 1.3	17 0.8 ± 1.1	39 1.6 ± 2.6	11 0.5 ± 1.2
LATE RESORPTIONS		N 0	0	0	0
DAMS WITH ANY RESORPTIONS		N(%) 8 (34.8)	10 (50.0)	12 (50.0)	5 (22.7)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED		N(%) 0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DAMS WITH VIABLE FETUSES		N(%) 23 (100.0)	20 (100.0)	24 (100.0)	22 (100.0)
PLACENTAE APPEARED NORMAL		N(%) 22 (95.5)	20 (100.0)	24 (100.0)	22 (100.0)

a. Includes values for dams 1808 and 1900, which did not have confirmed mating dates.

* Significantly different from the control group value ($p < 0.05$).

F2 Generation

Survival: The numbers of live fetuses were similar among groups
Body weight: There was no treatment effect on BW of fetuses (F2 generation)
External evaluation: There were no drug-related external variations
Male/Female ratio: The male to female ratio was similar among groups

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0	II 5	III 15	IV 50
LITTERS WITH ONE OR MORE LIVE FETUSES		N 23a	20	24	22a
IMPLANTATIONS	MEAN±S.D.	16.6 ± 2.6	15.2 ± 1.7	15.2 ± 3.1	16.2 ± 1.7
LIVE FETUSES	N	365	287	325	345
	MEAN±S.D.	15.9 ± 2.8	14.4 ± 1.9	13.5 ± 4.3	15.7 ± 2.2
LIVE MALE FETUSES	N	183	149	161	176
‡ LIVE MALE FETUSES/LITTER	MEAN±S.D.	50.6 ± 12.0	51.7 ± 15.1	47.2 ± 15.9	51.1 ± 13.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.21 ± 0.30 [22]b	5.28 ± 0.29	5.18 ± 0.36	5.23 ± 0.31 [21]b
MALE FETUSES	MEAN±S.D.	5.35 ± 0.30 [22]b	5.41 ± 0.29	5.33 ± 0.34 [23]c	5.39 ± 0.34 [21]b
FEMALE FETUSES	MEAN±S.D.	5.04 ± 0.32 [22]b	5.12 ± 0.30	5.06 ± 0.38	5.09 ± 0.29 [21]b
‡ DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.2 ± 8.3	5.5 ± 7.3	10.8 ± 17.9	3.2 ± 7.9

[] = NUMBER OF VALUES AVERAGED

- a. Includes values for litters 1808 and 1900; the dams did not have confirmed mating dates.
- b. Excludes values for litters 1808 and 1900; the dams did not have confirmed mating dates.
- c. Litter 1851 had no male fetuses.

PROTOCOL AVA00057: APD356: ORAL PRE- AND POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN RATS (SPONSOR'S STUDY NUMBER: TX06025)

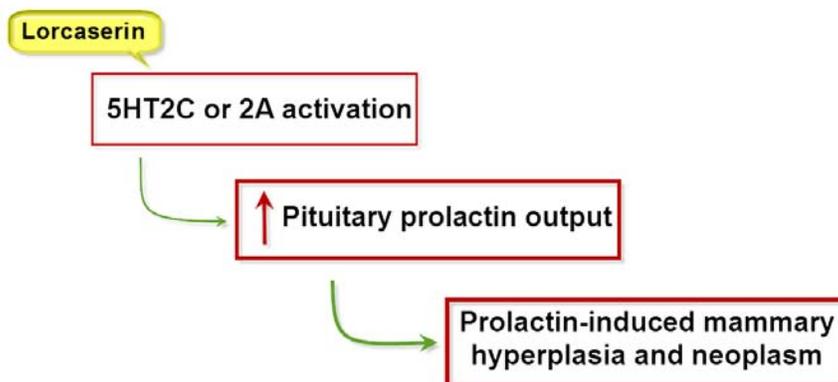
FETAL GROSS EXTERNAL ALTERATIONS		SUMMARY		F2 GENERATION LITTERS/FETUSES	
MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0	II 5	III 15	IV 50
LITTERS EVALUATED	N	23a	20	24	22a
FETUSES EVALUATED	N	366	287	325	345
LIVE	N	365	287	325	345
DEAD	N	1b	0	0	0
ANUS: NO OPENING PRESENT					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)c	0(0.0)	0(0.0)	0(0.0)
TAIL: ABSENT					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)c	0(0.0)	0(0.0)	0(0.0)

- a. Includes values for litters 1808 and 1900; the dams did not have confirmed mating dates.
- b. Dead fetus was excluded from summarization and statistical analyses.
- c. Fetus 1824-3 had other gross external alterations.

10 Special Mechanistic Studies

Mechanistic Studies Designed To Explore the Role of Prolactin in Lorcaserin Induced Mammary Neoplasia in Rats

The sponsor had performed series of mechanism studies to support the hypothesis that lorcaserin induced increase in mammary gland neoplasia in rats was due to lorcaserin related increase in serum prolactin levels. The studies were designed to show that the lorcaserin effect is via prolactin similar to several approved antipsychotic drugs which are recognized to increase prolactin and prolactin-related increase in mammary tumors in rats.



Lorcaserin is a 5HT_{2C} selective agonist (K_i=13 nM). Binding and functional tests have shown lorcaserin to be at least 5 and 11 fold more selective to 5HT_{2C} than to 5HT_{2A} (K_i=92 nM) and 5HT_{2B} (K_i=147 nM), respectively. The EC₅₀ for human 5HT_{2A}, 5HT_{2B} and 5HT_{2C} is estimated to be 133, 811 and 9 nM, respectively. Lorcaserin had no notable binding to dopamine receptors in the ^{(b) (4)} assay. Oral administration of lorcaserin (36 mg/kg) did not alter intracellular dopamine or serotonin in nucleus Accumbens in rat while dexfenfluramine (2.6 mg/kg), a nonselective serotonin agonist increased intracellular serotonin but not dopamine, suggesting both compounds may low abuse potential (a dopamine effect in nucleus accumbens). Lorcaserin is extensively metabolized in all species but the prominent inactive metabolites are M1 (lorcaserin sulfamate) and to some extent M5 (N-carbamoyl glucuronide).

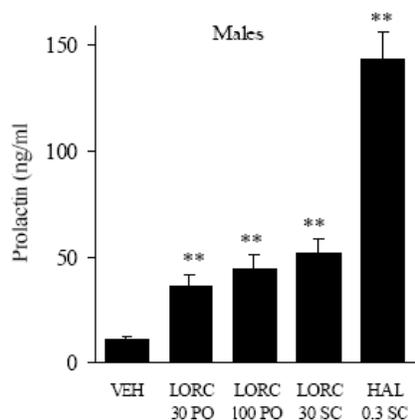
Study Title: Acute Effects of Lorcaserin on Serum Prolactin Levels in Rats (DBR-08-031)

In an acute non-GLP study, single dose of lorcaserin (30-100 mg/kg, PO) and neuroleptic haloperidol (positive control, 0.3 mg/kg, SC) were administered to 12 week old male and female SD rats (Arena Discovery Labs, March 20, 2008). Haloperidol is a dopamine antagonist known to increase serum prolactin levels. Prolactin levels were measured 30 min after administration of each compound via cardiac puncture under CO₂ anesthesia. A subset of females were bled (1 ml) prior to drug administration via

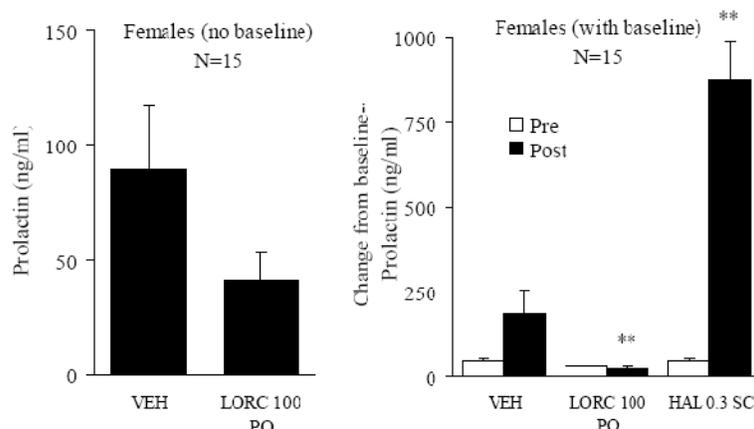
tail vein. Serum samples were sent to (b) (4) for prolactin analysis.

In this single dose study, lorcaserin increased prolactin levels in male rats but not in females. In female rats, serum prolactin levels appeared to decrease while haloperidol consistently increased serum prolactin levels in both male and female rats suggesting that haloperidol is a very potent stimulator of prolactin release. Since the lorcaserin induced increase in prolactin in male rats was relatively weak compared to haloperidol, the finding may be related to an acute effect possible stress related. For example, even administration of vehicle increased prolactin in females by ~5 fold. However, based on the study, the sponsor concluded that ovarian hormones may have been interfering with the prolactin release in female rats. Since haloperidol was able to increase prolactin levels in males by 15 fold and in females by as much as 80 fold, the role of lorcaserin in prolactin release in male rats thus was likely incidental.

Effects of lorcaserin and haloperidol on serum prolactin levels in male Sprague Dawley rats



Effects of lorcaserin and haloperidol on serum prolactin levels in female Sprague Dawley rats **p<0.01 compared to vehicle controls



In summary acute administration (single dose) of lorcaserin increased prolactin levels in male rats but not in female rats. In contrast, haloperidol, a dopamine antagonist, robustly increased prolactin levels in both males and female rats.

Study Title: Sub-chronic Effects of Lo on Serum Prolactin Levels in Ovariectomized Female Rats (DBR-08-032)

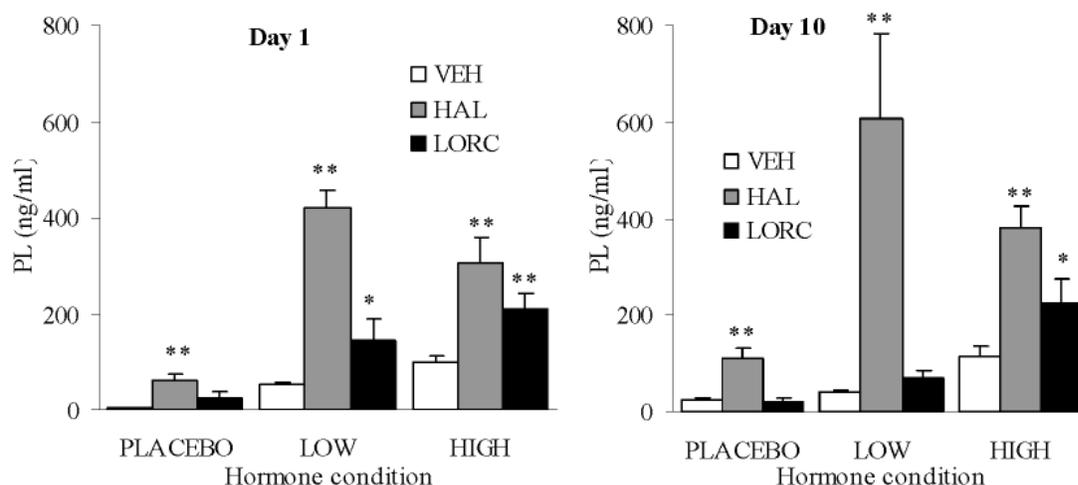
In a follow up sub-chronic non-GLP study, the role of multiple doses lorcaserin on prolactin levels was evaluated in ovariectomized female rats (May 28, 2008, Area Discovery Labs). The study was similar to the single dose study described above except that lorcaserin dose of 100 mg/kg was administered by gavage for 10 days to ovariectomized rats replenished with ovarian hormones (0.1 mg estradiol- β + 15 mg progesterone or 2.5 mg estradiol- β +35 mg progesterone). Haloperidol (0.3 mg/kg, SC) served as positive control.

As expected, haloperidol increased serum prolactin levels after acute and 10 day dosing in ovariectomized female rats replenished with ovarian hormones regardless of hormone replacement regimen. In contrast, lorcaserin increased prolactin levels only in animals receiving hormone replacement and not in placebo implanted ovariectomized

rats. Since lorcaserin's effect was minimal with low dose hormone replacement (normal levels) and only increased with supra-pharmacological doses of estradiol and progesterone, the relevance of this finding is questionable. The normal estradiol levels in female rats is about 2 to 50 pg/ml vs. estradiol levels with mega dose of implanted pellets. The reviewer estimated estradiol levels were up to 20x the normal levels. The study conditions do not resemble the conditions in the carci study. The estradiol levels in the ovariectomized rats were closer to high normal (next study) than it should have been and hormone replenishment itself produced a significant increase in serum prolactin.

Effect of lorcaserin and haloperidol on serum prolactin levels after acute and sub-chronic treatment in ovariectomized female rats implanted with placebo or hormone pellets

*p<0.05; **p<0.01

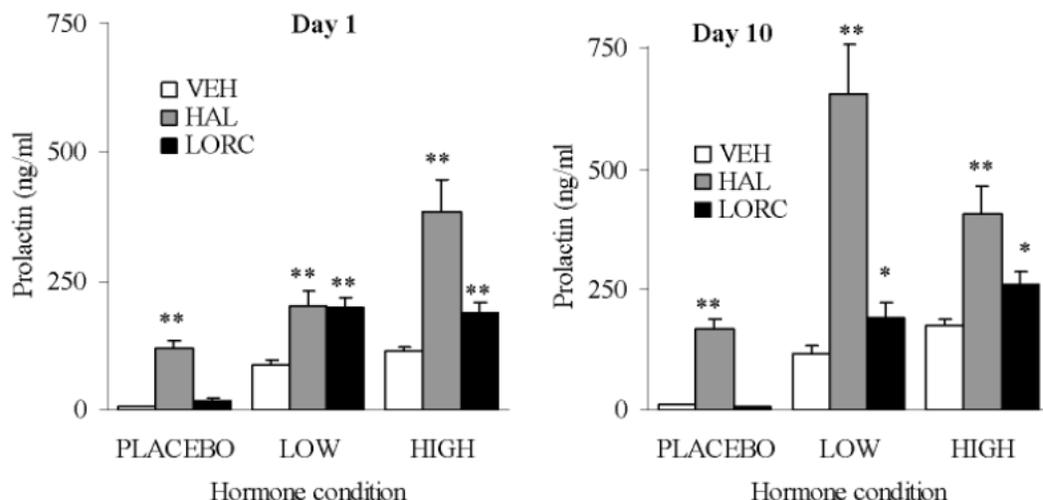


Study Title: Sub-chronic Effects of Lorcaserin on Serum Prolactin Levels in Ovariectomized Female Rats (DBR-09-001)

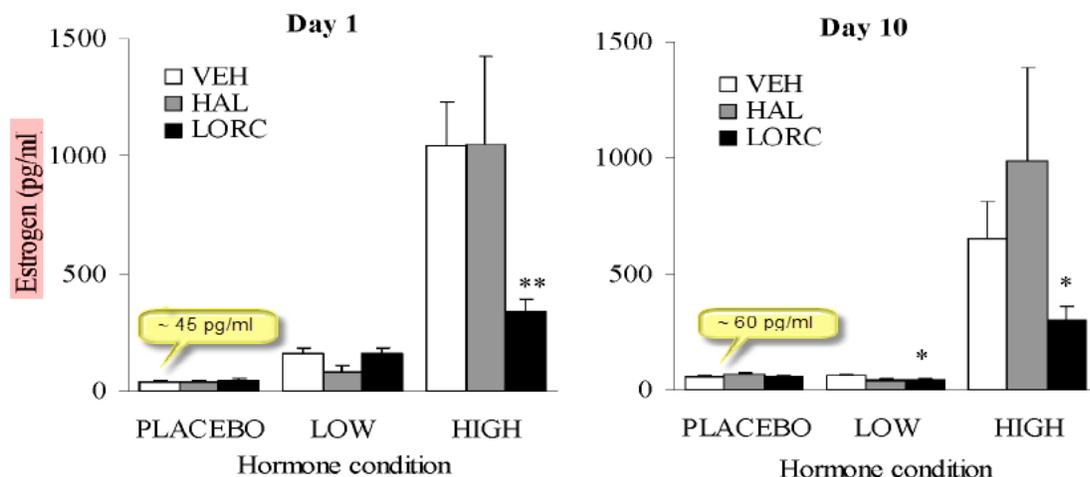
The sponsor repeated the same study above (Nov 4, 2008) in the same animal model as described in study # DBR-08-032 (Arena Discovery Labs). The study findings were pretty much similar to those in the earlier study. As discussed earlier, the relevance of ovariectomized rats replenished with supra-pharmacological doses of estradiol/progesterone is questionable and not similar to rats in the carci study. For prolactin to be the intermediary hormone between lorcaserin and mammary tumors there should have been persistent elevation in prolactin levels in intact rats in the chronic studies as was the case for most antipsychotic drugs.

Effect of lorcaserin and haloperidol on serum prolactin levels after acute and sub-chronic treatment in ovariectomized female rats implanted with placebo or hormone pellets

*p<0.05; **p<0.01 vs. vehicle



Serum estrogen levels in ovariectomized female rats implanted with hormone pellets



Study Title: A preliminary pharmacokinetic evaluation of prolactin release and concentration of other hormones in female SD rats after treatment with APD356 (b) (4) 670001 & TX08001)

In a mechanistic study, the effects of single and multiple doses of APD356 (100 mg/kg, 10 days) on several endogenous hormones were evaluated in ovariectomized Crl:CD(SD) rats. The positive control groups (intact and ovariectomized) received S⁺ fenfluramine (10 mg/kg, IP), a nonselective serotonin agonist. This GLP study was

initiated on Jan 16, 2008 and was carried out at (b) (4) for the sponsor.

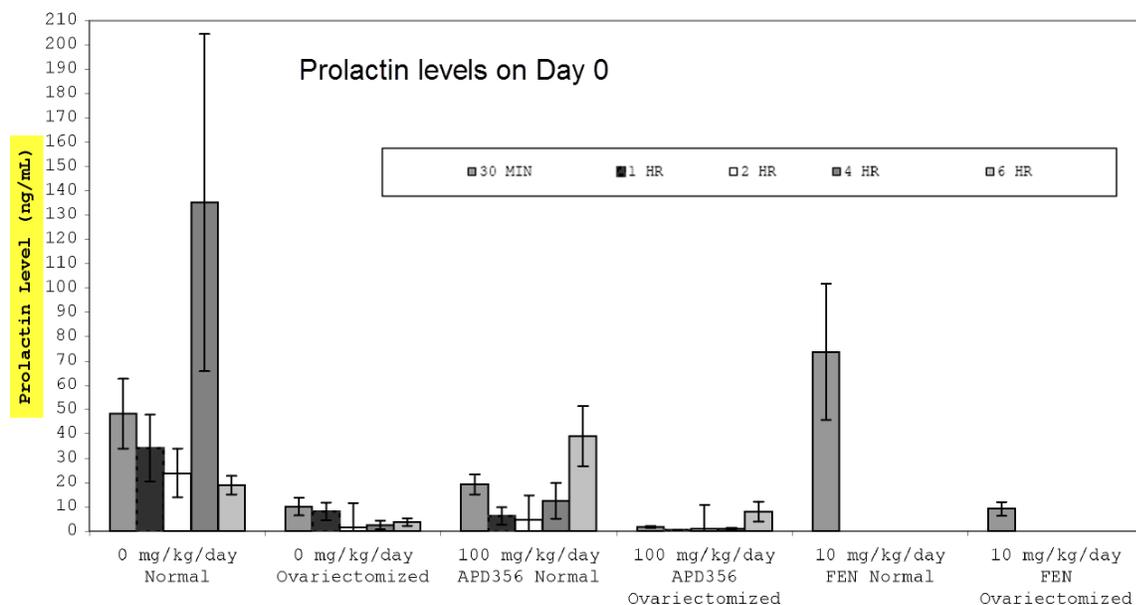
Group Number	Test Article	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals ^a	
				Normal Females	Ovariectomized Females
1	Vehicle	0	10	50	50
2	APD356	100	10	50	50
3	S ⁺ Fenfluramine	10	1	10	10

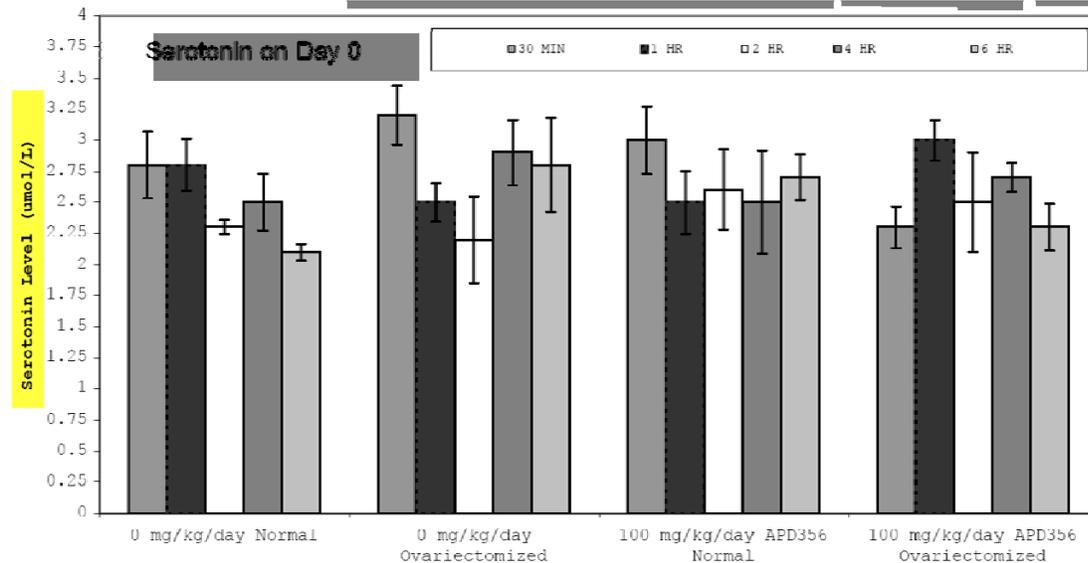
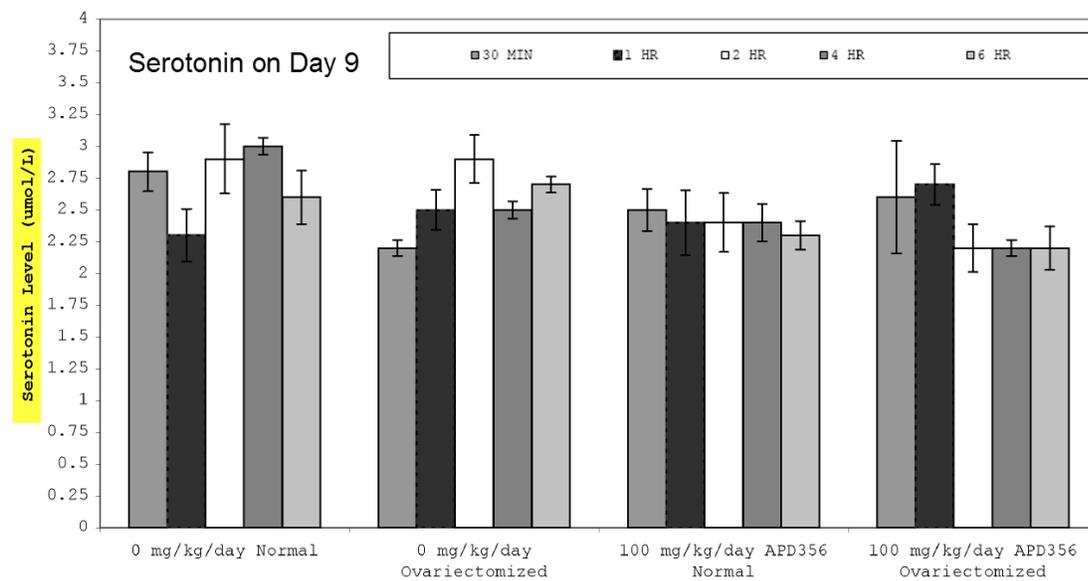
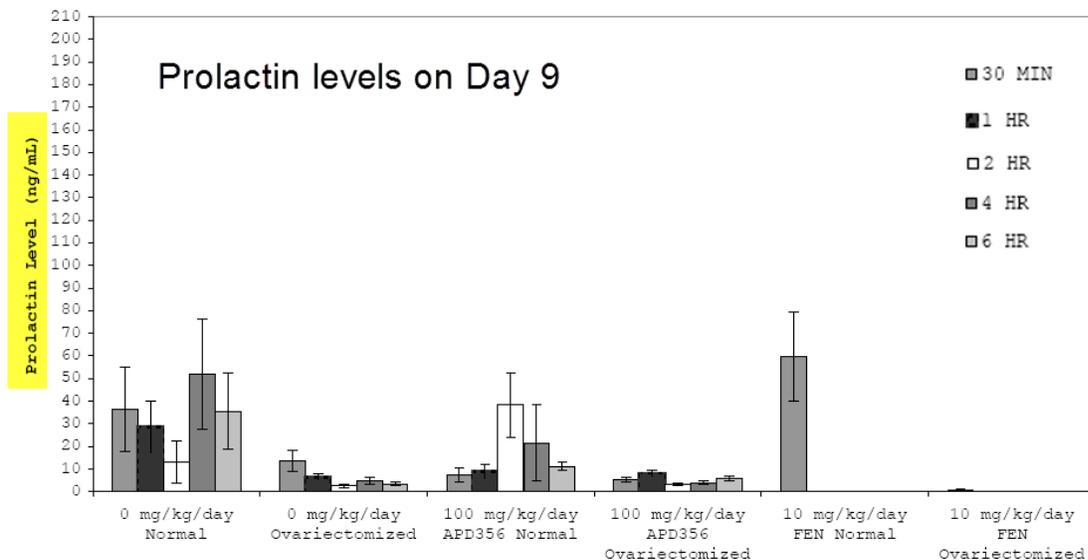
^a = One-half of the normal and ovariectomized animals from each group were exsanguinated (under anesthesia) during a 6-hour time course after the first dose on study day 0 and the remaining animals were exsanguinated, during a 6-hour time course on study day 9, after the tenth dose administration.

