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RESEARCH**

APPLICATION NUMBER:

203505Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on N203505 ospemifene

From A. Jacobs, AD

Date: January 15, 2013

1. I concur that there are no outstanding pharm/tox issues
2. I concur with the proposed pregnancy labeling.
3. I have conveyed some editorial suggestions to the reviewer and they will be addressed as appropriate.

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/s/

ABIGAIL C JACOBS
01/15/2013

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: 203505
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Product: Ospemifene
Indication: Vulvar and Vaginal Atrophy in Postmenopausal Women
Applicant: Shionogi, Inc.
Review Division: Division of Reproductive and Urologic Products
Reviewer: Jeffrey Bray, Ph.D.
Expert Reviewer/Team Leader: Alex Jordan, Ph.D.
Division Director: Hylton Joffe, M.D.
Project Manager: George Lyght

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1 Executive Summary

1.1 Introduction

NDA 203505 was submitted for ospemifene for the proposed indication of treatment of moderate to severe vulvar and vaginal atrophy (VVA) in postmenopausal women. VVA is a condition characterized by vaginal thinning and dryness that may result in vaginal itching and burning and painful sexual intercourse. Ospemifene is a new molecular entity submitted as a 505(b)(1) application. The proposed clinical regimen is once daily oral ospemifene given as a 60 mg tablet. Nonclinical pharmacology, pharmacokinetic, and toxicology studies were submitted to support approval.

1.2 Brief Discussion of Nonclinical Findings

Overview: Ospemifene is a mixed estrogen receptor agonist/antagonist that has tissue selective activities- a drug class commonly known as SERMs (selective estrogen receptor modulators). Ospemifene demonstrated the expected pharmacology of a mixed estrogen agonist/antagonist with no unexpected nonclinical safety signals. Ospemifene is a reproductive toxicant and is tumorigenic in rodents (**see below**) at or below comparable human exposure levels. However, the reproductive findings are expected and not relevant for the indicated population and the tumor signal in rodents was expected and was observed with other SERMs and estrogens. Most tumors observed are not relevant to humans; post-marketing experience for other SERMs has not shown an increased risk for tumors.

Ospemifene is chemically related to clomiphene, toremifene, and tamoxifen displaying similar, but not identical, pharmacological profiles to these SERMs. Ospemifene potently binds to both Estrogen Receptor α and β types of nuclear receptors. Ospemifene has pharmacological activities in rats and monkeys consistent with estrogen agonism in the vagina, ovary, and bone, mixed agonism/antagonism in the uterus, and antagonism in the mammary gland. There were no significant findings from a battery of safety pharmacology assays designed to evaluate neurological, cardiovascular, pulmonary, and renal effects.

The overall PK/ADME profiles among species are comparable. Ospemifene is highly protein bound in all species. Ospemifene is not a substrate for P-glycoprotein in vitro, is rapidly but poorly absorbed in rats, monkey and humans, and distributes widely in rats and monkeys. Excretion is predominantly through the feces in rats, monkeys, and humans with very little excreted in urine, consistent with elimination of ospemifene in rat and monkey predominantly through the liver with hepatobiliary recirculation. Ospemifene is extensively metabolized in the liver by CYP enzymes in all species, and most metabolites are hydroxylated and glucuronidated products. The predominant CYPs involved in metabolism are CYP3A4, CYP2C9, CYP2C19 and CYP2B6. In humans, ospemifene is primarily metabolized by CYP3A4, CYP2C9 and CYP2C19. Nonclinical and clinical drug interaction studies show that ospemifene is not an inducer or inhibitor of CYP activity, but can be affected by prototypical CYP inducers or inhibitors.

The metabolites 4-hydroxy ospemifene (designated M1) and 4'-hydroxy ospemifene (designated M2) are the predominant metabolites in humans, mice, rats, and monkeys. M1 and M2 have similar pharmacological profiles as ospemifene. M1 is present at levels that exceed the parent ospemifene in rodents and monkeys, whereas levels in humans are 25% of parent. M2 is present at low, comparable levels in all species. M1 and M2 were measured and qualified by use in the repeat dose toxicology and carcinogenicity studies since exposure in rodents and monkeys exceeded human exposure.