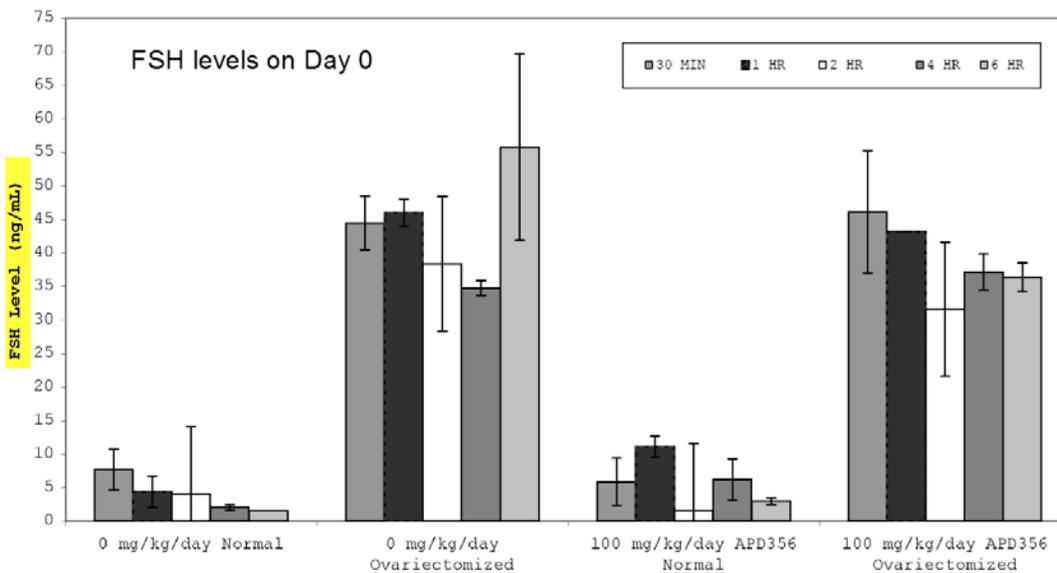
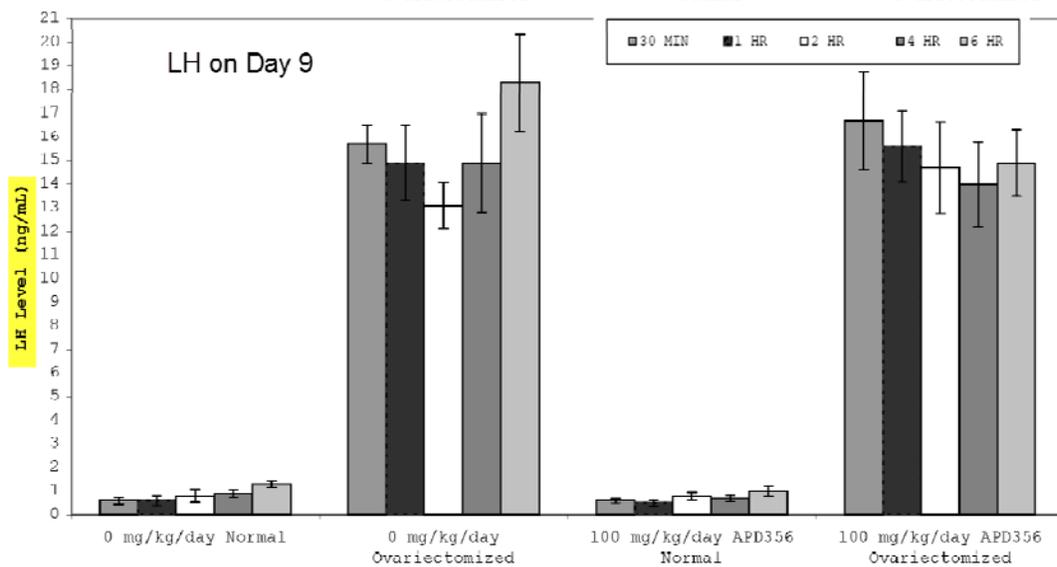
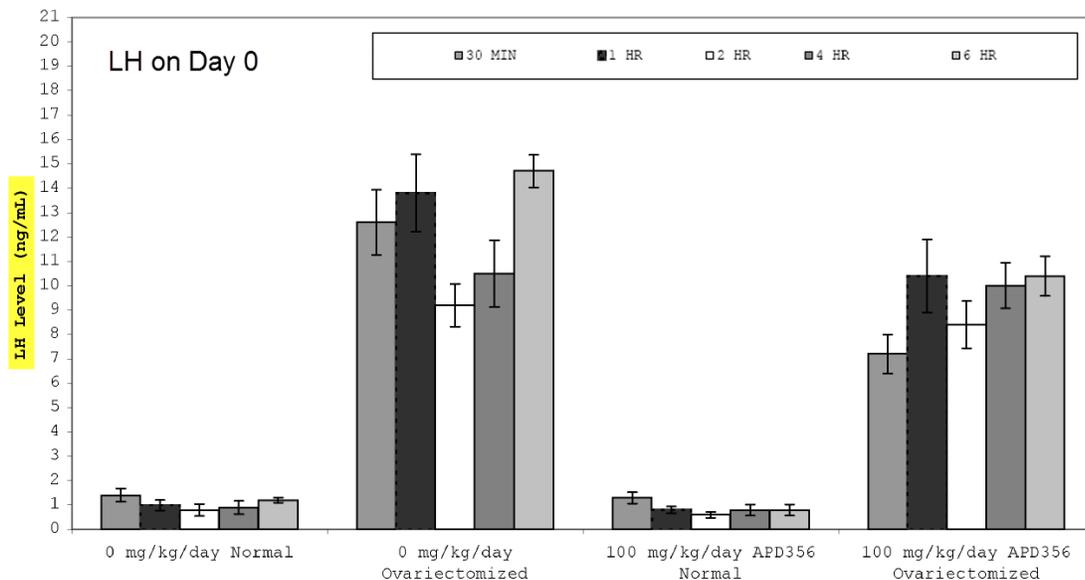
Study Findings

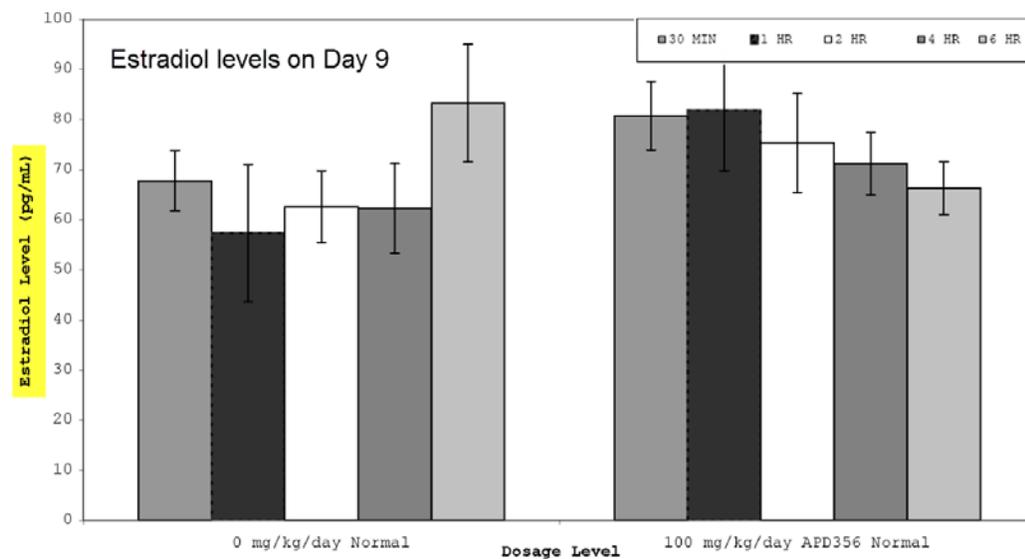
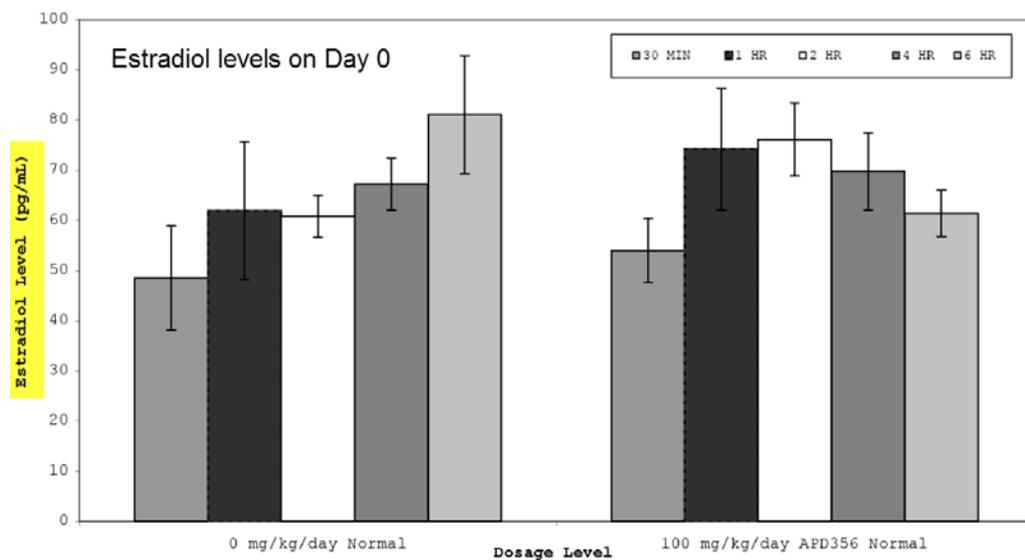
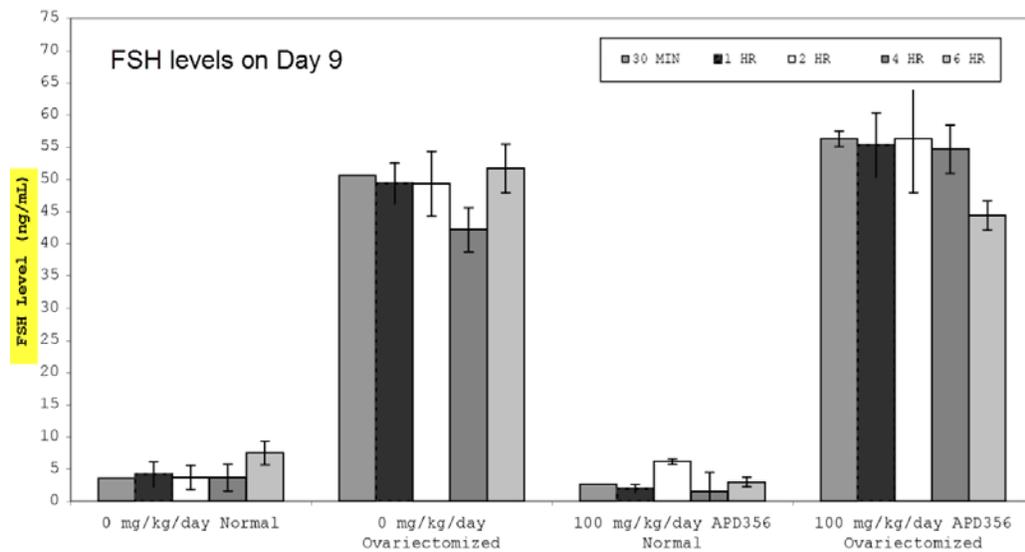
Lorcaserin had no significant effect on prolactin, LH or estradiol levels in normal or ovariectomized female rats. Occasional changes were noted in serotonin, FSH, progesterone, GH, IGF-1 and corticosterone levels with no statistical significance on Day 9. Variation in these hormone are shown in the bar plots on Day 0 and Day 9 for reference.

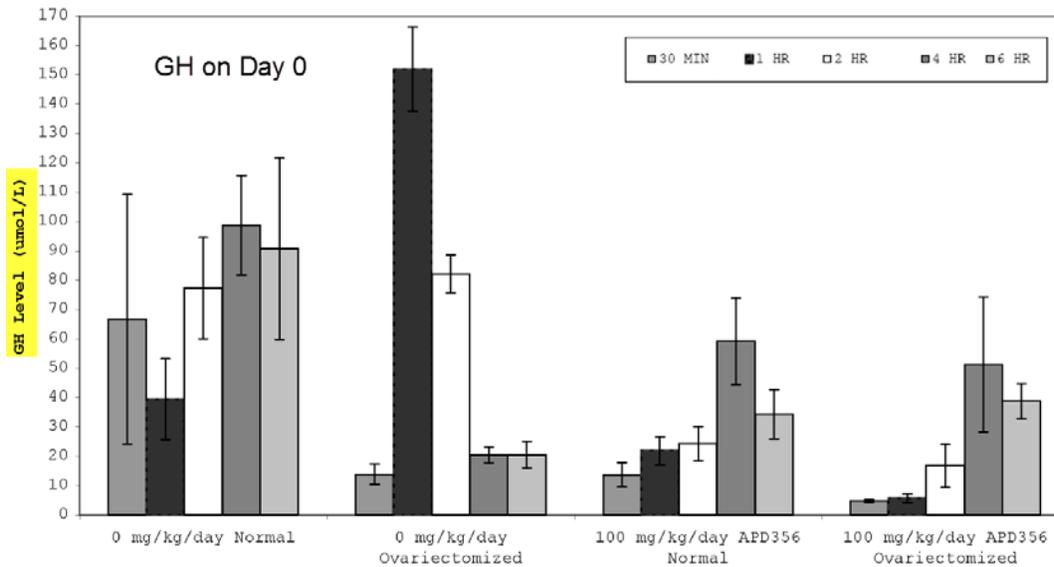
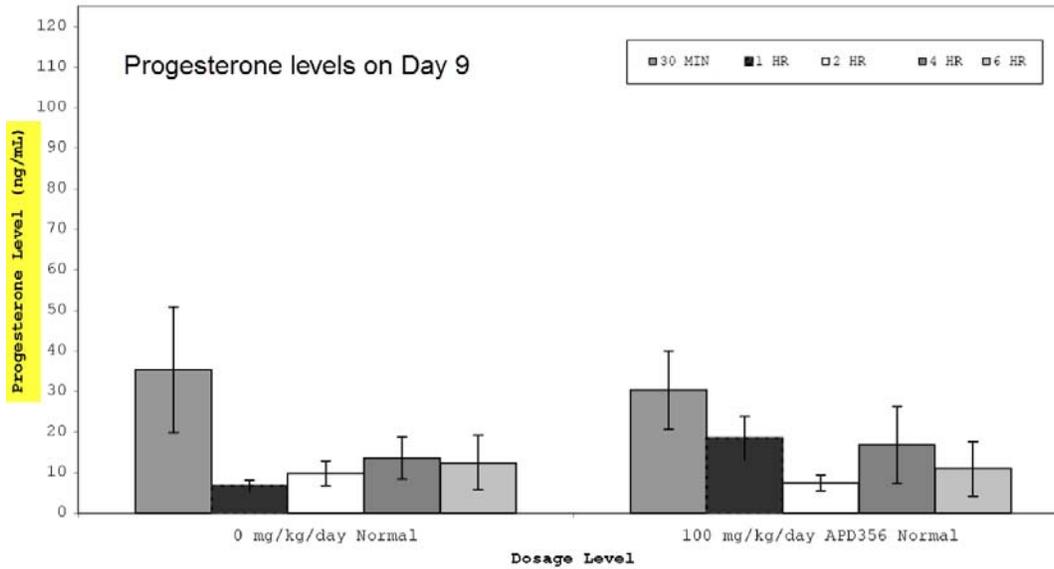
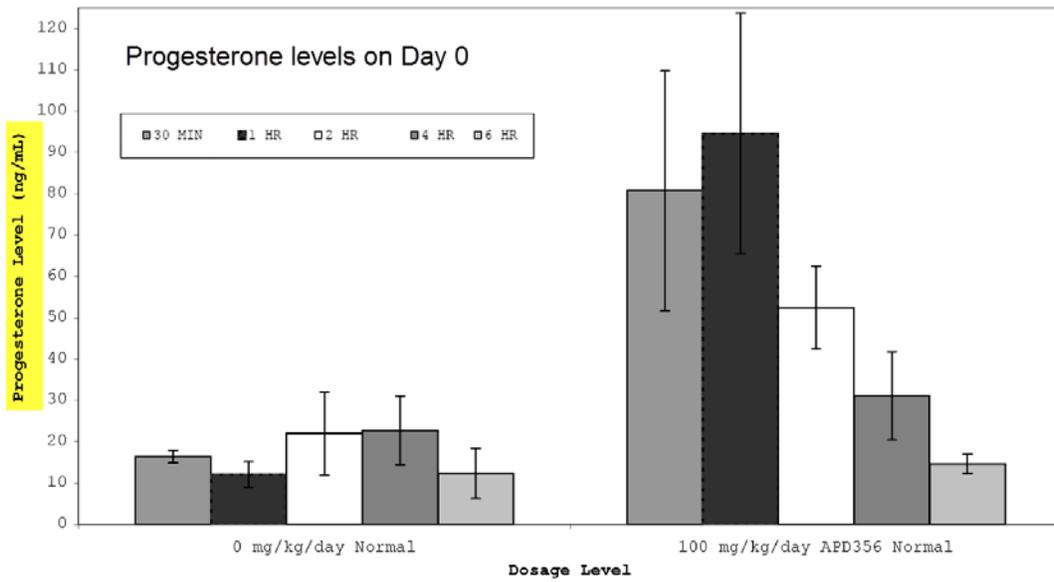
Fenfluramine increased prolactin levels relative to the control normal rats ($p > 0.05$). Of the 10 females in the study 4 were in proestrus while others were in diestrus or estrous at the time of analysis. The C_{max} at 2 hours on Day 0 and at 1 hr post dose on Day 9 after 100 mg/kg of lorcaserin were 1.45 and 4.2 µg/ml in normal female rats, respectively. The AUC on Day 0 and 9 were 6.91 and 17.9 µg.h/ml, respectively. In the ovariectomized rats the C_{max} at 1 hr post dose on Day 1 and Day 9 were 1.25 and 4.23 µg/ml. The lorcaserin AUC in ovariectomized rats was increased by 2.7 fold on Day 9 (16.3 µg.h/ml) relative to Day 0 (5.93 µg.h/ml).

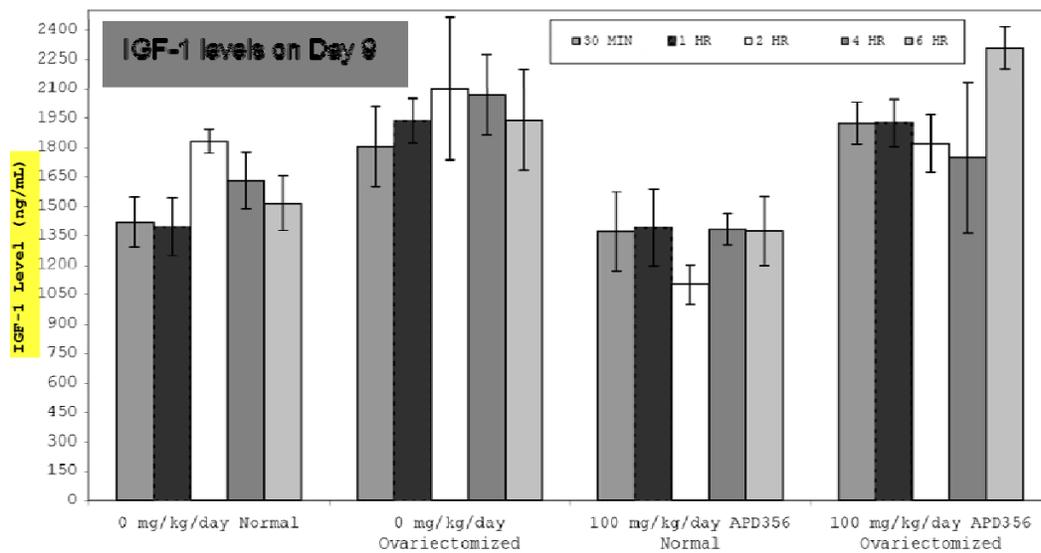
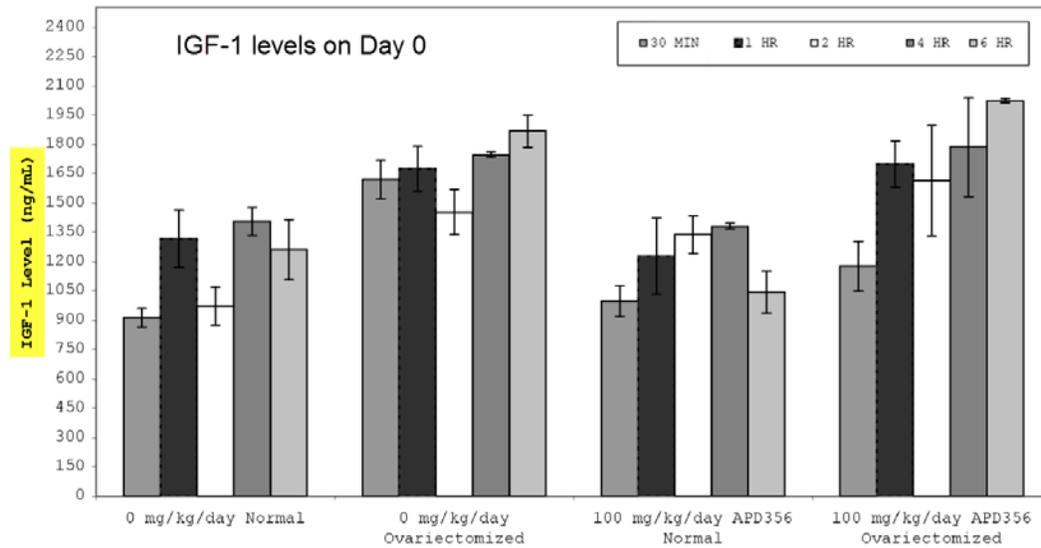
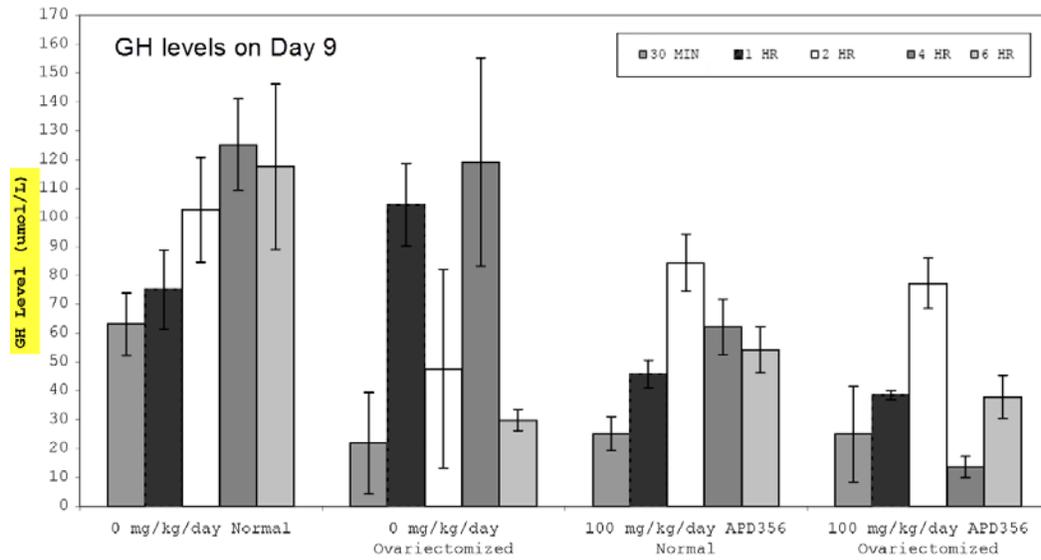


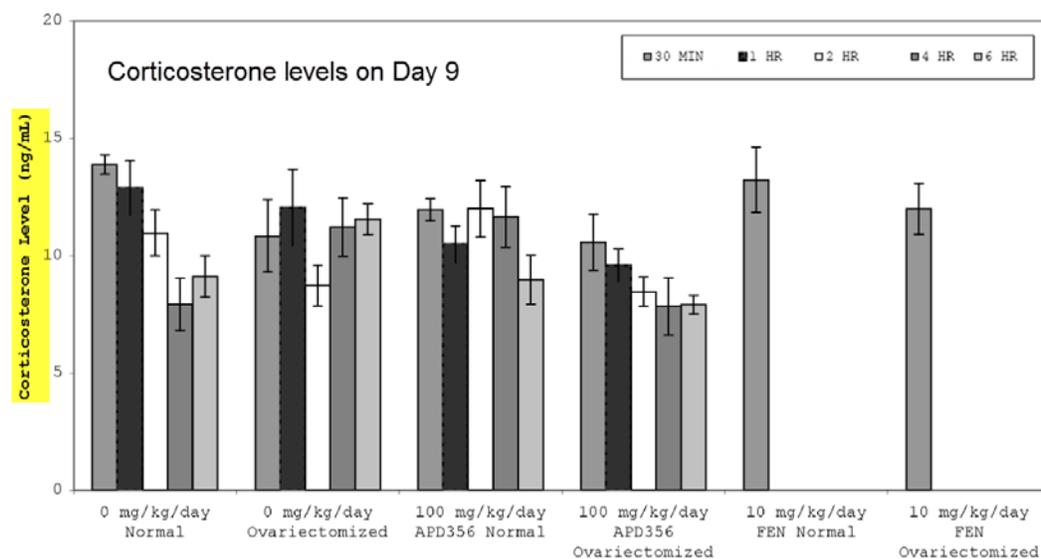
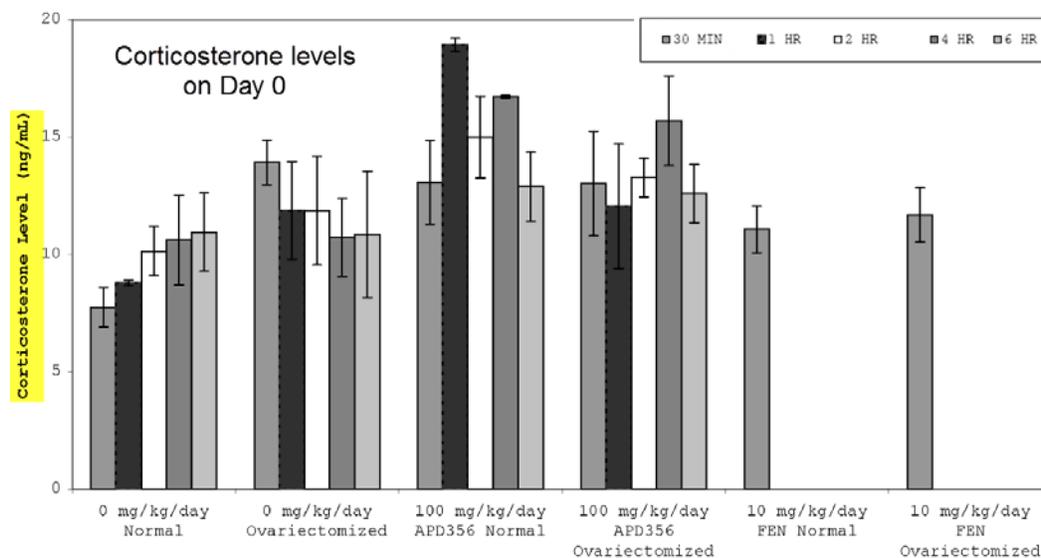












In summary, oral administration of lorcaserin 100 mg/kg of lorcaserin had no significant impact on serum prolactin, LD or estradiol concentrations in intact or ovariectomized rats in the course of 10 day study.

Study Title: APD356: Assessment of serum prolactin and estradiol in female rats during daily dosing for 28 days (MPI 900-101 & TX07035)

In this 28-day GLP comprehensive mechanistic study carried out for the sponsor at MPI facility (July 3, 2007), the role of lorcaserin on serum prolactin and several other parameters were evaluated in female CD[®][CrI:CD[®](SD)] rats. The primary objective of the study was to examine the changes in serum prolactin on different days at different time intervals post lorcaserin administration in female SD rats. The study also examined the pituitary and mammary prolactin positive staining cells. The lorcaserin treated group received 100 mg/kg (n=20) of lorcaserin while control animals received water (n=20, 5 ml/kg). Additional TK animals were used for plasma

lorcaserin exposure. The study examined the mortality, food intake and BW through out the study. Serum prolactin and estradiol levels were measured on Day 1, 15 and 28 at 2, 6, 12 and 24 hrs post dose. Prolactin immunohistochemistry performed on the pituitary gland in all untreated rats.

Lorcaserin initially reduced BW of female rats by 7.5% compared to control rats. The final BW was similar to the control which is consistent with other studies in female SD rats.

There were no macroscopic or microscopic changes attributable to lorcaserin dose of 100 mg/kg. The mean estrous cycle length was increased from 4.6 days to 7.2 days with corresponding decreased in the number of cycles from 5 to 3.5 cycles. There was no notable change in serum estradiol levels among groups. Lorcaserin dose of 100 mg/kg had no effect on serum prolactin in female SD rats. The prolactin staining intensity was similar between pituitary gland of treated and untreated females except in 1 female where the prolactin intensity was mildly decreased compared to untreated rat. The prolactin labeling index during the estrous cycle and proestrous was similar between control and treated rats. In mammary gland, the prolactin positive staining cells were unevenly scattered through out the mammary gland. The mammary prolactin positive cells were identical among treated and non-treated females. According to the report, the ovaries of both treated and non-treated females contained developing follicles and corpora lutea in various stages of maturation and regression. The estrous cycle determined histologically were consistent with estrous cycle determined by vaginal lavage. The body weights of lorcaserin treated females were less than the control at WK 1, 2 and 3 but not at week 4.

Summary of Body Weight Values - FEMALE

Study Interval (Week)	0 mg/kg/day			100 mg/kg/day		
	Mean	SD	N	Mean	SD	N
-1	238.0	7.60	20	240.4	9.71	20
1	248.5	9.71	20	229.9 ^b	12.78	20
2	261.1	9.31	20	252.8 ^a	11.47	20
3	268.8	13.00	20	259.5 ^a	13.55	20
4	273.9	16.69	20	265.0	15.17	20

(b) (4) Study Number 900-101

APD356: Assessment of Serum Prolactin and Estradiol in Female Rats During Daily Dosing for 28 Days

Summary of Microscopic Observations - FEMALE

Tissue	Observation	Severity	0 mg/kg/day	100 mg/kg/day
	Number of Animals Examined		20	20
mammary gland (ihc)			(19)	(18)
	prolactin stain, increased	- minimal	9	9
	within normal limits		10	9
pituitary gland (ihc)			(19)	(20)
	prolactin stain, decreased	- mild	0	1
	within normal limits		19	19
vagina			(20)	(20)
	diestrus		4	2
	estrus		5	6
	metestrus		6	6
	proestrus		5	6

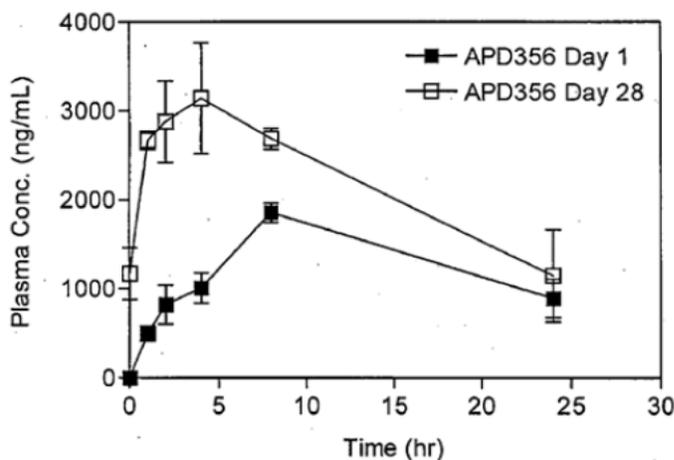
Summary of Prolactin Positive Stained Cell Counts in the Pituitary Gland

Endpoint	Estrous Stage	0 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N
Prolactin Labeling Index (%)	All Stages	40.54	6.711	19	36.92	8.830	20
	Proestrus	37.06	6.601	4	30.59	8.239	6

N - Number of measures used to calculate mean
SD - Standard Deviation

Analysis of plasma lorcaserin levels found significant exposure at all time intervals with Tmax ranging from 8 hrs on Day 1 to 4 hrs on Day 28. Repeated administration of lorcaserin increased AUC by 1.8 fold from Day 1 to Day 28. **The AUC on Day 1 and 28 were 30.4 and 53 µg.h/ml, corresponding to AUC observed in the 13-week rat study but less than the exposure in the 2-year rat carcinogenicity study.** Since serum prolactin and estradiol levels were measured at several time points on Day 1, 15 and 28, the reviewer calculated the mean estradiol and prolactin levels for 2, 6 and 24 hrs to cover the Tmax of lorcaserin.

APD356 Concentration versus Time Curves after Oral Administration of APD356 at 100 mg/kg/day to Female Sprague-Dawley Rats for 28 Days



Toxicokinetic Parameters for APD356 after 100 mg/kg/day in Female Sprague-Dawley Rats

Parameter	100 mg/kg/day	
	Day 1	Day 28
t _{max} (hr)	8.0	4.0
C _{max} (µg/mL)	1.85	3.14
AUC ₀₋₂₄ (hr·µg/mL)	30.4	53.0

Serum estradiol and prolactin concentrations in the vehicle and 100 mg/kg of lorcaserin treated female rats at different time intervals on Day 1, 15 and 28 is shown in table below. Although estradiol levels were generally below 2 pg/ml, several rats had elevated estradiol levels. Overall both prolactin and estradiol levels were variable within a group and with in a time interval during the 28-day study. There was no difference in prolactin levels between control and lorcaserin treated female SD rats.

Day and time of measurements	Estradiol, pg/ml		Prolactin, ng/ml	
	0 mg/kg	100 mg/kg	0 mg/kg	100 mg/kg
Day 1, at 2hr	< 2 to 6	< 2 to 6	49	62
Day 1, at 6 hr	< 2 to 18	< 2 to 33	378	213

Day 1, 12 hr	< 2 to 6	< 2 to 8	59	95
Day 1, 24 hr	< 2 to 15	< 2 to 3	87	215
Day 15, 2 hr	< 2 to 26	< 2 to 13	109	31
Day 15, at 6 hr	<2 to 18	< 2 to 24	659	505
Day 15, at 24 hr	<2 to 13	< 2	76	109
Day 28, 2 hr	< 2 to 15	<2 to 16	569	167
Day 28, at 6hr	< 2 to 20	< 2 to 13	409	882
Day 28, at 24 hr	< 2	<2 to 11	294	205

In summary, oral administration of 100 mg/kg lorcaserin for 28-days had no effect on serum estradiol, prolactin or prolactin positive staining cells in the pituitary or mammary gland in female SD rats. Lorcaserin however, reduced body weight and estrous cycle length relative to control females. Although both estradiol and prolactin levels were variable within a group and time interval data was collected, there was no overall trend to suggest any meaningful change in estradiol or prolactin as consequence of lorcaserin treatment. Overall, the study found no evidence of change in serum prolactin with top dose of 100 mg/kg in the 28-day study in female SD rats.

Study Title: Prolactin Release in Female Sprague-Dawley Rats after Treatment with APD356 for 10 and 21 Days (b)(4)-670002 & (b)(4)08007

A new study was designed to examine the role lorcaserin administered on normal and ovariectomized female SD over 10 to 21 days. This GLP study was initiated on Feb 15 and completed on Dec 19, 2008). Another group of rats received S⁺ fenfluramine HCl (D-Fen, 20 mg/kg, IP). Blood samples were collected to measure serum prolactin, estradiol, progesterone and free estradiol on Day 9 and 20.

Group Number	Test Article	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals ^a	
				Normal Females	Ovariectomized Females
1	Vehicle	0	10	20	20
2	APD356	100	10	20	20
3	S ⁺ Fenfluramine	20	10	10	10

^a One-half of the normal and ovariectomized animals from each group were necropsied on study day 9 (following the tenth dose) and the other half on study day 20 (following the twenty-first dose). Ten positive control animals (5 normal and 5 ovariectomized) had blood samples collected following 1 dose on study day 9 and the remaining positive control animals had blood samples collected following 1 dose on study day 20.

Administration of 20 mg/kg of fenfluramine HCl increased serum prolactin levels in both ovariectomized and intact female rats. Prolactin levels in lorcaserin treated rats were similar to placebo control intact and ovariectomized rats. Animals in proestrus generally did not show high levels of prolactin. Both estradiol and progesterone levels were similar among vehicle and lorcaserin treated ovariectomized and intact female rats. These hormones were not measured in fenfluramine treated rats. Interestingly, ovariectomized rats had moderate pituitary hypertrophy (chromophobe cells) consistent

with castration. Immunohistochemical staining of adenohypophysis and mammary tissue for prolactin positive staining cells found no significant difference between vehicle (water) and lorcaserin treated rats. Ovariectomized females had vaginal morphology similar to anestrus and slight glandular atrophy of mammary gland on Day 20.

Prolactin Analysis.

Group:	Sexually Intact Females			Ovariectomized Females		
	Vehicle	APD356	D-FEN	Vehicle	APD356	D-FEN
Prolactin (ng/mL)						
Day 9 Mean	15.0	6.2	42.1	10.7	3.1	21.5
% Difference		-58.7	180.7		-71.0	100.9
SD	16.42	6.05	41.75	8.73	1.96	15.42
Range	0.4-47.8	1.1-18.1	3.8-105.4	2.2-28.8	0.4-7.0	4.2-38.1
N	10	10	5	10	10	5
Day 20 Mean	11.7	9.1	98.1*	4.6	4.7	12.6*
% Difference		-22.2	738.5		2.2	173.9
SD	17.03	6.73	143.74	2.79	3.06	11.92
Range	0.8-44.7	0.4-18.3	14.3-354.2	0.4-9.1	0.4-9.8	1.7-27.8
N	10	10	5	10	10	5

* = Significantly different from the control group at 0.05 using Dunnett's test

Incidence Of Selected Histopathologic Findings, Study Day 9 Interim Necropsy

Group:	Normal Females			Ovariectomized Females		
	Vehicle	APD356	D-FEN	Vehicle	APD356	D-FEN
Pituitary gland^a	10	10	0	10	10	0
Hypertrophy, chromophobe	0	0	NA	10	10	NA
Mild	-	-	NA	10	10	NA
Mean prolactin- positive cells	50.9%	46.1%	NA	24.3%	26.8%	NA
Vagina^a	10	10	5	10	10	5
Anestrus	0	0	0	10	10	5
Proestrus	1	2	1	0	0	0
Estrus	3	2	1	0	0	0
Diestrus	6	3	3	0	0	0
Mucification	0	3*	0	0	2	3
Mammary gland^a	10	10	0	10	10	0
Normal	9	8	NA	10	10	NA
Hyperplasia, Glandular (minimal)	1	2	NA	0	0	NA

^a = Number of tissues examined from each group

* = mucification of the vaginal epithelium precluded staging of the estrus cycle.

Incidence Of Selected Histopathologic Findings, Study Day 20 Primary Necropsy

Group:	Normal Females			Ovariectomized Females		
	Vehicle	APD356	D-FEN	Vehicle	APD356	D-FEN
Pituitary gland^a	10	10	0	10	10	0
Hypertrophy, chromophobe	0	0	NA	10	10	NA
Mild	-	-	NA	4	5	NA
Moderate	-	-	NA	6	5	NA
Mean prolactin-positive cells	47.6%	46.8%	NA	37.0%	38.2%	NA
Vagina^a	10	10	5	10	9	5
Anestrus	0	0	0	10	9	5
Proestrus	2	2	3	0	0	0
Estrus	1	2	1	0	0	0
Diestrus	7	6	1	0	0	0
Mucification	0	0	0	0	0	0
Mammary gland^a	10	10	0	10	10	0
Normal	10	9	NA	1	1	NA
Atrophy (minimal)	0	0	NA	9	9	NA
Hyperplasia, Glandular (minimal)	0	1	NA	0	0	NA

^a = Number of tissues examined from each group

* = mucification of the vaginal epithelium precluded staging of the estrus cycle.

In summary, oral administration of 100 mg/kg of lorcaserin (to achieve highest drug exposure) for 10 and 20 days had a minimal effect on prolactin and estradiol levels in intact or ovariectomized SD rats. In contrast, D-fenfluramine significantly increased prolactin levels. Immunohistochemistry evaluation of the pituitary (adenohypophysis) and mammary gland found no significant lorcaserin-related change in percent prolactin positive staining cells in intact or ovariectomized rats.

The rationale for using fenfluramine was to test whether a non-selective 5HT_{2C} would have any effect on prolactin since earlier studies were failing to show a lorcaserin effect. The increase in prolactin was supposedly through the fenfluramine induced increase in brain serotonin levels. Since fenfluramine does not increase mammary tumors (NDA 20-344) in rats, the slight increase in prolactin by fenfluramine but not by lorcaserin suggests that prolactin levels have to increase significantly and persistently in order to result in mammary tumors in rodents which is the case with haloperidol.

Integrated Summary and Safety Evaluation

Lorcaserin is a serotonin 2 C receptor (5HT_{2C}) agonist under development for treatment of obesity. Lorcaserin is highly pure (99.9%) and predominantly an R-enantiomer (>98%) with minimal potential for chiral inversion *in-vivo*. The safety of lorcaserin was assessed in series of *in-vitro* and *in-vivo* nonclinical studies. Lorcaserin was not genotoxic in series of tests carried out for the parent drug. The toxicity of lorcaserin was evaluated in mice, rats, rabbits and monkeys.

Lorcaserin is rapidly metabolized by several liver microsomal and non-microsomal enzymes to numerous metabolites, with two prominent being M1 (lorcaserin sulfamate) and M5 which were identified in circulation in nearly all the species tested including humans. The exposure to M1 metabolite was 15 to 160x the parent drug in blood, while exposure to M5 metabolite was 2 to 3x the parent drug in nonclinical studies. All the metabolites identified in humans were found in at least one of the species used in the nonclinical evaluations thus sufficient coverage is provided for all the major and minor lorcaserin metabolites.