In rats, mice, female dogs, and female monkeys, there were no unexpected toxicities noted. The main effects noted were related to the exaggerated pharmacological effect of ospemifene on reproductive organs. Organ weight, gross pathological and histopathological effects were noted on the ovary, uterus, vagina, mammary gland, liver, prostate, testis, and epididymis in most species and studies. The ovary, mammary gland, and male reproductive organs showed a predominantly antagonistic profile, whereas the vagina and liver showed agonism. However, some studies showed cell- and tissue-selective agonism. This was most clearly observed in the uterus of monkeys; in the rat, the profile was predominantly antagonistic. There were decreased ovarian weights with ovarian cysts with species- and cell-specific effects noted. Vaginal mucification was noted in rats and monkeys. The mammary gland showed sex- and species-specific effects, considered to be pharmacological and predominantly antagonistic in female rats and monkeys. In rats and female monkeys, increased liver weight correlated with centrilobular hepatocyte hypertrophy and enzyme changes. These findings are consistent with induction of CYP enzymes that metabolize ospemifene and M1. Sporadic findings were reported in the adrenal and pituitary and on hematopoiesis in rodents. In rodents and dogs, decreased male reproductive organ weights with atrophy of the prostate, testis, and epididymis were noted. All findings were at exposures comparable to human exposure at the proposed dose.

Ospemifene was embryotoxic and adversely affected parturition. There were development effects noted in the offspring of treated pregnant rats. These effects were noted at exposures significantly lower than the human exposure. In rabbits, the exposure was 10-fold over proposed clinical dose based on body surface area.

Embryofetal toxicity (EFT) studies for ospemifene were conducted with rats and rabbits. In rats, an increase in placental weight and an increased number of testicular displacements among pups was noted. In rabbits, an increase in total resorptions was noted that correlated with decreased number of live fetuses and an increase in post-implantation loss. In a pre- and post-natal development study in rats, there was increased maternal mortality and total litter loss preceded by clinical signs of difficult parturition such as dystocia, vaginal bleeding, ruffled fur, lethargy, hypothermia, and/or uterine prolapse. Gestational duration increased, consistent with mortality, prolapse, and dystocia. There was a significant decrease in viable pups born and increased post-implantation loss (total and %), and non-significant increase in number of litters with dead pups compared to control. The highest exposures obtained in reproductive

toxicology studies were only 4% of human exposure with higher doses precluded by significant maternal toxicities and fetal losses. No fertility and early embryonic development study was conducted or necessary for the indicated population of post-menopausal women.

The weight of evidence suggests that ospemifene is not genotoxic. Ospemifene was negative in the in vitro Ames and mouse lymphoma cell assays and in the in vivo mouse micronucleus and rat liver DNA adduct assays. There were no structural alerts for ospemifene or the M1 and M2 metabolites.

Ospemifene is carcinogenic to rodents based on the findings from the rat and mouse 2-year carcinogenicity studies. All treated rat and mouse groups had lower body weight gain and greater survival rates than control groups.

Hepatocellular and ovarian neoplasm were considered to be clearly related to drug in rats and ovarian and adrenal neoplasms were considered to be clearly related to drug in female mice. The male mouse study was terminated very early and not evaluable. In the carcinogenicity studies, increasing doses did not result in corresponding increases in drug blood levels, which explain the lack of dose-relationship for neoplastic findings. The exposure multiples in rats and mice were 1- and 5-fold, respectively, over clinical exposure at the proposed dose.

Except for skin, both neoplastic and non-neoplastic treatment-related effects in estrogen target organs, such as testes, epididymides, ovary, uterus, mammary gland, bone, liver, adrenal, pituitary, spleen, thymus and thyroid were consistent with the established ospemifene pharmacology/toxicology, or other mixed estrogen agonist/antagonists. Adrenal, ovary, and pituitary tumors may be related to endocrine steroidogenic feedback loop disruption.

1.3 Recommendations

1.3.1 Approvability

The nonclinical findings support Approval for the treatment of moderate to severe VVA in post-menopausal women at a daily oral dose of 60 mg.