Lorcaserin appears to have minimal inhibitory effect on liver CYP1A1, CYP2C9, CYP2C19 or CYP3A4 enzymes in human. However, a slight inhibition of human CYP2D6 (K_i of 2.03μM =397 ng/ml) may occur. Using rat liver, lorcaserin induced CYP1A, CYP2B and CYP1A1 and CYP2B1/2. In contrast, no such induction was seen in humans. Lorcaserin had no effect on human CYP1A2, CYP2C9 and CYP3A4 enzymes suggesting that lorcaserin is unlikely to enhance metabolism of coadministered drugs metabolized by CYP1A2, CYP2C9 and CYP3A4 enzymes. Pharmacokinetic studies in rats found no food effect on lorcaserin bioavailability. Since lorcaserin is primarily excreted by the kidneys (70 to 82%) with small fraction lost to feces (14 to 25%) in rats suggests that renal condition might be an important factor in drug exposure in humans.

As a CNS active drug, distribution of lorcaserin to the brain was measured in the brain and CSF of male mice, rats and monkeys. Lorcaserin partitions significantly more to the brain tissue than plasma. The partitioning to the brain tissue does not appear to be specific to a region in the brain. The ratio of brain to plasma ranged from 13 in the initial studies in rats to 35 fold in recent multiple dose rat studies. In mice, the brain to plasma ratio was about 25 fold and about 10 fold in monkeys (range of 10 to 23). Lorcaserin concentration in the human brain is unknown. Interestingly, the CSF concentrations of lorcaserin is a fraction of plasma levels. It appears that lorcaserin partitions to brain rapidly against a large concentration gradient suggesting perhaps a role for an active transport system since both the CSF and plasma levels are significantly less than the brain levels. The brain distribution studies found no meaningful levels of the metabolites in the brain and CSF, again pointing to some active transport system specific to lorcaserin (5HT_{2C}). Protein binding studies found lorcaserin to be moderate (mice:66%, rats:70%, monkeys:73%, humans:74%). Whether high free fraction (26 to 34%) in the circulation was contributing to high brain partitioning is not clear. The toxicity of lorcaserin was assessed up to 6 months in rats (1, 5 and 50 mg/kg), and up to 12-months in monkeys (2, 10, 50 and 125 mg/kg). Lorcaserin significantly reduced BW gain in male rats at 50 mg/kg at the end of first week (M: -29%, F:- 49%) but most

of the weight loss was recovered by the end of the study (M:-15%, F:-7%). The decrease in BW gain was result of CNS suppression of appetite, marked by significant decrease in food intake in HD male rats. There was no change in BW gain in female rats at any dose level which is a consistent phenomenon in all studies in female SD rats. The absence of appetite suppression and BW in female rats is puzzling since plasma lorcaserin exposure in female rats at 50 mg/kg is about 60% higher than males. Since appetite suppressive effect of lorcaserin is via 5HT_{2C} receptors in the CNS, one might conclude that females have potentially lower 5HT_{2C} receptor concentrations and/or lower lorcaserin levels in the brain. Both are plausible since the brain lorcaserin and 5HT_{2C} receptor numbers are unknown in female rats. Since the brain distribution studies had only examined the brain lorcaserin levels in males which ranged from 13 to 35x the plasma, determination of brain lorcaserin exposure in females is warranted. Potential brain exposure differences may explain some of disparity in the rat studies (male vs. female SD rats).

With up to 35 fold partitioning of lorcaserin to the brain, the brain concentrations at pharmacodynamically effective dose of 50 mg/kg (AUC 37.7 μ g.hr/ml) in male rats might have been as high as 1320 μ g.hr/ml. This suggests that chronic weight loss in male rats is achieved by very high brain concentrations of lorcaserin. Interestingly, the few deaths seen in rats of unknown causes occurred mostly in HD males in the main (M: 2 C, 5 HD, F:1HD) and TK study (M:3 HD, F:1 HD).

Consistent with the 13-week rat study, lorcaserin decreased erythroid parameters (RBC, Hgb, Hct) in HD rats with corresponding increase in reticulocytes. Hypertrophy of liver (relative to BW) was seen in HD males (28%) and to some extent in HD females (19%) which may have been due to metabolic responses to drug load. Lorcaserin resulted in a dose-related hepatocellular vacuolation at all doses in male rats. Liver hypertrophy due to metabolic load was likely the reason for liver tumor in male rats. Minimal to moderate centrilobular hepatocellular hypertrophy was noted in most of the HD rats. Minimal to mild increase in incidence of extramedullary hematopoiesis and pigmented macrophages in HD rats is consistent with the findings in the 2-year rat carci study. Except for incidence of 2 HD females with mammary lobular hyperplasia, there was no notable change in mammary gland pathology to suggest that lorcaserin would result in significant increase in mammary tumors in rats. It is highly likely that longer treatment would have resulted in more mammary tubular hyperplasia. In the 2-year carcinogenicity study, mammary tumors were palpable at 9 months.

In the 12-month monkey study (2, 10, 50 and 125 mg/kg) the two deaths at 125 mg/kg which occurred within a short time after dosing on Day 15 (F, replaced) and Day 64 (M) were attributed to apparent gavage error. Histopath data appear to support this explanation. However, there were also 2 deaths at 125 and 75 mg/kg in the 13 week study (1 F on day 3 at 125 mg/kg, 1 M on day 43 at 75 mg/kg) which raises the possibility that other factors may have contributed such as tremor, emesis (aspiration) and seizure which were seen at lorcaserin doses \geq 75 mg/kg. Clinical signs of emesis and tremor appeared to be more common in female than male monkeys. Unlike in rats, the greatest effect of lorcaserin (\geq 50 mg/kg, \geq 30x the MRHD) on BW was observed in female monkeys. Only a small decrease in BW gain was noted in HD male monkeys. Lorcaserin doses of 2 and 10 mg/kg (up to 5 to 8x the MRHD) had no effect on BW or

BW gain in monkeys. The slight decrease in BW at HD was associated with lower LDL, HDL and cholesterol. Interestingly, the triglyceride levels were increased in HD (36 to 58%) at 52 wks likely due to mobilization of lipids. However, similar to rats, lorcaserin reduced erythroid parameters with associated increase in reticulocytes. As expected, the decrease in BW gain was associated with a decrease in food intake. Since only higher doses reduced BW, chronic weight loss with lorcaserin may require greater drug exposure in animals. The clinical signs noted above (lower BW and food intake at ≥ 50 mg/kg, reduced erythroid parameters and reduced lipid profile at ≥ 50 mg/kg) were similar to those in the 13-week monkey study.

ECG evaluations of monkeys at near T_{max} found no notable changes to suggest a lorcaserin related hemodynamic effect in monkeys. In the *in-vitro* tests, lorcaserin prolonged action potential duration and inhibited hERG current at concentrations ≥ 14 μ M. Since these concentrations are unlikely to be achieved in plasma in humans, the potential QT effect is minimal. Histopath analysis of the heart found no evidence of valvulopathy, although some questionable findings were noted in couple of monkeys such as aortic intima fibrosis (1 M at 50 mg/kg) and atrial epicardial inflammation (1 F at 125 mg/kg). In the 13-week study, there was a single incidence of fibrosis of the heart (1 HMD male) and myocardium fibrosis and necrosis (1 LMD female). Whether these incidences were related to drug or coincidental is unknown.

Two of the organs most affected by lorcaserin in monkeys were the liver (hypertrophy, vacuolation) and kidneys (hypertrophy, degeneration/regeneration). The liver signal was relatively weak (necrosis in 1 HD male, vacuolation in 1 female at 10 mg/kg and occasional incidences of lipidosis) relative to the 13-week monkey and 26-week rat study considering the duration of the treatment. In the 13-week monkey study, lorcaserin was associated with vacuolated liver cells marked by swelling which increased in a dose-dependent manner in both male and female monkeys (absent during recovery). It is highly possible that the liver effect was due to initial weight loss and fatty acid utilization/mobilization and subsequent adaptation to the treatment. Since the liver subsided and recovered, it is possible that this effect is reversible upon drug withdrawal and has no long-term consequences. Lorcaserin's effect on the kidneys in monkeys was the primary concern for the reviewer. The incidence of renal tubular regeneration increased with dose (≥ 10 mg/kg) while renal tubular degeneration (minimal to moderate) was noted at 125 mg/kg. The renal histopathology was associated with cellular casts in the tubules at doses ≥ 50 mg/kg. The renal findings were also supported by white blood cells and protein in the urine in the HD monkeys. Since some of the renal signals persisted even during the recovery, renal tubular regeneration/degeneration is apparently not readily reversed. The renal signal in monkeys was unexpected since it was not seen consistently observed in rats. The renal effect of lorcaserin was peer reviewed. Both the original and peer review pathologists agreed that renal signal in monkey was treatment related. The sponsor objected to the CRO's interpretation and hired two external renal pathologists to examine the slides. The external pathologist concluded that the renal tissue findings were an artifact and common to monkeys, and thus unrelated to lorcaserin. The external reviewer's assessments are not as credible as the original pathologist's report since the external pathologists had examined new semi-blinded slides. Furthermore, the renal data is supported to some degree by the renal findings in the 13-week monkey

study (i.e. renal tubular regeneration in 1 HD male, tubular protein casts in 1LD female, 2HD males and 1HD female).

The 13-week and 12-month monkey studies overall appear to suggest that renal signal in monkeys is a treatment effect albeit at $\geq 8x$ the clinical dose and the effect becomes more established and prominent with time and dose of lorcaserin. Other noteworthy histopath findings include bone marrow lymphoid nodules (minimal) in 1 male at 50 and 125 mg/kg, ovarian follicular cysts (minimal to mild) at ≥ 10 mg/kg and thyroid follicular degeneration (minimal to mild) at ≥ 10 mg/kg and arteriolar focal necrosis in the brain of 1 HD female.

Carcinogenicity Assessment of Lorcaserin

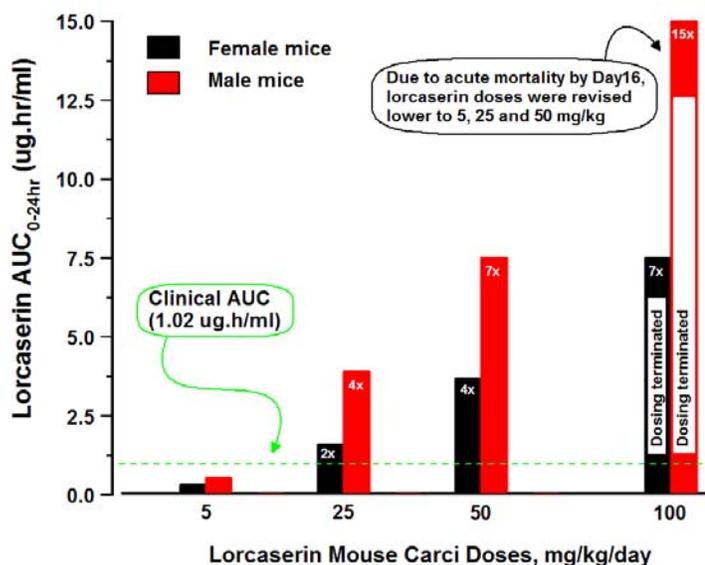
Lorcaserin and its major sulfated metabolite (APD244208) showed no evidence of genotoxicity in *in-vitro* and *in-vivo* tests. Since lorcaserin was carcinogenic in rats, non-genotoxic mechanisms are therefore thought to underlie lorcaserin-induced tumors observed in the rat.

Mouse Carcinogenicity study

The carcinogenicity study in mouse was initiated with 25, 50 and 100 mg/kg of lorcaserin and a vehicle control. Despite the evidence of tolerability of 100 mg/kg in the acute and 13-week dose-ranging study, excessive mortality was seen within the first 16 days of dosing at 100mg/kg. The deaths were clearly related to lorcaserin of undetermined causes. Since lorcaserin can partition to the brain up to 25x the plasma levels and there was an incidence of convulsion in 1 male on Day 1 at 100 mg/kg, the deaths may have had a neural origin. In consultation with the FDA, the doses of lorcaserin were decreased and treatments were reintroduced on Day 19 at 5, 25 and 50 mg/kg.

Lorcaserin exposure in male mice achieved a 0.5x, 4x, and 7x multiple at the LD, MD, and HD compared to the clinical dose. Exposure in female rats was higher, achieving a 0.3x, 1.5x, and 4x multiple of the clinical dose of 10 mg BID based on AUC. The lowered doses were well tolerated and survival in lorcaserin-dosed groups was similar to the control group for the remainder of the 2-year study.

Lorcaserin doses up to 50 mg/kg had no effect on BW or food intake in mice. There was no statistically significant treatment related increase in non-neoplastic or neoplastic findings. The only notable finding was the numerical increase in the number of adenocarcinoma in female mice at 50 mg/kg relative to controls. The incidence of adenocarcinoma in control, LD, MD and HD female rats were 2/75, 1/65, 1/65 and 4/75, respectively. The lack of pharmacodynamic and neoplastic response in the rat study appears to be due to lower lorcaserin exposure, since the



exposure at the NOAEL dose of 50 mg/kg in the mouse carcinogenicity study was 4 to 7x the clinical dose of 10 mg/kg BID. As noted earlier, most of the changes in BW in rats and monkeys were seen at ≥ 50 mg/kg with exposure multiples of ≥ 22 x the MRHD. The primary safety concern in the mouse study was the apparent and unanticipated steep dose response curve for toxicity. Whereas mice tolerated a 50mg/kg dose for 2 years without apparent adverse effects, a doubling of exposure to 100mg/kg resulted in rapid and unexplained deaths in a number of mice. As discussed earlier, 100 mg/kg had not resulted in sharp increase in mortality in earlier studies.

Rat Carcinogenicity Study

Sprague-Dawley rats were treated with 10, 30 and 100 mg/kg of lorcaserin for 2-years in the main study. After collection of plasma samples for TK analysis at WK 52, lorcaserin treatment was extended for 2 to 4 weeks for analysis of serum prolactin, estradiol, TSH and immunohistochemistry staining of prolactin positive cells in the mammary and pituitary gland. Lorcaserin exposure in male rats achieved a 5x, 17x, and 55x multiple at the LD, MD, and HD compared to the clinical dose. Exposure in female rats was higher, achieving a 7x, 24x, and 82x multiple of the clinical dose. Oral administration of lorcaserin significantly decreased the survival rate in the male and female rats. The number of live rats and survival rate at the end of the 2-year rat carcinogenicity study is shown in table below.

2-Year Rat study	Sex	Lorcaserin Dose, mg/kg			
		Controls (H ₂ O)	10	30	100
Survival rate, %	M	33.8%	24.6%	30.7%	5.3%
	F	35%	18.4%	7.7%	0%

Surviving females of all dose groups and the HD males were necropsied around week 96/99, in accordance with ECAC's recommendations. Clonic convulsion occurred early in the study more in females (~1.5 fold higher exposure) than in males (2C male, 1 LD, 3 HD male, 1 LD female and 13 HD females), corresponding to higher lorcaserin exposure. Deaths in females were primarily due to mammary tumors palpable as early as WK 42 while the first evidence of palpable tumors in male rats occurred much later around WK 61. Deaths in HD males were due to brain, skin, mammary tissue, and neuronal tissue (schwannomas) tumors. These tumors occurred much earlier in lorcaserin treated rats. The first appearance of nodules in lorcaserin treated female rats was 11 to 13 weeks earlier than controls while in male rats they occurred 10 to 23 weeks earlier than control males.

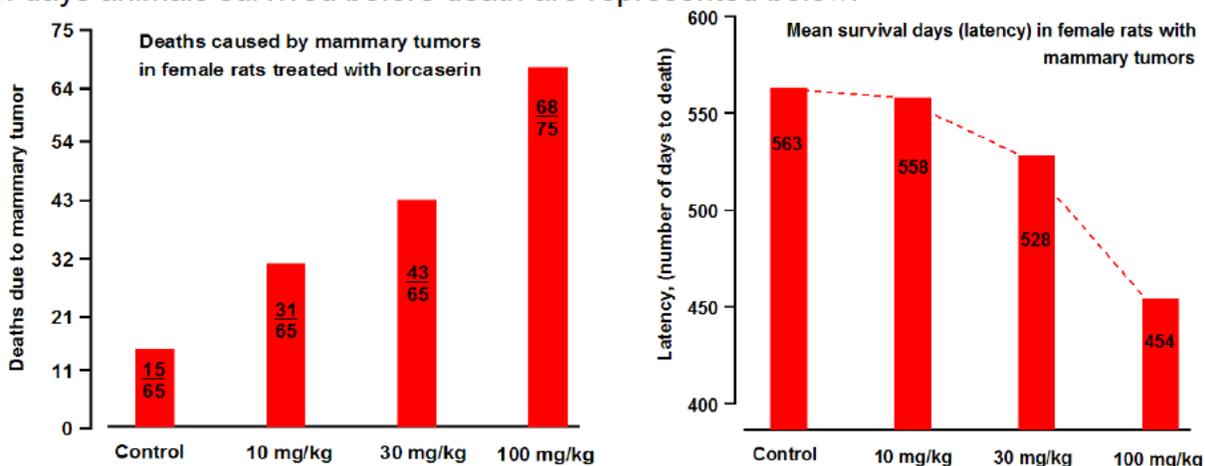
Lorcaserin Dose, mg/kg	0	10	30	100
First tumor appearance, Weeks				
Female rats	33	24	20	20
Male rats	50	40	38	27

The number of deaths caused by mammary tumors increased in females in a dose-dependent manner while latency decreased with increase in lorcaserin dose. The higher the lorcaserin dose, the more females died of mammary tumor and at an earlier

time. Furthermore, higher doses were associated with multiple tumor sites for both fibroadenoma and adenocarcinoma in female rats. The number of deaths due to both fibroadenoma and adenocarcinoma in control, LD, MD and HD are shown in table below. Looking at the table below closer, one can readily see that even the low dose of lorcaserin (31 deaths) was causing significant more deaths than control females (15 deaths due to mammary tumor). Two HD females (#4202 and 4212) that were euthanized in extremis due to mammary tumor (official cause of death) had no record of mammary tumors in the histopath evaluation.

Lorcaserin Dose, mg/kg	0	10	30	100
Number of death due to mammary tumors in female rats				
Number of animals per group	65	65	65	75
Death due to adenocarcinoma and or fibroadenoma	13	25	29	50
Death due to fibroadenoma	2	6	14	10
Combined	15	31	43	68

Graphical representation of deaths due to mammary tumors in female rats and number of days animals survived before death are represented below.



Lorcaserin resulted in significant BW reduction in male at 100 mg/kg (10% at WK 51 to 28% at WK 99) but with little effect in females. The sensitivity of male rats to lorcaserin is consistent with other rat studies. The decrease in food intake was variable in both sexes but slightly lower in males. Since the decrease in BW and food intake is an expected pharmacological effect of lorcaserin, the significant decrease in BW was not regarded as sign of toxicity.

Oral administration of 10, 30 and 100 mg/kg of lorcaserin resulted in significant lorcaserin related tumors in the 2-year rat carcinogenicity study. The prominent tumors identified in the study were mammary (fibroadenoma/ adenocarcinoma) and brain tumors (astrocytoma) in rats. Other notable tumors in male rats included hepatocellular adenoma/carcinoma, skin fibroma (subcutis) and squamous cell carcinoma, schwannoma (all sites) and follicular cell adenoma in the thyroid gland.

Neoplastic tumors in male rats treated with lorcaserin ^a
(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in male rats		Lorcaserin dose, mg/kg				Trend Analysis
		0	10	30	100	
Brain	astrocytoma	1	0	4 NS	8 ^b p=0.0019	< 0.0001
Liver	hepatocellular carcinoma	1	3	2	4	NS
	hepatocellular adenoma	1	1	2	6 p=0.0302	p=0.0033
	combined	2	4	4 NS	10 p=0.0048	p=0.0012
Mammary	adenocarcinoma	0	0	2	2 NS	p=0.0464
	fibroadenoma	0	1	4 NS	6 NS	p =0.0001
	combined	0	1	6 p=0.0131	8 p=0.0009	p=0.0003
Skin, subcutis	benign fibroma	3	7 NS	11 p=0.0175	17 p<0.0001	p <0.0001
Skin	squamous carcinoma	0	0	4 NS	5 p=0.014	p=0.0030
Schwannoma, all sites		0	0	2 NS	9 p<0.0037	p< 0.0001
Thyroid	follicular cell adenoma	0	5 p=0.028	4 NS	8 p=0.0011	p=0.0035

^a The statistical analysis and p values in the table were provided by the FDA statistician, Dr. Mathew Jackson.

^b One of the astrocytomas in the HD males was reclassified as infarct due to lymphocytic leukemia in an amendment to the NDA
NS = not significant (p > 0.05)

Neoplastic tumors in female rats treated with lorcaserin
(n= 65/sex/C, LD, MD and n=75/sex/HD)

Tumors in female rats		Lorcaserin dose, mg/kg				Trend Analysis
		0	10	30	100	
Brain	astrocytoma	0	2	0	1	NS
Mammary	adenocarcinoma	28	34 NS	35 NS	60 p<0.0001	p < 0.0001
	fibroadenoma	20	47 p<0.0001	53 p<0.0001	45 p<0.0001	p < 0.0001
	combined	40	56 p=0.0004	61 p<0.0001	70 p<0.0001	p<0.0001

^a The statistical analysis and p values in the table were provided by the FDA statistician, Dr. Mathew Jackson.

NS = not significant (p > 0.05)

Mammary tumors

Lorcaserin dose-dependently increased both mammary adenocarcinoma (females) and fibroadenoma (female and male rats, trend analysis). The incidence of adenocarcinoma was significantly increased in female rats at 100 mg/kg (80%, HD) relative to the concurrent control. The incidence of adenocarcinoma in females at 10 (52%, LD) and 30 mg/kg (54%, MD) was above the concurrent control (43%) and (b) (4) historical control values (8.3 to 37%, x=24%). The incidence of fibroadenoma alone was significantly increased at all doses of lorcaserin (LD: 52%, MD: 53% and HD: 60%) relative to the control (31%) with safety margin less than 7x the clinical dose of 10 mg BID based on AUC. The (b) (4) historical control for fibroadenoma in females ranged from 22 to 54% with mean value of 36%. The combined incidence of adenocarcinoma and fibroadenoma was statistically significant ($p < 0.0001$) at all lorcaserin doses (C:61%, LD:86% MD:94% and HD:94%). The combined incidence of fibroadenoma and adenocarcinoma was significantly increased with all doses of lorcaserin in female rats. Both adenocarcinoma and fibroadenomas were the predominate causes of deaths in lorcaserin treated female rats. Although the incidence of adenocarcinoma in the interim TK female rats was not included in the analysis, there were more incidences of adenocarcinoma in the lorcaserin treated than control TK female rats.

Lorcaserin Dose, mg/kg	0	10	30	100
Mammary tumors in TK female SD Rats (n = 5-14/group)				
Adenocarcinoma	0/5	7/14	6/14	7/10
Fibroadenoma	3/5	5/14	8/14	5/10

Interestingly, two HD females (4202 and 4212) that had nodules in the auxiliary area with the official cause of death due to consequence of mammary tumor, had no evidence of mammary tumor in the histopath results. It appears that the nodules were lost during data collection.

In male rats, the incidence of mammary fibroadenoma was significantly increased at only at 100 mg/kg (8%) but was above the control (0%) and (b) (4) historical controls data (0 to 2%) in the MD males (3%). The combined incidence of fibroadenoma and adenocarcinoma was significantly increased in both MD and HD male rats. A peculiar finding in male rats was the high incidence of feminization defined as partial or complete replacement of typical lobulo-alveolar appearance of the mammary gland with ductuloalveolar appearance was high in all treated males (LD: 64%, MD: 69% and HD: 63%) as well as controls (48%). The significance and possible cause of this is not clear.

Mid way through the rat study, the sponsor submitted a 15-day safety report regarding higher than normal incidence of mammary tumors in lorcaserin treated female rats. This led to bimonthly updates from that time forward. With each report, the pathologist identified a higher incidence of adenocarcinoma in groups treated with lorcaserin (Main and TK) compared to the control group. At week 96, when nearly all HD and the majority of MD female rats were evaluated histologically, there was an apparent dose-related increase in incidence of adenocarcinoma in female rats. The Division considered putting the on-going phase 3 clinical studies on hold in 2008. But since the

rat study was incomplete and the number of tumors could balance out as more animals necropsied and the ongoing mouse study was negative, the plausible prolactin-based hypothesis for the tumorigenic mode of action appeared logical, the Division decided to continue the ongoing phase 3 clinical trials and asked for mechanistic studies exploring the role of prolactin. In the final tally the incidences of adenocarcinoma (WK 104) in the MD and HD females were revised lower than earlier reports (WK 96). It appears that the decrease in the number of adenocarcinoma after week 96 were due to reclassification of adenocarcinoma to fibroadenoma which was itself also down graded in the later updates.

Changes in diagnosis of adenocarcinoma and fibroadenoma over time
(from Wk 55 until the final NDA submission)

Mammary Adenocarcinoma Incidence over time in Female Rats (main study)				
Data Update (Week)	Control	10 mg/kg	30 mg/kg	100 mg/kg
Week 55 update	0 / 1	2 / 4	5 / 7	13 / 15
Week 68 update	2 / 5	6 / 6	16 / 18	45 / 46
Week 88 update	16 / 28	27 / 38	36 / 45	72 / 74
Week 96 update	20 / 39	34 / 50	43 / 57	72 / 75
Week 104 update	30 / 65	35 / 65	35 / 65	63 / 75
Final update	29 / 65	35 / 65	36 / 65	62 / 75
Final NDA	28 / 65	34 / 65	35 / 65	60 / 75

Mammary Fibroadenoma Incidence over time in Female Rats (main study)				
Data Update (Week)	Control	10 mg/kg	30 mg/kg	100 mg/kg
Week 55 update	0 / 1	1 / 4	3 / 7	2 / 15
Week 68 update	1 / 10	1 / 11	5 / 18	20 / 46
Week 88 update	4 / 28	16 / 38	24 / 45	35 / 74
Week 96 update	10 / 39	27 / 50	36 / 57	36 / 75
Week 104 update	20 / 65	47 / 65	60 / 65	53 / 75
Final update	20 / 65	48 / 65	56 / 65	51 / 75
Final NDA	20 / 65	47 / 65	53 / 65	45 / 75

The reclassification of the mammary tumors from adenocarcinoma to fibroadenoma by the CRO pathologist suggests that a) there are significant histological similarities between fibroadenoma and adenocarcinoma that are not easily distinguishable, b) a reclassification rule was applied biased toward fibroadenoma, c) tumors were initially classified in error.

Prolactin has been known to be the intermediary hormone in the development of mammary tumors in rodents. Several drugs including CNS active antipsychotic anti-dopaminergic compounds cause mammary tumors in rats by indirectly altering pituitary dopamine in rodents. Although these compounds also increase serum prolactin in humans, a relationship between hyperprolactinemia and mammary tumors in humans has not been established.

To address the hypothesis that the lorcaserin-related increase in mammary tumors in rats was due to lorcaserin-induced increase in serum prolactin, the sponsor evaluated serum prolactin and the number of prolactin positive staining cells in the pituitary and

mammary tissue in the TK rats in the carci study. Serum analysis found prolactin levels to be similar among groups in female SD rats (~115 ng/ml). The levels in treated males was reduced by 50 % relative to control males. The numbers of pituitary prolactin positive staining cells were similar among males while MD and HD females had slightly higher incidence than control females. The incidence of mammary prolactin positive cells in the HD was lower than the corresponding control.

Additional single dose (males) and multiple dose mechanistic studies in intact and ovariectomized female rats with or without hormone supplement were conducted. The single and multiple doses of lorcaserin (10 to 100 mg/kg) consistently failed to show a significant rise in serum prolactin levels in female rats at any time period (2 to 24 hrs post dose) whether intact or ovariectomized. In contrast, animals treated with positive control, haloperidol (dopamine antagonist) saw a robust and significant rise in serum prolactin levels in intact and ovariectomized rats, consistent with prolactin's role as a central hormone for haloperidol-induced mammary tumors. Contrary to lorcaserin, dexfenfluramine, a non-selective 5HT agonist, increased serum prolactin presumably by increasing brain serotonin levels. However, dexfenfluramine does not increase mammary tumors in rats (albeit a different strain), suggesting that perhaps a robust chronic increase in prolactin is needed for rats to develop mammary tumors as is the case for haloperidol. The lack of lorcaserin on prolactin was further supported by the absence of any change in pituitary and mammary immunohistochemistry staining in the 28-day study in female SD rats.

There is some evidence from the single dose study that lorcaserin may have a small acute effect on prolactin in male but not female rats, even though there was a 50% decrease in prolactin in males after 54 weeks of dosing. The acute rise in prolactin in the single dose male rat study lead to the hypothesis that reproductive hormones in females were masking detection of an increase in prolactin with lorcaserin. To achieve a controlled level of reproductive hormones, females were ovariectomized then replenished with specified doses of estradiol+progesterone. Under these conditions, lorcaserin increased prolactin levels a marginal degree over the robust increase observed with the hormones. The relevance of the finding is questionable because the degree of increase in prolactin was marginal, and occurred under experimental conditions that bear little resemblance to those encountered by the rats tested in the carcinogenicity study. Interestingly, the estradiol level (~ 50 pg/ml) in the ovariectomized rats before hormone replenishment for some reason was equivalent or higher than normal estradiol levels in intact animals (2 to 50 pg/ml), raising concern regarding the validity of these non-GLP studies carried out at the sponsor's own labs, which appears inconsistent with the expectation of reduced estrogen with ovariectomy.

Overall, the effect of lorcaserin on serum prolactin in rats is consistent with the clinical observation that a small increase in prolactin may occur for a short time following a dose of lorcaserin but is not sustained under chronic conditions. The acute effect of lorcaserin is consistent with the published literature showing that repeated dosing with a serotonin 5HT_{2A/C} agonist can quickly lead to rapid tolerance in rats (Aulakh CS et al 1994) and humans (Greenberg J et al, 1996). Together these studies suggest that a lorcaserin related increase in prolactin, if any, is likely to be short lived with minimal consequences. It is the reviewer's opinion that the sponsor failed to show a meaningful

role for prolactin in the development of mammary tumors with lorcaserin in rats. With no role ascribed to prolactin, human risk becomes difficult to predict. The sponsor has to show a link between prolactin and lorcaserin if they want to explain mammary tumors via this mode of action. Since lorcaserin's effect may be independent of prolactin (e.g., a direct effect on serotonin receptors in mammary tissue), the sponsor is advised to explore other modes of action to explain the drug-related increase in mammary tumors and their clinical relevance.

Brain Astrocytoma

Lorcaserin also significantly increased brain astrocytoma in male rats in a dose-dependent manner (trend $p < 0.003$). The incidence of astrocytoma was significant in HD (10.67%, 55x the clinical dose of 10 mg BID based on AUC) and numerically higher in the MD male rats (6.15%) than concurrent (1%) and ^{(b) (4)} historical control data (0-5%). The increase at the MD and HD is considered related to lorcaserin. The total number of astrocytoma was unusually high in the lorcaserin treated rats overall (19 incidences) than the control rats (1 incidence).

Brain astrocytoma in 2-year carcinogenicity study in SD rats		Lorcaserin dose, mg/kg			
		0	10	30	100
Main study, astrocytoma	M	1/65	0/65	4/65	8/75
	F	0/65	2/65	0/65	1/75
TK study, astrocytoma	M	0/6	0/14	0/11	1/14
	F	0/5	0/14	1/14	2/10
Combined		1	2	5	12

In an attempt to identify the lineage of the brain astrocytoma in male rats, 19 identified brain tumors in the carci study were processed by immunohistochemistry staining (ED1, GFAP and MHCII). Out of 19 tumors, only 13 of the new slides had astrocytoma. Seven of the tumors were missed in the new slides. This finding alone suggests that these small tumors can be easily missed and perhaps more sections of brain tissue should be prepared if the slides are re-examined. All the 13 slides stained positive for ED1 and none for GFAP and only one was positive for MHCII suggesting a macrophage/histiocyte lineage (ED1). There are published reports suggesting that astrocytomas in rats are from macrophage lineage vs. glial lineage in humans. The issue of lineage is unresolved, although this has been known for over 20 years and they are still classified as astrocytoma. Brain levels of lorcaserin can be as high as 35x the plasma levels in rats (13 - 35x) and monkeys (10x), which raises overall concern. With no clinical data, use of monkey brain exposure as a surrogate for humans is reasonable but since the brain exposure in monkeys can vary from 10 to 23 fold, the safety margin is not much improved. Wide variability in brain partitioning among and within species makes calculation of safety margins based on monkey brain exposure unreliable. Lorcaserin significantly increased the incidence of astrocytoma in HD male rats (55x the clinical dose, based on plasma AUC), and numerically in the MD males (greater than background). A safety assessment based on brain levels between animals and humans is the most appropriate, but with no human brain exposure data available and variability in brain exposure among species, a more conservative approach to safety assessment

should be considered. If the brain levels in humans are similar to monkeys, the safety margin is about 17x the MRHD but if the brain exposure in humans is similar to rats, the safety margin would be about 5x the MRHD, and is thus a clinical concern.

Exposure multiple based on estimated brain concentrations of lorcaserin in humans		
Brain : Plasma Ratio	10 mg/kg (No astrocytoma)	30 mg/kg (astrocytoma)
Assuming 10x →	14 x	50x
Assuming 25x →	5 x	17x

Skin fibroma and Squamous cell carcinoma

There was a statistically significant positive dose-dependent trend for both skin fibroma and squamous cell carcinoma in males but not in females. In the pairwise comparison the incidence of skin fibroma was statistically increased in MD (16.9%) and HD (22.7%), the incidence in the LD males (10.8%) was above the concurrent control (4.6%) and the (b)(4) historical control data (0 to 5%). The number of squamous cell carcinoma in MD (4/65, 6.15%) and HD (5/75, 6.67%) males were above the control and (b)(4) historical control data (0 to 5%) but significant only in the HD males. The skin in these animals was visibly ulcerated and increased with dose. The skin effect may represent an off-target activation of receptors by lorcaserin (i.e. 5HT_{2A}). It is unclear why they were more common in males. Whether shortened duration of exposure in female rats had a role is not clear. Skin tumors can be easily monitored and treated, so the clinical risk compared to a brain tumor is lower, but nevertheless of concern given the clinical indication being considered.

Malignant schwannomas

Lorcaserin also resulted in higher incidence of malignant schwannomas (all sites combined) in male rats at 30 (2/65) and 100 (9/75, p<0.004) mg/kg. There was no schwannomas in the control or LD males (NOAEL = 10mg/kg, or 4.8x the clinical dose of 10 mg BID). Schwannomas across all locations (kidney, eyes thoracic and abdominal cavity, bone, skin subcutis) were characterized as small round neoplastic cells with unclear border. In at least 3 of the HD male rats their metastasis were seen in the lungs and thymus. Overall, the incidence of combined schwannomas in the HD males (p=0.06) was above the (b)(4) historical control suggesting that schwannomas were lorcaserin-related. The acceptability of a 5x safety margin must be weighed against the clinical benefits afforded by lorcaserin.

Liver tumors

Lorcaserin dose-dependently increased the incidence of hepatocellular adenoma and carcinoma in male rats but the increase was significant only in the HD males in the pairwise comparisons. There was no drug-related increase in hepatic tumors in females. The incidence of both adenoma and carcinoma in HD (adenoma 8% and carcinoma 5.3%) which were greater than the (b)(4) historical data for adenoma (0-5.7%) and carcinoma (0 to 1.7%) were likely drug metabolism related. The increase in hepatic tumors was likely to metabolic adaptation to high drug load in liver leading significant induction of liver metabolizing enzymes, as evidenced by the greater degree of

hepatocellular hypertrophy in HD males. Females at the high dose had a lesser degree of hepatocellular hypertrophy and displayed basophilic foci of cellular alterations (preneoplastic). The disproportionate effect on the liver in males may reflect greater induction of drug-metabolizing enzymes, as reflected by the 1.5 fold decrease in drug exposure in males. With no significant increase in pair wise comparison and high exposure multiples (> 55x the clinical dose of 10 BID on AUC basis), the potential risk to humans is deemed minimal.

Thyroid Tumors

The trend for incidence of thyroid follicular cell adenoma was significant in lorcaserin treated males (0/65, 5/65, 4/65 and 8/75 for C, LD, MD and HD male, respectively). No significant thyroid tumors were noted in females. The profile of thyroid and liver tumors appears similar, as a possible adaptation to increased T3 turnover and high liver drug load.

To summarize carcinogenicity studies, lorcaserin significantly increased the incidence of mammary tumors (fibroadenoma and/or adenocarcinoma) at all doses in females and MD and HD males and brain tumors (astrocytoma) in HD males in the 2-year rat carcinogenicity study. There was no safety margin for mammary fibroadenoma in females (< 7x the MRHD). Both fibroadenoma and adenocarcinoma were fatal. Since the mechanistic studies failed to persuasively demonstrate prolactin as the intermediary hormone as is the case for antipsychotic drugs, the mechanism remains unresolved and clinically relevant. With regards to astrocytoma in males, a safety margin to the NOAEL was identified (5x to 17x the MRHD) based on plasma exposure. A safety margin based on comparative brain levels of lorcaserin is most appropriate because lorcaserin significantly accumulates in the brain (drug pharmacology target). Because brain levels of lorcaserin in human subjects is not known and there is a significant variability in brain exposure among species, the estimated safety margin of 5x to 17x is somewhat unreliable and may be greater or smaller depending on the degree of drug partitioning in the human brain. Lorcaserin also dose-dependently increased liver adenoma, benign skin fibroma, benign thyroid adenoma and malignant schwannoma in male rats. The NOAELs for these tumors provides a safety margin of 5x to 17x. Although lorcaserin did not result in neoplasm in mice, the AUC exposure at the high dose (50 mg/kg, 4 to 7x the MRHD) was less than the exposure at the lowest dose of lorcaserin in rat study, therefore absence of neoplastic tumors in mice might be due to low lorcaserin exposure.

Reproductive Studies

The reproductive effect of lorcaserin was evaluated in rats and rabbits. Lorcaserin doses of 5, 15 and 50 mg/kg were given to male (before and after mating) and female rats (before and after mating DG 7) for fertility assessment. Lorcaserin slightly reduced BW gain (~7%) in males at 50 mg/kg but was unremarkable in female rats. Lorcaserin doses up to 50 mg/kg had no effect on fertility parameters in male or female rats. The NOAEL for embryonic development and fertility was 50 mg/kg.

In the Seg II rat study, lorcaserin doses of 10 and 50 mg/kg (DG7 to DG17) resulted in slight but significant decrease in maternal BW. The decrease in BW correlated with a decrease in food intake in dams. There was no statistically significant change in fetal external, visceral or skeletal malformations. Minor fetal variations were considered

incidental. The maternal NOAEL was selected as 10 mg/kg (8x the MRHD) in rats due to weight loss at 50 mg/kg, which was also selected as fetal NOAEL (48x the MRHD).

The fetal developmental study in New Zealand white lorcaserin doses of 20, 60 and 200 mg/kg were administered to pregnant rabbits from DG 7 through DG 19. One HD and one LD dam aborted on Day 23 and 26, respectively while one MD female delivered prematurely on GD 28. The incidence of spontaneous abortion in LD and HD was within the historical control (0 to 6.9%). Lorcaserin significant decreased BW and food intake of dams at 200 mg/kg. Gross necropsy findings were limited in one LD, MD and HD dam. These animals had either empty implantation sites or early resorption in the case of MD and LD females. Overall, there were no statistically significant differences in C-section parameters, total fetal external, visceral or skeletal malformations or developmental variations between lorcaserin treated animals and control. However, there was incidence several variations i.e. heart and greater vessel anomaly in 2 HD fetuses were greater than historical background. Based on significant decrease in BW, the NOAEL for maternal toxicity was 60 mg/kg (0.6x the MRHD). Even though fetal variations in the HD were not significant, the reviewer selected 60 mg/kg (0.6x the MRHD) as the NOAEL for fetal toxicity due to slightly higher incidence fetal variations such as heart and greater vessel anomaly at high dose.

The pre- and post-natal development studying rats were performed with 5, 15 and 50 mg/kg of lorcaserin. There were no drug-related deaths; however, lorcaserin reduced BW gain of dams in a dose-dependent manner during gestation and lactation which lead to lower pup (F1) weight at all doses but reaching statistical significant at 50 mg/kg. The percentage of live pups was reduced and the number of pups found dead was increased by lorcaserin dose of 50 mg/kg resulting in reduced viability index (87.3% vs. 98% in control). The gestation index was similar while lactation index. The slightly lower postweaning BW of the F1 generation recovered as the terminal BW was similar to control. There were no notable differences in F1 behavioral tests. Pups generation from mating of F1 generation had no significant gross alterations. The NOAEL dose of 5 mg/kg was selected for dams (weight loss). The reproductive NOAEL in dams was 15 mg/kg due to reduction in lactation index, increased stillborn pups at higher lo dose.

In summary, lorcaserin was not teratogenic in rat and rabbit reproductive studies. Lorcaserin appeared to reach fetal plasma in rats at concentrations equivalent to 1/3 of the maternal exposure. Surprisingly, the relative bioavailability of lorcaserin in rabbits was very poor, less than that in mice and significantly less than those in rats and monkeys. Therefore, it wasn't surprising when no measurable drug levels were detected in fetal plasma due to poor systemic maternal exposure in rabbits. Lorcaserin had no notable effect on fertility and mating in female and male rat. Lorcaserin dose-dependently reduced BW in pregnant rats and rabbits. The maternal and fetal NOAEL in rats was 10 and 50 mg/kg (1.3x and 48x the MRHD), respectively. The maternal and fetal NOAEL in rabbits was 60 mg/kg (0.6x the MRHD). In the pre- and post-natal developmental study in rats, lower BW gain at ≥ 5 mg/kg resulted in lower initial pup weight at 50 mg/kg. By the study termination, the BW of the affected was recovered matching those in the controls. The maternal NOAEL in dams was 5 mg/kg (4x the MRHD) while reproductive NOAEL was 15 mg/kg (12x the MRHD) in F1 generation rats.

Exposure margins

Species	Daily Dose, (mg/kg)	lorcaserin AUC ₀₋₂₄ (µg.h/ml)	NOAEL, (mg/kg) M/F	Exposure margins based on AUC (Animal/Human)	
				male	Female
13-Week mouse Study	25	M:3.4 F:1.0		3.3	1
	50	M:7.6 F: 2.3	50/50	7.4	2.2
	250	M:34.8 F:9.2		34.1	9
	350	M:25 F:27		24.5	26.4
13-Week rat study	1	M:0.143 F:0.33	5/1	<1	<1
	5	M:0.75 F:1.71		<1	2
	50	M:16.6 F:32.5		16	32
	100	M:33.6 F:55.8		33	55
6-Month rat study	1	M:0.20 F:0.31		0.2	0.3
	5	M:1.19 F:2.87	5/5	1.2	2.8
	50	M:22.0 F:34.4		22	34
12-Month cynomolgus monkey study	2	M: 1.0 F: 0.6	2 / 2	1	0.6
	10	M: 7.9 F: 4.5		7.7	4.4
	50	M:43.6 F:31.4		43	30.8
	125	M: 50.9 F: 51		50	50
104-Week Mouse Carci Study	5	M:0.55 F:0.32		0.5	0.3
	25	M:3.9 F: 1.6		3.8	1.5
	50	M:7.5 F:3.7	50/50	7.3	3.6
104-Week Rat Study	10	M:4.78 F:6.7	5 / <7	4.7	6.6
	30	M:16.9 F:24.1		16.6	24
	100 ^b	M:55.9 F:83.8		55	82
Fertility and early embryonic development in rats	5	M:2.68 F: 4 ^a		2.6	4
	15	M:9.91 F:12 ^a	15/50	9.7	12
	50	M: 29.3 F:48.7 ^a		28.7	48
Oral Embryo-fetal development in rats	2	F:1.34			1.3
	10	F:7.99	10		7.8
	50	F:48.7			47.7
Oral Embryo-fetal development in rabbits	20	F: 0.155			0.15
	60	F: 0.443	60		0.43
	200	F:19.3			18.9
Pre- and postnatal development in rats	5	F:4 ^a	<5		4
	15	F:12 ^a			12
	50	F:48.7 ^a			48
Clinical Dose: lorcaserin, 10 mg BID		1.02			

^a The AUC value is derived from other existing similar studies.

^b The lorcaserin AUC in females in the 2-year rat study was about 50% higher than the AUC in female rats in the 13-week toxicology and 28-day prolactin mechanistic study (AUC 53 µg.hr/ml).

12 Appendix/Attachments

Appendix A

Meeting Minutes from FDA Executive Carcinogenicity Assessment Committee

Executive CAC

Date of Meeting: August 10, 2010

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Haleh Saber, Ph.D., DHP, Alternate Member
Todd Bourcier, Ph.D., Team Leader
Fred Alavi, Ph.D., Presenting Reviewer

NDA 22-529

Drug Name: Lorcaserin HCl

Sponsor: Arena Pharmaceuticals

Background:

Lorcaserin is a first-in-class serotonin 5HT_{2C} receptor agonist. The sponsor is seeking an indication for the treatment of obesity.

Mouse Carcinogenicity Study

Carcinogenic assessment in CD1 mice was initiated at doses of 25, 50, and 100mg/kg, in accordance with the Committee's dosing recommendations. High mortality within two weeks of dosing initiation prompted a reduction in doses to 5, 25, and 50mg/kg, and the addition of 10 mice/sex to the control and 50mg/kg groups on day 19. The survival rate across the dose groups was similar to control for the remainder of the study. Drug exposure at the 5, 25, and 50mg/kg dose groups provided multiples of 0.5x, 4x, and 7x in males and 0.3x, 1x, and 4x in females relative to the clinical dose of 10mg bid.

Rat Carcinogenicity Study

Carcinogenic assessment in Sprague Dawley rats was initiated at doses of 10, 30, and 100mg/kg, in accordance with the Committee's dosing recommendations. Survival declined significantly at all doses in females due to the emergence of drug-related mammary tumors. Survival also declined significantly in high dose males, due to the emergence of drug-related tumors in the brain, skin, mammary tissue, and nerve sheaths (schwannoma). Drug exposure at the 10, 30, and 100mg/kg dose groups provided multiples of 5x, 17x, and 55x in males and 7x, 24x, and 82x in females relative to the clinical dose of 10mg bid.

Because excess mortality was due to drug-induced tumors rather than dose-limiting toxicity, the high dose of 100mg/kg is not considered to have exceeded the MTD.

Mechanistic studies were presented showing, at most, a small and non-sustained increase in serum prolactin in rats administered lorcaserin. Immunohistochemical staining of pituitary and mammary tissue failed to establish a correlation between prolactin and mammary tumors. Conversely, the anti-dopaminergic compound haloperidol readily

increased prolactin in these studies, and is associated with rodent mammary tumors via this mechanism.

Immunohistochemical staining of astrocytoma in thirteen sections showed a lack of staining with GFAP, and occasional staining with MHCII and an anti-CD68 marker, suggesting that the cellular lineage of the astrocytomas was not astrocytic but rather monocytic. The literature reports an absence of GFAP staining in rat astrocytoma, but this lack of staining is not necessarily evidence of a non-astrocytic origin of the tumor (Nagatani M et al; Toxicol Path, 2009). Regardless of cell lineage, the mechanism of tumor induction was not assessed and the relevance to human risk cannot be dismissed.

The incidence of mammary adenocarcinoma and fibroadenoma was reported on a quarterly basis in response to the Division's request starting at week 55. The Division expressed concern that the number of adenocarcinoma in the mid- and high-dose groups decreased from week 96 to the final study report, whereas the incidence in the control and low dose groups either increased (control) or stayed the same (low dose) over the same time period. Additionally, the Division identified 2 cases of high dose females suspected of having a mammary tumor that were not counted as such in the study report.

Executive CAC Recommendations and Conclusions:

Mouse:

- The Committee agreed that the study was acceptable, as mortality was encountered at doses higher than 50mg/kg.
- The Committee concluded that the study was negative for any statistically significant drug-related tumor findings.

Rat:

- The Committee expressed some concern about the conduct and evaluation of the study. Specifically, concern was expressed about a large number of diagnostic changes of mammary tumor type in the evaluation for the mid and high dose group.
- The Committee noted that because high-dose animals died due to drug-induced tumors, the MTD was not exceeded in this study.
- The Committee was not persuaded by the sponsor's argument that mammary tumors were caused by increased prolactin levels. Specifically, the sponsor's data failed to demonstrate an increase in prolactin in repeat-dose mechanistic studies and in the 2 year carcinogenicity study.

- A mechanism for the induction of astrocytomas was not identified. Drug-induced astrocytomas were observed at exposures equal to 17x the clinical exposure, with a NOAEL that provides a 5x multiple to the clinical dose.

The Committee concluded that the following tumors were drug-related:

Males

Brain: Astrocytoma at HD. Numerical, non-statistically significant increase in astrocytoma at mid-dose also considered drug-related.

Liver: Hepatocellular adenoma and carcinoma combined, at HD.

Mammary: Adenocarcinoma and fibroadenoma combined, at MD & HD.

Skin, subcutis: Fibroma at MD & HD

Skin: Squamous Carcinoma at HD. Numerical, non-statistically significant increase in squamous carcinoma at MD also considered drug-related.

Schwannoma (all sites) at HD. Numerical, non-statistically significant increase at the MD also considered drug-related.

Thyroid: Follicular cell adenoma at HD.

Females

Mammary: Adenocarcinoma + fibroadenoma at LD, MD, HD

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

cc:\n
/Division File, DMEP
/Todd Bourcier, DMEP
/Fred Alavi, DMEP
/Pat Madara, DMEP
/ASeifried, OND IO

Appendix B

Receptor Profile of Lorcaserin, Lorcaserin Enantiomer and Metabolites for a Collection of Human GPCRs, Ion Channels and Neurotransmitter Transporters

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
Adenosine A ₁	-11	13	-6	6	-5
Adenosine A _{2A}	7	1	-11	21	14
Adenosine A ₃	-1	1	4	13	14
α ₁ Adrenergic (nonselective) (rat)	18	16	27	22	9
α ₂ Adrenergic (nonselective) (rat)	30	41	13	65	9
β ₁ Adrenergic	21	52	-8	56	7
β ₂ Adrenergic	19	22	-3	47	10
Angiotensin AT ₁	-12	3	11	-18	-13
Angiotensin AT ₂	1	13	1	1	3
Benzodiazepine (central) (rat)	0	6	-2	6	11
Benzodiazepine (peripheral) (rat)	-3	-1	6	-1	1
Bombesin (nonselective) (rat)	-16	2	-10	6	1
Bradykinin B ₂	-3	-15	11	1	-4
CGRP	-10	-8	-9	-4	-2
Cannabinoid CB ₁	3	8	-9	5	-2
Cannabinoid CB ₂	-8	ND	-3	-6	-8
Cholecystokinin CCK _A	-2	-9	-11	29	18
Cholecystokinin CCK _B	3	-1	6	-2	3

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
Dopamine D ₁	-2	14	-1	-3	-7
Dopamine D _{2S}	-1	0	9	2	1
Dopamine D ₃	7	23	2	21	-2
Dopamine D _{4,4}	-1	-4	-5	4	4
Dopamine D ₅	-3	5	-3	8	-1
Endothelin ET _A	-8	3	-23	-2	2
Endothelin ET _B	-14	-8	-7	1	9
GABA (nonselective) (rat)	9	-3	-15	9	2
Galanin GAL ₁	5	-6	4	-1	4
Galanin GAL ₂	-18	-10	3	-10	-2
PDGF (mouse)	-9	ND	-13	1	-7
CXCR2	8	-14	12	-12	-2
TNF-α	ND	4	6	-21	-1
CCR1	-3	-2	0	-4	-3
Histamine H ₁	-4	10	8	9	8
Histamine H ₂	6	16	-12	-3	-1
Melanocortin MC ₄	-7	1	1	2	4

Receptor Profile of Lorcaserin, Lorcaserin Enantiomer and Metabolites for a Collection of Human GPCRs, Ion Channels and Neurotransmitter Transporters

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
Melatonin MI ₁	-5	5	1	6	9
Muscarinic M ₁	12	1	-27	9	6
Muscarinic M ₂	3	9	-1	-4	-7
Muscarinic M ₃	3	11	-29	10	5
Muscarinic M ₄	4	18	-2	7	2
Muscarinic M ₅	0	14	-4	4	-3
Neuronal nACh (rat)	30	ND	4	20	13
NMDA (rat)	-4	ND	17	5	1
Neurokinin NK ₁	10	-4	-4	4	2
Neurokinin NK ₂	-5	10	4	1	-3
Neurokinin NK ₃	7	-3	-6	13	11
Neuropeptide Y ₁	-9	-4	21	-6	-10
Neuropeptide Y ₂	-1	-18	-15	-3	-2
Neurotensin NT ₁	-2	-1	4	2	0
δ ₂ Opioid	4	5	10	10	0
μ Opioid	26	5	0	28	7
κ Opioid (rat)	6	24	0	18	19
Nociceptin ORL1	1	9	-2	17	10

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
PPARγ	-9	8	ND	ND	ND
PAC ₁ (PACAP)	-15	-3	-12	-18	6
PCP (rat)	8	3	-22	-14	-6
Prostanoid EP ₂	ND	7	ND	ND	ND
Prostanoid EP ₄	-3	ND	ND	-3	0
Prostanoid TP	0	11	-2	30	32
Prostanoid IP	-5	0	ND	-2	-3
P2X (rat)	9	-2	-5	-3	0
P2Y (rat)	1	-5	-4	5	11
Serotonin 5-HT _{1A}	85	92	22	72	22
Serotonin 5-HT _{1B} (rat)	69	92	13	49	4
Serotonin 5-HT _{2A}	29	50	-3	22	-6
Serotonin 5-HT _{2B}	78	78	ND	51	9
Serotonin 5-HT _{2C}	67	62	-7	32	15
Serotonin 5-HT ₃	7	14	-3	10	8
Serotonin 5-HT _{5A}	7	10	-4	17	24
Serotonin 5-HT ₆	15	47	1	17	1
Serotonin 5-HT ₇	61	82	7	40	1

Receptor Profile of Lorcaserin, Lorcaserin Enantiomer and Metabolites for a Collection of Human GPCRs, Ion Channels and Neurotransmitter Transporters

Receptor	Lorcaserin % Inhibition ^a	Enantiomer % Inhibition ^b	M1 % Inhibition ^c	M2 % Inhibition ^d	M5 % Inhibition ^e
Sigma σ (nonselective)	14	23	6	8	14
Somatostatin (nonselective)	-12	6	-12	-3	-3
Glucocorticoid	-2	2	ND	-8	2
VIP ₁ (VPAC ₁)	-8	3	-25	-3	-2
Vasopressin V _{1a}	3	0	-5	2	-1
L-Type Ca ⁺⁺ Channel (rat)	15	19	-18	12	-8
K ⁺ _v Channel (rat)	-4	3	-11	0	2
SK ⁺ _{Ca} Channel (rat)	1	3	6	0	1
Na ⁺ Channel (rat)	3	13	-17	18	32
Cl ⁻ Channel (GABA-gated) (rat)	3	-3	8	1	-1
Norepinephrine Transporter	0	21	-2	11	8
Dopamine Transporter	-7	9	-20	6	-5
5-HT Transporter	20	29	-12	3	1

^a Data from DBR-09-004, Sections 9 & 10. Lorcaserin test concentration = 1 μ M

^b Data from DBR-09-008, Section 7. Lorcaserin enantiomer test concentration = 1 μ M

^c Data from DBR-09-005, Sections 6 & 7. Lorcaserin metabolite M1 test concentration = 10 μ M

^d Data from DBR-09-006, Sections 6 & 7. Lorcaserin metabolite M2 test concentration = 1 μ M

^e Data from DBR-09-007, Sections 6 & 7. Lorcaserin metabolite M5 test concentration = 1 μ M

^f Bold font indicates >50% inhibition of radioligand binding. ND = not determined.

All assays utilized human receptors except where indicated in the table.

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/s/

FRED K ALAVI

10/20/2010

Nonclinical review of lorcaserin NDA 22529
(IND 69,888)

TODD M BOURCIER

10/20/2010

Reviewer recommends non-approval. I concur with Dr Alavi's recommendation.

PHARMACOLOGIST REVIEW OF GLP EIR (CP 7348.808)

Firm Names:

- Arena Pharmaceuticals, Inc., San Diego, CA (inspection dates June 7-11, 2010)
- [REDACTED] (b) (4)

Inspection Highlights

- For the 2-year rat carcinogenicity study conducted by [REDACTED] (b) (4) there were some histopathological diagnostic errors discovered during the inspection; however, the incidence of mammary tumors (both malignant and benign) in APD356 treated female rats was affected minimally or not at all by these errors. Consequently, the reclassification of the tumor types at the completion of the study compared to the data provided to the Agency during the in-life phase is considered scientifically valid and DSI recommends the data in the final report should be accepted.
- The re-evaluation of the renal histopathology of the 1-year monkey toxicity study managed by Arena lacked necessary documentation to assure data integrity.
- Issues of GLP non-compliance were found at both firms. For example, Arena failed to follow test methods for the analysis of test article formulations and records of study conduct were incomplete and could not be readily retrieved. The [REDACTED] (b) (4) inspection found that the study director prepared draft final reports without the raw data of contributing scientists (i.e., without the signed and dated pathology report).

Studies Audited During This Inspection

NDA#: 22-529
Sponsor: Arena Pharmaceuticals, Inc
Drug name: Lorqess (lorcaserin HCl, APD356) Tablets, 10 mg
Indication: Obesity and weight management
Rev Division: DMEP
Primary P/T reviewer: Fred Alavi, Ph.D.

Studies: TX05070, 2 year carcinogenicity study in mice [REDACTED] (b) (4) 900-062)
TX05071, 2 year carcinogenicity study in rats [REDACTED] (b) (4) 900-063)
TX04039, 52 week study in monkeys [REDACTED] (b) (4) AVA00016)
TX05004, 13 week study in mice [REDACTED] (b) (4) 900-047)
TX04041, 13 week study in rats [REDACTED] (b) (4) 900-039)

Background

At the request of DMEP, DSI initiated inspections of portions of the aforementioned toxicology studies submitted in support of NDA 22-529. The review division raised several concerns, including those highlighted below:

1. Reclassification of tumor types in the 2 year rat carcinogenicity study at (b) (4)
2. The study pathologist involved in both carcinogenicity studies at (b) (4) left the CRO.
3. In the 1 year monkey toxicity study, the study pathologist and peer reviewer at (b) (4) expressed agreement regarding renal lesions; however, the sponsor contracted two external human pathologists to re-evaluate the histopathology of the renal slides.
4. Data quality and integrity of the bioanalytical assays, test article formulations, and toxicokinetic analysis at Arena and its contract CROs (b) (4)

Based on these concerns, DSI initiated inspections of the sponsor Arena Pharmaceuticals and the nonclinical CRO (b) (4) as key sites.

Arena Pharmaceuticals is the sponsor of NDA 22,529 and as such the inspection focused on the firm's overall oversight of nonclinical testing for lorcaserin as well as analytical work conducted in-house in support of GLP studies. No GLP study portions are currently carried out at Arena's San Diego facility and the last analysis of dosing formulations for toxicology studies was completed in 2008.

(b) (4) is a large contract testing facility, conducting a wide range of general toxicology, reproductive toxicology, safety pharmacology, bioanalytical, ADME, and research/development studies. Most of the firm's activities are subject to GLP regulations. Several pivotal toxicity studies supporting NDA 22-529 were conducted by this firm, including the 13-week general toxicity studies in mice and rats and 2-year carcinogenicity studies in mice and rats. The inspection at (b) (4) focused on the carcinogenicity studies.

Inspections were not initiated at the contract CRO (b) (4) as the major concerns of DMEP involved issues at Arena and (b) (4)

Inspectional Findings

The inspections resulted in issuance of Form FDA 483 to both the sponsor Arena Pharmaceuticals Inc. and the CRO (b) (4). Our evaluation of the inspectional findings and the firm's respective responses to the FDA 483 items (Arena response dated 6/29/2010 while (b) (4) response is pending up to date) are the following:

Findings at (b) (4)

- The inspection at (b) (4) verified the histopathology diagnosis of the mammary gland tumors after treatment of APD356 in the 2-year carcinogenicity study in rats (b) (4) and examined the rationale for reclassification of the mammary gland adenocarcinoma and fibroadenoma in the MD and HD groups. In this regard, more than 600 mammary gland tissue slides, including all female

rats from control, MD and HD groups, were carefully reviewed by Dr. Dylan Yao of DSI under microscope with the (b)(4) pathologists. Please refer to "Attachment 1" (Review and Verification of Histopathology Tissue Slides) and "Attachment 2" (Selected Photomicrographs of the Mammary Gland Tumors) for detailed information. The following points are noted:

- There were some errors in the mammary tumor diagnosis discovered through our microscopic slide review; most of these errors are considered "over-estimated" for the malignancy rather than "under-estimated" (see details in Attachment 1).
- In the MD group, mammary gland adenocarcinoma should be corrected to be 34 of 65 female animals (originally 35 of 65) due to the correction of animal #3265; the total number of animals bearing mammary gland fibroadenoma did not change (still 53 of 65 animals as reported).
- In the HD group, the total numbers of animals bearing fibroadenoma are adjusted to 46 of 75 (instead of 45 out of 75 reported) due to the tumor verification in animal #4209. The total numbers of animals bearing adenocarcinoma did not change (60 of 75) after the audit.
- Tumor incidence in the final study report was determined based on evaluations by the study pathologist (b)(6) peer review pathologists did not adjust the incidence markedly, i.e. both peer review pathologists agreed in general with the diagnosis made by the study pathologist.
- Adjustment of the adenocarcinoma- and fibroadenoma-bearing animal numbers in the MD and HD groups from Week 96 and thereafter was apparently conducted by (b)(6) herself. There was no correspondence in the study record to suggest that her scientific decisions to re-classify the diagnoses were affected by the peer review pathologists (internal and external), the sponsor, and (b)(4) management. However, the inspection also found that (b)(4) uses unsigned draft reports from contributing scientists to prepare final reports and invites sponsors to comment on the drafts. This practice fails to assure individual accountability for accurate reporting and does not meet regulatory requirements¹. Although (b)(4) needs to correct this objectionable finding, DSI's re-evaluation at the inspection found only minimal changes of the tumor incidences across groups as compared to the final conclusions of the study pathologist.
- According to (b)(4) management, the study pathologist (b)(6) for female animals in the rat study (and the mouse carcinogenicity study as well), was laid off in early 2010, as well as some other employees. Her departure appears unrelated to the specific studies she conducted.
- Issues of non-compliance cited on Form 483 are summarized in Attachment 3 and include the study director's failure to assure that all applicable GLP

¹ Only the signed and dated final report of the pathologist comprises raw data respecting histopathological evaluation of tissue specimens. See Attachment 3 for additional details.

regulations were followed and transfer study-specific email correspondence to the archives during or at the close of the study.

Findings at Arena Pharmaceuticals:

For (b) (4) Study AVA00016, the 1 year monkey toxicity study conducted at (b) (4) the inspection at Arena found the following:

- Integrity of the external human pathologists' re-evaluation of the renal pathology was not assured in that Arena lacked documentation for the project procedure (rationale, objective, personnel involved, methodology, data collection, and interpretation etc.). Arena's response to the Form 483 dated 6/29/2010, acknowledged that specific documentation for the blinding was not available and instead provided memos from the pathologists dated June 2010 (four years after the fact) that stated the slides were blinded. Their response is inadequate in that these memos do not reflect or replace contemporaneous documentation of study conduct (i.e., the blinding code).
- As contributing scientist reports from the external human pathologists re-evaluating the monkey kidney histopathology were missing from the submitted final study report (only a signature page with date and signatures included, page 13 of 13), the sponsor provided during the inspection a joint report signed by the sponsor and the two contributing pathologists (b) (6). In the 483 response Arena also promised to submit this report with amendment to (b) (4) Study AVA00016 to the agency. The individual report of each pathologist was not provided during the inspection or in Arena's response.

There were other deficiencies discovered related to the conduct of analytical studies of test article formulations, TK analysis, documentation and archiving issues. For example:

- Arena failed to follow HPLC test method calculations for dosing formulation analyses. In response to the Form 483, Arena acknowledged the failure and stated that their subsequent review of the data found errors in system suitability calculations but no errors in sample concentration calculations.
- Freezer temperature records were not maintained to document conditions of frozen storage of dosing formulation samples (Studies TX04039, TX05004, and TX04041) or showed daily excursions to 0°C for the duration of sample storage (Study TX05071). Although it is objectionable that frozen conditions were not verified, the measured concentrations of lorcaserin in the dosing formulations were within ±10% the nominal value.
- For Study TX05004, QAU failed to assure that Tables 6-21 in TK report PDR-05-205 accurately reflected the raw data. Arena acknowledged that the report stated incorrectly that Day 42 samples at 0.25 hours were not available due to animal mortality; the protocol did not require a sample at this time point for any animal. Arena stated that the amended TK report was submitted to (b) (4) for inclusion in the final report.

- Arena’s archival practices were poor in that records of study conduct (laboratory notebooks, loose data records such as printed chromatograms) were not readily retrieved. Furthermore, the firm lacked a procedure for archiving study-specific email correspondence. This concern affected not only study portions carried out at Arena but their oversight of contracted activities (e.g., the re-evaluation of renal tissue for the 1 year monkey study).
- Several additional issues of non-compliance cited on Form 483 are summarized in Attachment 4. Although objectionable and corrective action is required for future studies, the findings in Attachment 4 do not impact study outcome.

Conclusions and Recommendation

Following the above inspections, DSI concludes that:

1. For the 2 year rat carcinogenicity study with APD356 conducted at (b) (4) the discrepancy in the incidence of malignant and benign mammary tumors (namely, adenocarcinoma and fibroadenoma) in the MD and HD treated female animals between the final data and that provided to the Agency during the in-life phase (study Weeks 88 and 96) resulted from reclassification by the study pathologist (b) (6)
 2. For the incidence of mammary gland tumors of female rats in the 2 year carcinogenicity study, some errors and a few cases of misdiagnosis were discovered through the inspection. However, these errors or misdiagnosis were mostly “over-estimation” rather than “under-estimation” of the malignancy incidences in the mid- and high-dose treated female animals. They were not considered to be affecting the study conclusions of the two-year carcinogenicity bioassay in rats; DSI review and evaluation of slides during the inspection found only minimal or no change on the reported benign or malignancy incidences. DSI concludes that the study should be accepted for the NDA review.
 3. For the one-year monkey toxicity study conducted at (b) (4) DSI recommends that the kidney histopath findings reported by (b) (4) be accepted as the valid data. The process of the tertiary review by the two external human pathologists managed by Arena lacks documentation to assure the data integrity of the re-evaluation.
 4. Although Arena needs to improve their record keeping practices and adherence to written procedures for future studies, the analytical portions for dosing formulation analyses conducted by the sponsor are acceptable for review.
 5. The in-life portions of Studies, (b) (4) 900-062 and (b) (4) 900-063, conducted at (b) (4) are acceptable for review.
- **Recommended HQ Classification:**
Arena Pharmaceuticals – Voluntary Action Indicated (VAI)
(b) (4) - Voluntary Action Indicated (VAI)

Jacqueline A. O'Shaughnessy, Ph.D.
Pharmacologist/Team Leader

Carol Rivera-Lopez, Ph.D.
Pharmacologist

Dylan D. Yao, M.D., Ph.D.
Pharmacologist

Supervisory Concurrence:

Concur: _____ Date: _____

Nonconcurrence: _____ Date: _____
(see attached supervisory memorandum)

Date Assigned: 4/1/2010
EI Dates: 6/7 - 6/11/2010 (Arena)
(b) (4)

District Office: LOS-DO (Arena)
(b) (4)

FDA Investigators: Arena Pharmaceutical, Inc.
Jacqueline A. O'Shaughnessy, Pharmacologist, DSI
Dylan D. Yao, Pharmacologist, DSI
Courtney Long, Investigator, Los Angeles District Office

(b) (4)

Inspection Type: ___ Routine Surveillance X Directed
FDA-483 Issued: ___ No X Yes
Letter Issued: ___ None (CDER) X PI Letter (b)(4)
 ___ Warning Letter ___ Rejection of Study

Date EIR Received DSI: (b)(4) (b)(4) Arena EIR Pending
Date EIR Received by Reviewer: (b)(4) (b)(4) Arena EIR Pending
1st Draft Review Completed: 8/24/2010

FEI: 3001433984 (Arena)
(b)(4)

FACTS: 1161598 (Arena)
(b)(4)

Inspection Conclusion: VAI (b)(4)

District Decision: VAI (b)(4)

Final HQ Classification: VAI for both Arena and (b)(4)

cc: via DARRTS

DMEP/Fred Alavi/Todd Bourcier/Patricia Madara (NDA 22529)/Mary Parks

DSI GLPBB/Ball/Haidar/Rivera-Lopez/O'Shaughnessy/Patel/YaoD

HFR-PA2535 Long

HFR-CE7535 Pittman

HFR-CE750 Haynes

Draft: DY 8/24/10, 8/26/10; JAO 8/27/10; (b)(4) 8/30/10; DY 8/30/10; JAO 8/31/10; SH 8/31/10

DSI File: GLP0748

O:GLP\EIRcover\FY10\22529Arena (b)(4) 10.doc

ATTACHMENTS

1. Review and Verification of Histopathology Tissue Slides (all mammary gland tissues and masses in selected groups of the female rats) in (b)(4) Study 900-063
2. Selected photomicrographs of the mammary gland tumors
3. Additional Inspectional Findings at (b)(4)
4. Additional Inspectional Findings at Arena Pharmaceuticals
5. Form FDA 483 for (b)(4)
6. Form FDA 483 for Arena Pharmaceuticals

Attachment 1

Review and Verification of Histopathology Tissue Slides (all mammary gland tissues and masses in selected groups of the female rats) at (b) (4)

The priority of this inspection was to verify the histopathology diagnosis of the mammary gland tumors after treatment of APD356 in the 2-year carcinogenicity study in rats, and to discover the rationale for reclassification of the mammary gland adenocarcinoma and fibroadenoma in the MD and HD groups at the site.

More than 600 mammary gland tissue slides, including all female rats from control, MD and HD groups, were carefully reviewed under microscope with the (b) (4) pathologists. Please refer to “Attachment 1” (Review and Verification of Histopathology Tissue Slides) and “Attachment 2” (Selected Photomicrographs of the Mammary Gland Tumors) for details.

The findings through microscopic review of the mammary tissue slides are summarized in this report. Some representative fibroadenoma cases that might have previously been counted as malignancy during early updates to the agency in the three groups of female rats audited are also highlighted:

METHODS

From June 29 through July 1, 2010, DSI's Dylan Yao reviewed histopathology slides from Study (b) (4) 900-063 under a multi-head light microscope. All histology slides of the mammary gland tissues in female rats from the vehicle control, mid-dose and high-dose groups were reviewed accompanied by (b) (4) veterinary pathologists (b) (6) and both are DVM and Diplomat A.C.V.P.

Tissue slides of mammary gland in the control, mid-dose and high-dose groups were reviewed and evaluated (female animals only) for verifying the original diagnosis.

AUDIT RESULTS

Control Group (0 mg/kg/day):

Animal No.	No. Masses	No. Fibroadenoma (FA)	No. Adenocarcinoma (AC)	Diagnosis Modified by Audit (*) and photo shot (**)
1201				
1202	1		1	
1203				
1204	1		1	
1205	1	1		
1206	1	1		
1207				
1208				
1209				
1210	1		1	
1211	1	1		
1212	1		1	
1213	2	1	1	
1214				
1215	1		1	
1216				
1217	1	1		
1218				
1219	Multi		1+	
1220	1		1	

1221				
1222	1		1	
1223	1		1	
1224	1		1	
1225	1		1	
1226	1	1		
1227	Multi		1+	
1228				
1229	2	1	1	
1230				
1231	2	1	1	
1232				
1233				
1234	1	-	1	
1235	2	1	1**	** photo taken, 10x, verified to be a carcinosarcoma
1236				
1237	Multi		1+	
1238				
1239				
1240	Multi	1	1+	
1241	1		1	
1242	Multi		1+	
1243	1		1	
1244	1		1	
1245	1		1	
1246	1	1		
1247				
1248	Multi		1+	
1249	1		1	
1250	Multi	1+		
1251	Multi	1+		
1252	1	1		
1253				
1254				
1255				
1256	1	1		
1257				
1258	1	1		
1259	Multi	1+	1+	
1260				
1261	1	1		
1262				
1263				
1264				
1265	1		1	

Mid-Dose Group (30 mg/kg/day):

Animal No.	No. Masses	No. Fibroadenoma (FA)	No. Adenocarcinoma (AC)	Diagnosis Modified by Audit (*) and photo shot (**)
3201	5	5		
3202	5	5**		**photo taken, 4x, 10x,

3203	4	3	1**	**Photo taken, 4x, 20x
3204				
3205	3	3		
3206				
3207	3	2	1	
3208	1		1	
3209	3	Slide missing	1+1*	*Plus 1 mammary gland skin squamous cell carcinoma
3210	2	1	1	
3211	5	5		
3212	5	5**		**Photo taken, 4x, 20x
3213	5	3	2*&**	*1 is benign, but called malignant; **Photo taken, 4x
3214	1	1		
3215	4	2	2	
3216	4	2**	2	** photo taken, 4x
3217	2		2**	** 2x could have been called FA ** 20x typical AC
3218	6	6		
3219	4	3	1*&**	*Small focus had been called AC , should be corrected. **Photo taken 2x
3220	4	4		
3221	2	1	1	
3222	6	6		
3223	1		1	
3224				
3225	3	2	1	
3226	7	7		
3227	2	2		
3228	2	1	1	
3229				
3230	2	2		
3231	3	2	1	
3232	1		1**	** photo taken, 2x
3233	4	3	1	
3234	2		2	
3235	4	3	1	
3236	5	2	2	1 missing
3237	3	2	1	
3238	2	1	1	
3239	4	4		
3240	7	5	2	
3241	5	5**		**Photo taken, 4x, small focus could have been called AC
3242	5	5		
3243	3	1	2**	** photo taken, 2x, 20x, typical AC
3244	9	8**	1	** photo taken, 4x, small focus could have been called AC
3245	4	4		
3246	5	5		
3247	5	5		
3248	3	2		1 mass is a lymph node

3249	5	5		
3250	4	3	1	
3251	2		2	
3252	2	1	1	
3253	5	5		
3254	6	6		
3255	1		1**	** photo taken, 4x, 10x, 20x, typical AC
3256	4	4		
3257	2	2		
3258	3	2	1	
3259	3	2	1	
3260	4	4		
3261	4	4		
3262	7	5	2	
3263	4	3	1	
3264	3	2	1	
3265	3	3*	(3*)	*Reported "multiple AC", verified as FA for all 3
Total animal# bearing tumors		53	34	

- Animal #3209, one mass of the 2 reported mammary gland adenocarcinomas is now verified to be (mammary gland) skin squamous cell carcinoma, which is supposed not to be classified into the mammary gland tissue.
- Animal #3213, one mass of the 2 reported adenocarcinomas is now verified to be a benign fibroadenoma.
- Animal #3219, there is a small focus inside the tumor parenchyma showed atypical hyperplasia in one of the two previously diagnosed adenocarcinomas, now it is verified to be a fibroadenoma (benign), refer to the attached photomicrograph. This did not affect the total malignancy numbers since the animal still bear an adenocarcinoma.
- Both animals #3241 and #3244 had similar small focus of lesions like that in animal #3219 (this sort of small foci may have been called adenocarcinoma during the update time to DMEP before completion of the study at Week 104), refer to the attached photomicrographs.
- Animal #3265, all 3 masses of reported adenocarcinomas are now verified to be fibroadenomas, which reduced one of the total animal numbers bearing malignancy (adenocarcinoma) in this group.
- Mammary gland adenocarcinoma should be corrected to be 34 of 65 female animals (originally 35 of 65) due to the correction of animal 3265) in this group.
- The total number of animals bearing mammary gland fibroadenoma did not change (still count as 53 of 65 animals as reported) in this group.

High-Dose Group (100 mg/kg/day):

Animal No.	No. Masses	No. Fibroadenoma (FA)	No. Adenocarcinoma (AC)	Diagnosis Modified by Audit (*) and photo shot (**)
4201	6	5	1	
4202				
4203	1		1	
4204	3		3	
4205			2	
4206			1	

4207	4	3	(1*)	*a skin fibrosarcoma is now verified as malignant fibrous histiocytoma, and should not be count as mammary tumor
4208	2	2		
4209	4	2*	2	*Originally all 4 diagnosed as AC
4210	4	3	1	
4211			1	
4212				
4213			1	
4214			1	
4215	3	2	1	
4216	4	3	1	
4217	4	3**	1	** photo taken, 4x, small focus could had been called AC
4218			1	
4219	1		1	
4220	1		1	
4221	1		1	
4222			1	
4223	5	4	1	
4224	2	2		
4225	6	3	3	
4226	5	3	2	
4227	5	3	2	
4228	5	5		
4229	2	1	1	
4230	1		1	
4231	3	3		
4232	4	3	1	
4233	4	2	2	
4234	0		1	
4235	2	1	1**	** photo taken, 20x, was called AC, verified as carcinosarcoma
4236	1		1	
4237	7	2	4	1 FA was verified as hyperplasia
4238	4	2	2	
4239	5	3	2	
4240	2	1	1	
4241	2	1	1	
4242	4	1	3	
4243	1		1	
4244				
4245			2	
4246	3	2	1	
4247				
4248	2	1	1	
4249	4	2	2	
4250			1	
4251	1		1	
4252	5	5		
4253	2	1	1	
4254	1	1		
4255				

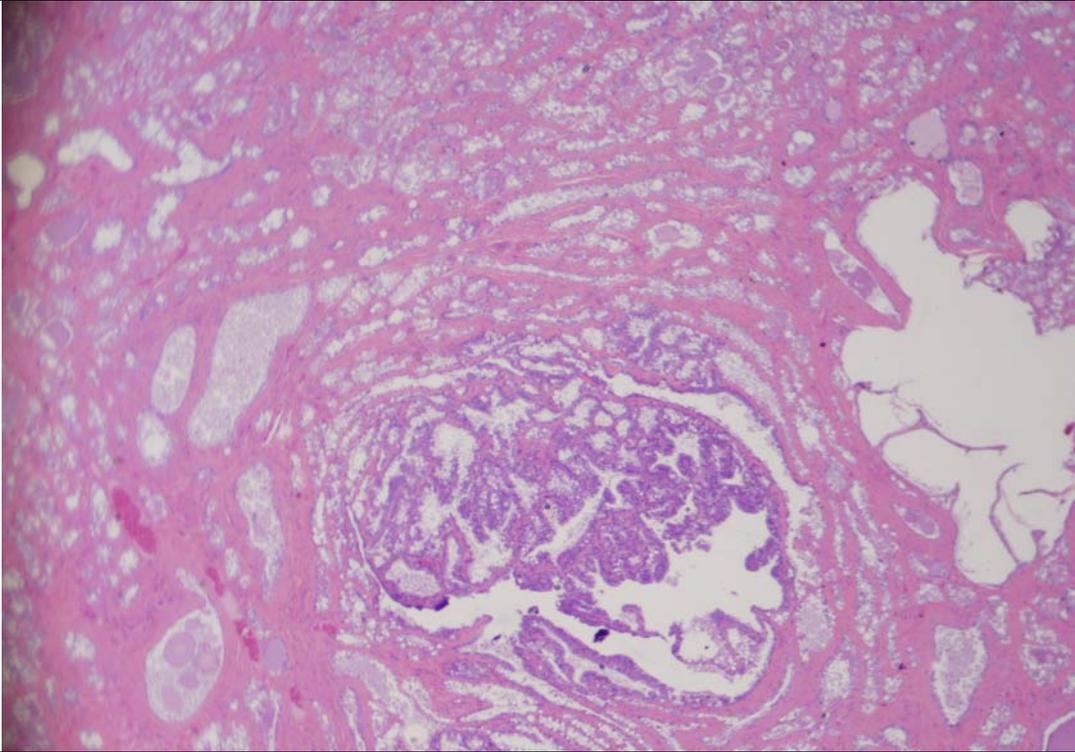
4256	1		1	
4257	5	5		
4258	8	4	4	1 hyperplasia
4259	2	2		
4260	6	5	1	
4261	7	7		
4262	4	3	1	
4263	3	2	1	
4264	3	1	2	
4265	4	1	3	
4266	2	1	1	
4267	1		1	
4268	2	1	1	
4269	5	4	1	
4270			1	
4271			2	
4272	4	2	2	
4273	7	6	1	
4274	2	1	1	
4275	4		4	
Total animal# bearing tumors		46	60	

- In animal #4207, a skin fibrosarcoma is now verified to be “malignant fibrous histiocytoma”, which was not misdiagnosed within the "Mammary Gland Individual Data" table in the report, but was archived (mixed?) within mammary gland slides.
- In animal #4209, originally all 4 masses were diagnosed as adenocarcinoma, now 2 of the 4 were corrected to be benign fibroadenoma. Therefore, the total animal # bearing benign tumors (fibroadenoma) is adjusted from 45 out of 75 to 46 out of 75.
- Animal #4217, a small focal lesion inside the tumor parenchyma showed atypical hyperplasia that could have been called adenocarcinoma previously, refer to the attached photomicrograph.
- The malignant tumor in animal #4235 was originally diagnosed as adenocarcinoma, now verified to be a carcinosarcoma (still malignant and therefore it did not change the reported results, see photomicrograph), refer to the attached photomicrograph.
- Animal #4237 has recorded 7 masses originally, 3 of which were diagnosed as fibroadenoma and 4 were adenocarcinoma; now one of the 3 benign tumors is verified to be hyperplasia (not a tumor).
- The total numbers of animals bearing fibroadenoma are adjusted to 46 of 75 (instead of 45 out of 75 reported) due to the tumor verification in animal # 4209 in this group.
- The total numbers of animals bearing adenocarcinoma did not change (60 of 75) after audit.

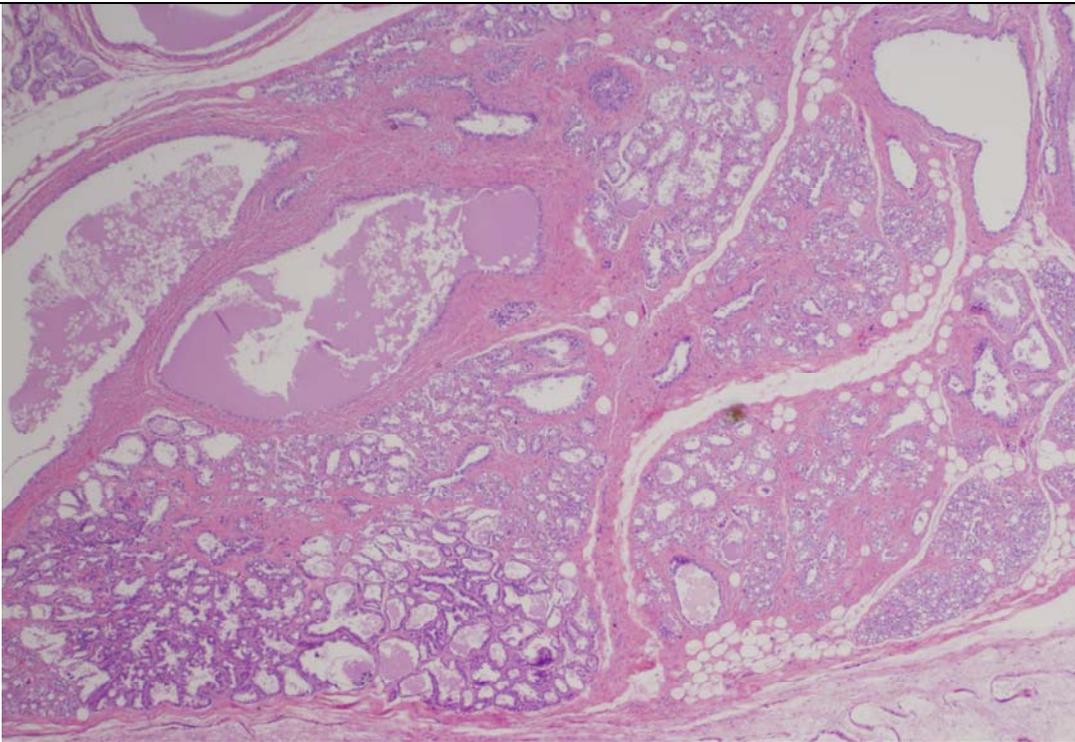
SUMMARY AND CONCLUSIONS

The results of the histopathology slides audit in the 2-year rat carcinogenicity study ((b) (4) 900-063) demonstrated that there were some errors in the mammary tumor diagnosis; most of these are considered "over-estimated" rather than "under-estimated" for the malignancy. Tumor incidence in the final study report was finalized based on the data the study pathologist (b) (6) evaluated, the internal and external peer review pathologists did not adjust the incidence markedly. There was no correspondence in the study record to suggest that the sponsor or the CRO management affected (b) (6) decision to re-classify the diagnosis. Based on the audit results, it is concluded that in spite of some errors in the tumor diagnosis, these errors did not affect the study results adversely, and no impact on the final study conclusions of the carcinogenic effect of Lorcaseerin in the female rats.

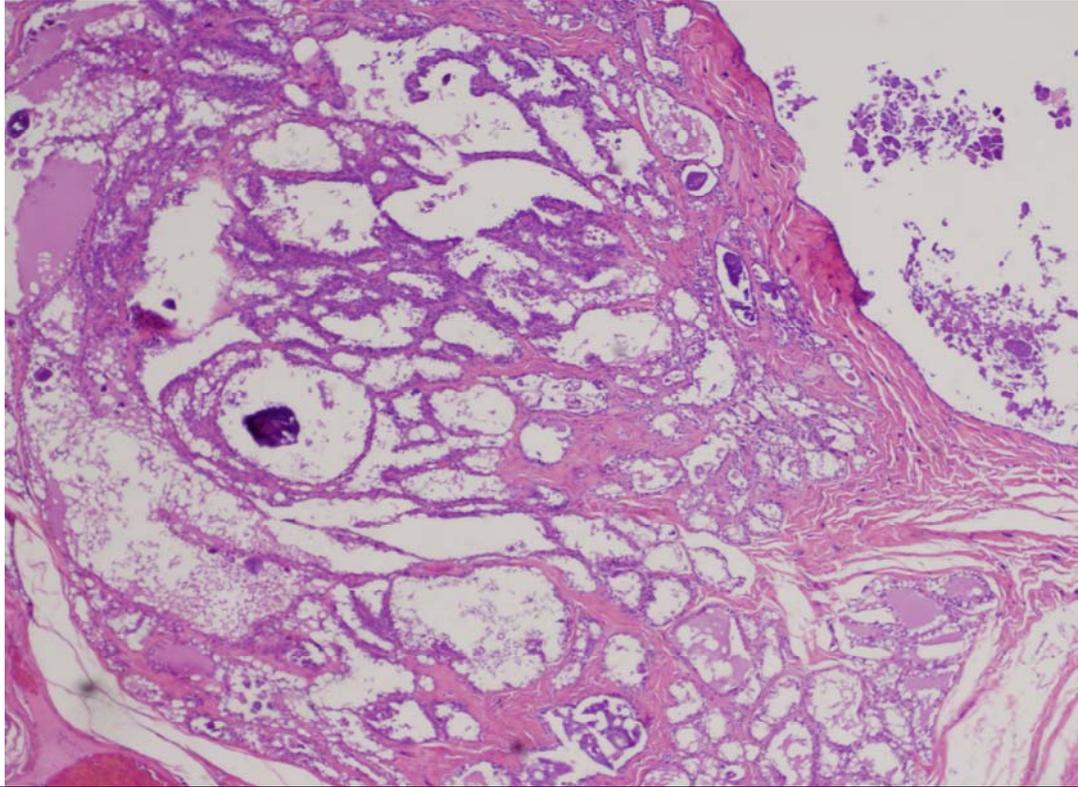
Attachment 2: Selected Photomicrographs of the Mammary Gland Tumors



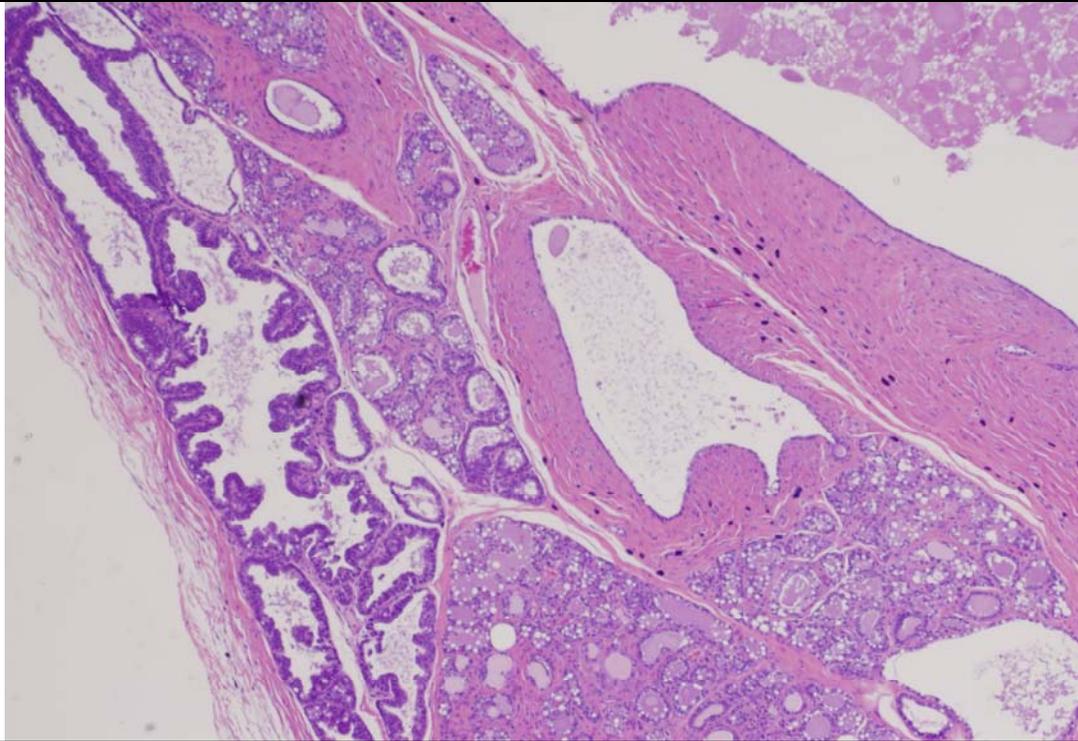
Animal #3213(MD group),Photomicrograph of a fibroadenoma, a small focus showing fast growth with atypical hyperplasia, originally may have been diagnosed as an adenocarcinoma. H&E, x20



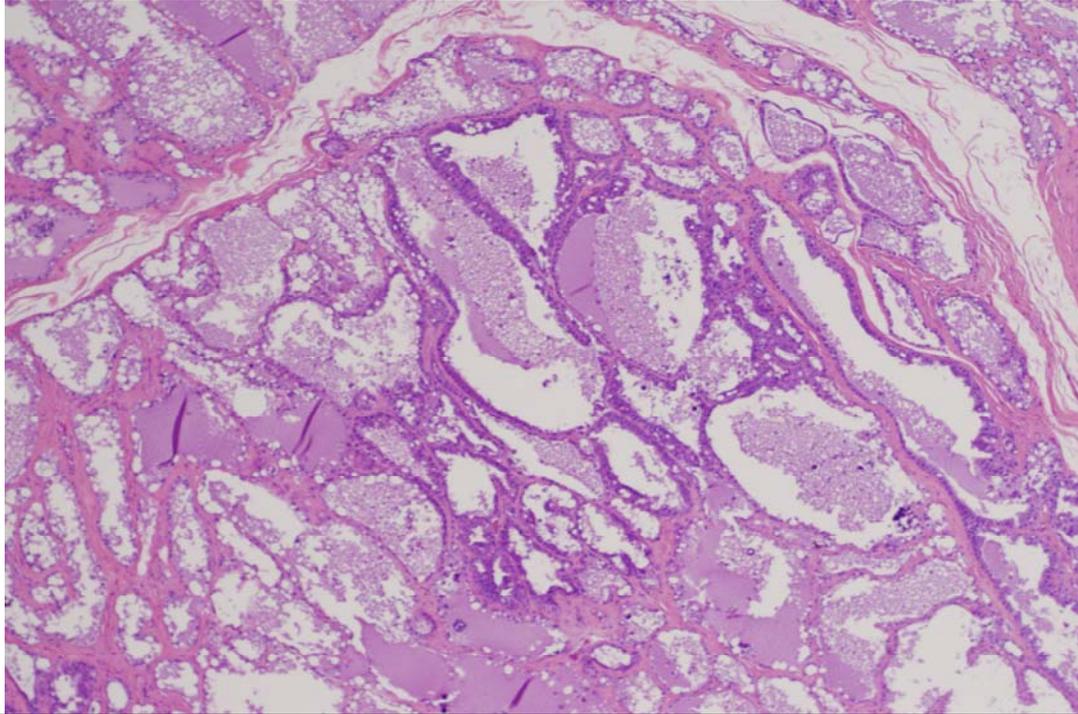
Animal #3219 (MD group). Photomicrograph of a fibroadenoma with a small focus of atypical hyperplasia, originally may have been diagnosed as an adenocarcinoma. H&E, x20



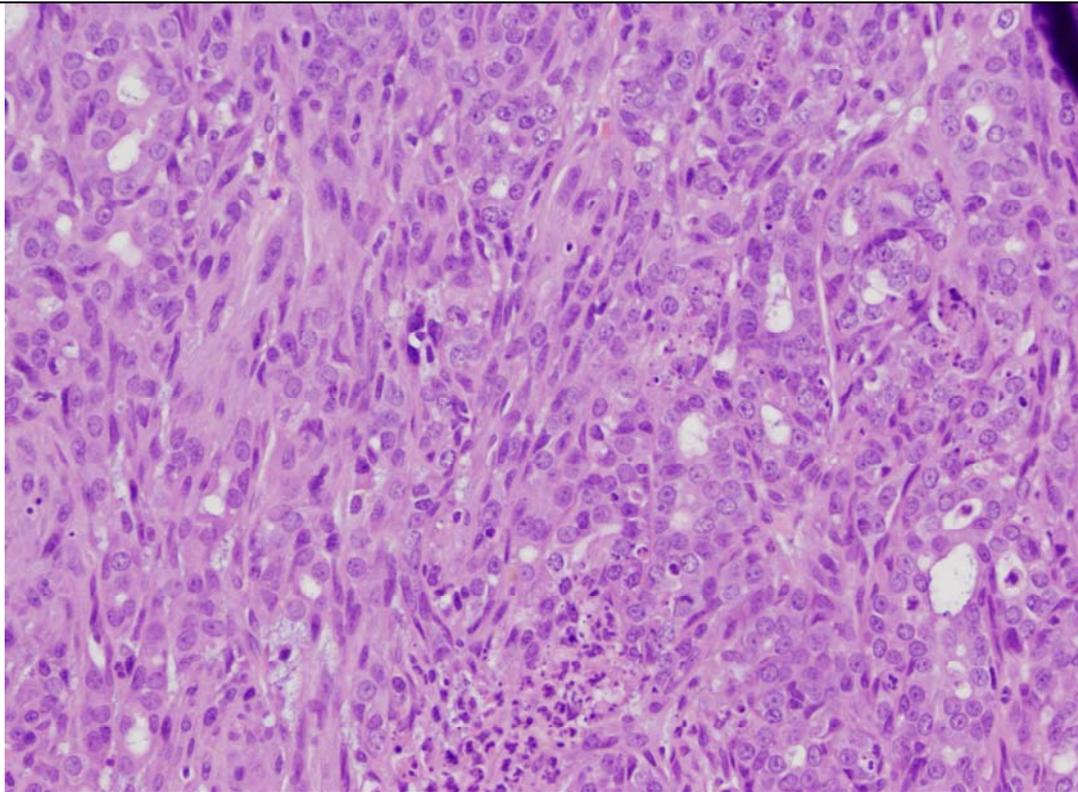
Animal #3241 (MD group). Photomicrograph of a fibroadenoma with a small focus of atypical hyperplasia, originally may have been diagnosed as an adenocarcinoma. H&E, x40



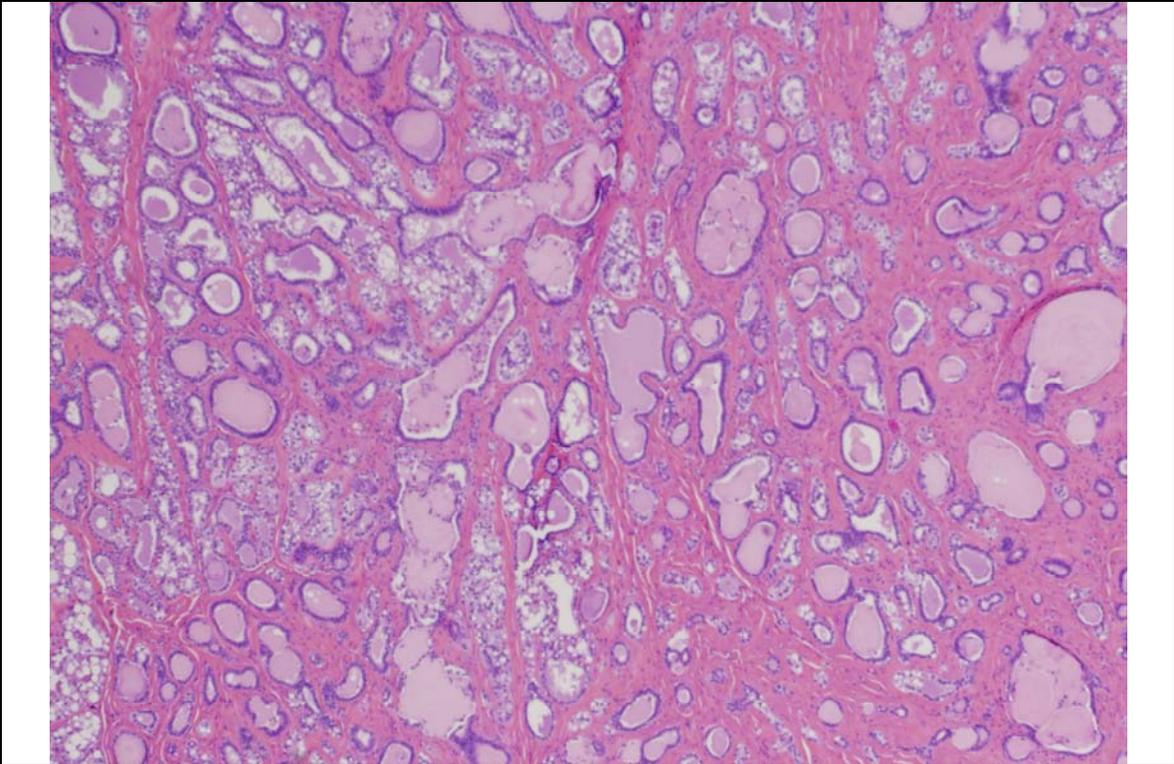
Animal #3244 (MD group). Photomicrograph of a fibroadenoma with a small focus of atypical hyperplasia, originally may have been diagnosed as an adenocarcinoma. H & E, x40



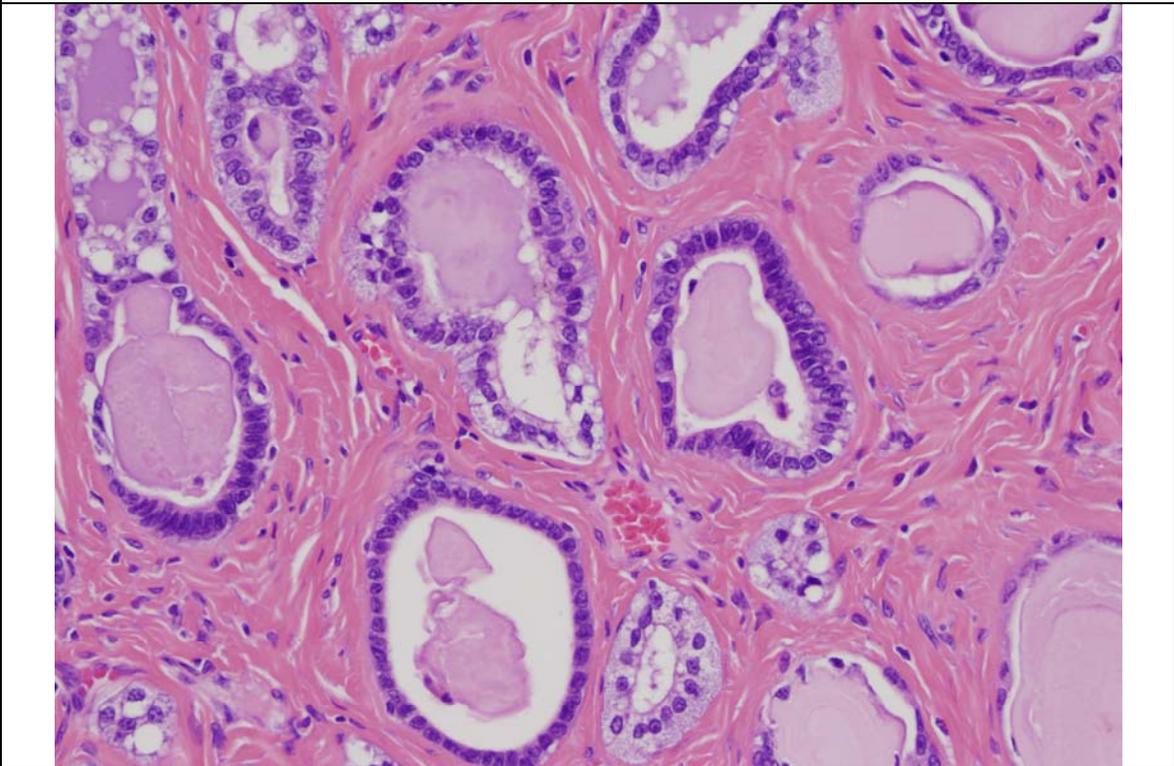
Animal #4217 (HD group). Photomicrograph of a fibroadenoma with a small focus of atypical hyperplasia, originally may have been diagnosed as adenocarcinoma. H&E, x40



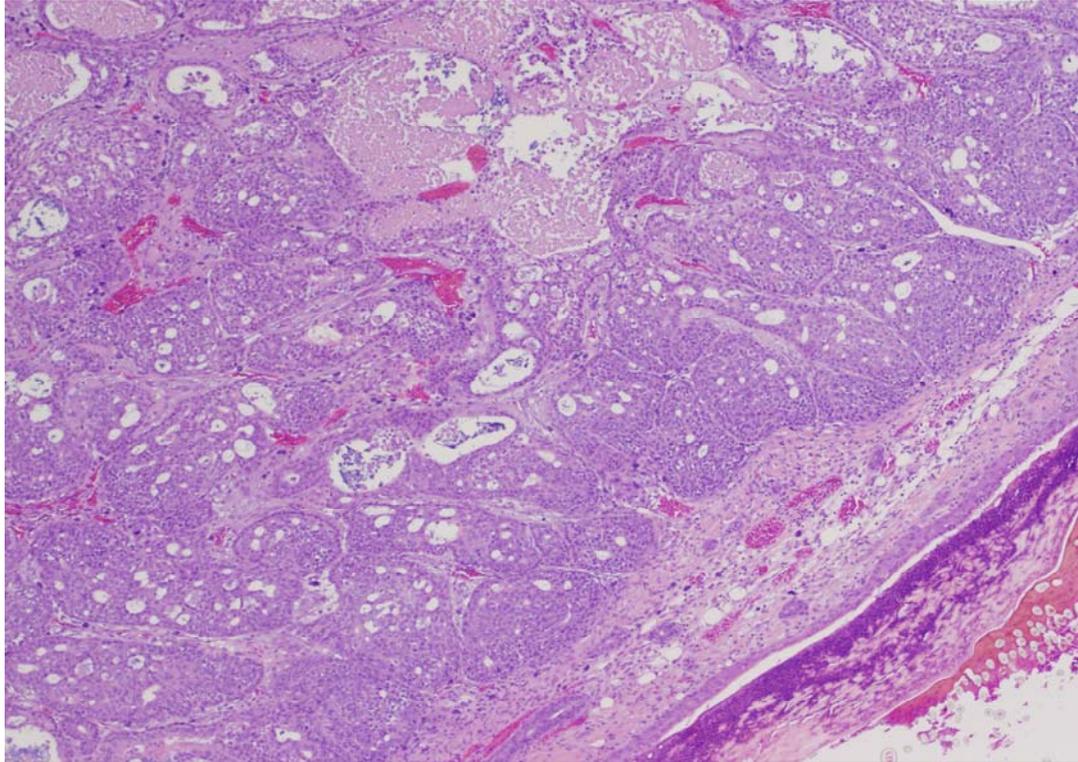
Animal #4235 (HD group). Photomicrograph of a carcinosarcoma (malignant), originally was diagnosed as adenocarcinoma. H&E, X200



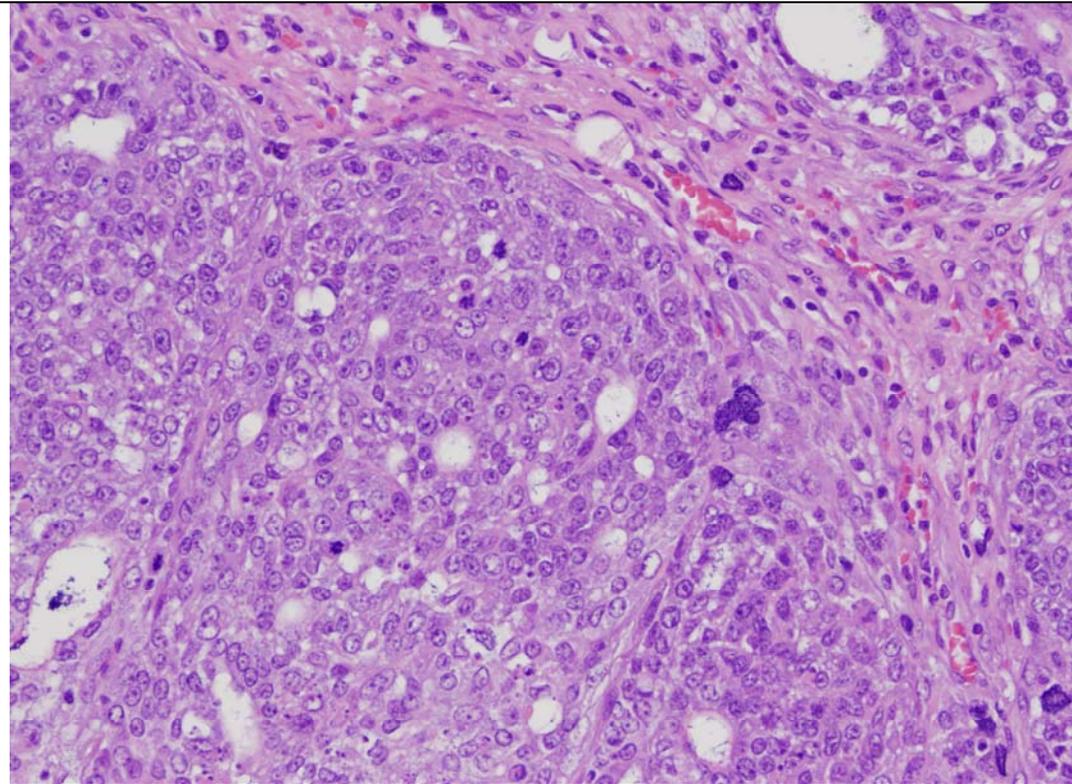
Animal #3202 (MD group). Photomicrograph of a typical fibroadenoma. H&E, x40



The same tumor of animal #3202 above, higher magnification, H&E, x200



Animal #3255 (MD group). Photomicrograph of a typical adenocarcinoma. H&E, x40



The same tumor of animal #3255 above, higher magnification, H&E, x200

Attachment 3. Additional Inspectional Findings at (b) (4)

The following pertains to the Form 483 observations for (b) (4). Please note that, as of the date of this review, DSI has not received a 483 response from (b) (4).

GLP Non-compliance issues:

- 1. The study director failed to assure that all applicable GLP regulations were followed. Specifically, the study director prepared the final report using unsigned draft reports from contributing scientists. 21 CFR 58.33(e)
For example:**

Study Number	Draft Study Report to Sponsor	Contributing Scientist Report	Final Study Report
900-062	12/19/2008	- Pathologist 5/6/2009 - Formulation analysis 4/2/2009	5/8/2009
900-063	8/7/2008	- Pathologist 6/9/2009 - Control plasma and metabolite report 3/3/2009 - Plasma and metabolite TK report 8/20/2008	6/10/2009

Our inspection found that (b) (4) uses unsigned draft reports from contributing scientists for preparation of final reports and submission to sponsors. According to firm management, the above examples are not isolated and this has been a standard practice. This is not an acceptable practice in that signed and dated contributing scientist reports are critical to assure individual accountability for accurate reporting (GLP Final Rules in 1978 and 1987)². In addition, draft final reports in the above examples were submitted to the sponsor (Arena) without having all the raw data from the contributing scientists. Furthermore, this finding concerns the possibility of biased data interpretation because (b) (4) submitted the draft final reports to Arena and invited them to comment and to edit the draft reports.

- 2. The study director failed to assure that all raw data, documentation, protocols, specimens, and final reports were transferred to the archives during or at the close of the study. For example, for study 900-062, email correspondence specific to study related activities was not maintained with study records. 21 CFR 58.33(f)**

Email correspondence specific to study related activities was not maintained with study records. For example, email correspondence between Arena and the study director regarding early deaths and dose reduction for Study 900-062 was kept only in the study director's personal email account. These records were provided by request during the inspection.

Recommendation (Surveillance portion at (b) (4))

- DSI should consider an Untitled Letter to (b) (4) to address the aforementioned non-compliance issues and to ensure (b) (4) is aware of the regulatory requirements.
- Based on the GLP workload, (b) (4) should be scheduled for routine surveillance at a two-year interval. The next inspection should verify whether corrective actions have been implemented for preparation of final reports and archiving of study records.

² 52 FR 33768; September 4, 1987.

Attachment 4. Additional Inspectional Findings at Arena Pharmaceuticals

The following issues were also cited on Form 483 issued at the Arena inspection:

- The amount of test article weighed to prepare the reference standard solution used to determine the concentration of APD356 in week 52 dosing formulations for Study # TX04039 was not recorded; however, the final solution concentration was documented.
- Unjustified re-assay of dosing formulation samples and selective reporting of valid study data. For example, the 6 mg/ml samples for week 78 in Study # TX05071 were repeated multiple times before a final result was accepted. The results of the various analyses are the following:

Analysis #	Result 1	Result 2
1	104.5	80.5
1 – Reinjection	104.9	80.5
2	119.4	97.7
2 – Reinjection	118.7	97.1
3	102.3	95.4

The firm accepted results of the third analysis and included the results of the first and second analysis as footnoted information in the data table; however, there was no justification at the time of analysis to reject the original results. Nevertheless, the remaining concentration results across the 104 week study were within 90-110% of label claim.

- Discrepant Laboratory Results Examination Forms to document repeat analysis of out of specification dosing formulation results were not completed contemporaneously with study conduct.
- Remedial actions for sample receiving deviations were not documented within the SOP-required time frame.

Although it is objectionable that the firm failed to properly document all aspects of study conduct and follow their own written procedures, these issues should not impact study outcomes.

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

NDA Number: 22-529

Applicant: Arena Pharmaceuticals

Stamp Date: Dec 22, 2009

Drug Name: lorcaserin

NDA/BLA Type: 505(b)(1)

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		The sponsor has done a good job of organizing and linking files and data.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		All PDF files for nonclinical studies are created rather than scanned therefore very legible.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		Nonclinical section is consistent with recommendations made at the pre-NDA meeting.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	x		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	x		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		1. Abuse potential: yes 2. Cardiac valve evaluation in tox studies: yes 3. Brain exposure after multiple dosing in rats and monkeys: Yes 4. Several mechanistic studies submitted to measure plasma prolactin levels in rats were not well executed. 5. Brain tissue samples were evaluated with immunostaining with glial fibrillary acidic protein (GFAP), anti-CD68 clone ED-1 (ED-1), and major histocompatibility complex II (MHCII) to further explore brain tumors in rat carcinogenicity study.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		There are at least 4 impurities identified by MultiCASE to be potentially genotoxic. According to the sponsor the concentrations of these potential genotoxic impurities are less than (b) (4) which is significantly less than 1.5 µg/day allowed to be ingested by humans on a daily basis. If the levels of the impurities as claimed by the sponsor are correct, no additional genotoxicity assessment are needed.
11	Has the applicant addressed any abuse potential issues in the submission?	n/a		Will be addressed by CSS staff
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	n/a		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___ Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Additional Internal Comments: None at this point

Reviewing Pharmacologist: Fred Alavi, Ph.D.
Team Leader/Supervisor: Todd Bourcier, Ph.D.

Date: Feb 16, 2010
Date: Feb 16, 2010

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22529	ORIG-1	ARENA PHARMACEUTICA LS INC	LORQESS (lorcaserin hydrochloride) Tablets

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

/s/

FRED K ALAVI
02/17/2010
PharmTox filing form for NDA 22529, lorcaserin HCl

TODD M BOURCIER
02/17/2010
Lorcaserin NDA fileable for pharm/tox