1.3.2 Additional Non Clinical Recommendations

none

1.3.3 Labeling

Recommended nonclinical revisions to the sponsor's proposed label are provided below:

1. INDICATIONS AND USAGE

[Trade Name] is an estrogen agonist/antagonist indicated for the treatment of vulvar and vaginal atrophy due to menopause.

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category X.

Osphena should not be used in women who are or may become pregnant [see Contraindications (4)].

There are no adequate and well-controlled studies using ospemifene in pregnant women. [TRADE NAME] should be used during pregnancy only if the potential benefit to the patient outweighs the risk to the patient and fetus. Women who become pregnant during ospemifene treatment are encouraged to contact their physician.

Risk Summary

Based on animal data, ospemifene is likely to increase the risk of adverse outcomes during pregnancy and labor. Adverse findings at maternally toxic doses included embryofetal lethality in rats and rabbits, and neonatal mortality and difficult labor in rats. The reproductive effects observed are consistent with and are considered to be related to estrogen receptor activity of ospemifene.

Animal Data

The effects of ospemifene on embryo-fetal development were studied in rats (0.1, 1 or 4 mg/kg/day) and rabbits (3, 10, or 30 mg/kg/day) when treated from implantation through organogenesis. In rabbits, there was an increase in the incidence of total resorptions at 30 mg/kg/day (10 times the human exposure based on surface area mg/m²). Drug-induced malformations were not observed in either rats or rabbits.

The effects of ospemifene on pre- and postnatal development were studied in pregnant rats (0.01, 0.05, and 0.25 mg/kg/day) treated from implantation through lactation. Pregnant rats given 0.05 or 0.25 mg/kg/day ospemifene (0.8 to 4% the human exposure based on surface area mg/m²), had a significantly prolonged and difficult gestation, increased post-implantation loss, increased number of dead pups at birth, and an increased incidence of postnatal loss. Ospemifene did not induce adverse effects in the surviving offspring of pregnant rats at drug exposures up to 4% the human exposure.

8.3 Nursing Mothers

[TRADE NAME] should not be used by lactating women. It is not known whether [TRADE NAME] is excreted in human breast milk. Ospemifene is excreted in rat milk, and is detected at concentrations higher than that in maternal plasma.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

No evaluation for carcinogenicity was conducted in male mice. In a 2-year carcinogenicity study in female CD-1 mice, ospemifene was administered at 100, 400,

or 1500 mg/kg/day. resulted in a drug-related increase in adrenal subcapsular cell adenomas at 4 and 5 times the human exposure based on AUC, and in adrenal cortical neoplasms at 5 times the human exposure. In the ovary, an increase in sex cord/stromal tumors, tubulostromal tumors, granulosa cell tumors, and luteomas were also seen. These drug-related findings occurred at doses 2 to 5 times the human exposure based on AUC, and are probably related to estrogenic/antiestrogenic effect of ospemifene in mice.

In a 2-year carcinogenicity study in Han Wistar rats, ospemifene administration of 10, 50, or 300 mg/kg/day resulted in drug-related thymomas for males and females at all ospemifene dose levels which were at 1/4 times the human exposure based on AUC. Hepatocellular neoplasms were drug related for females at all ospemifene dose levels.

Mutagenesis

Ospemifene was not genotoxic in vitro in the Ames test in strains of Salmonella typhimurium or at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells in both the absence and in the presence of a metabolic activator system. In in vivo testing, ospemifene was not genotoxic in a standard mouse bone marrow micronucleus test or in a determination of DNA adducts in the liver of rats.

Impairment of Fertility

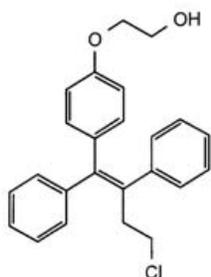
The effect of ospemifene on fertility was not directly evaluated. In female rats and monkeys, decreases in ovarian and uterine weights, decreased corpora lutea number, increased ovarian cysts, uterine atrophy, and disrupted cycles were observed when given repeated daily oral doses. In male rats, atrophy of the prostate and seminal vesicles was noted. The effects on reproductive organs observed in animals are consistent with the estrogen receptor activity of ospemifene and potential for impairment of fertility.

2 Drug Information

2.1 Drug

CAS Registry Number	128607-22-7
Generic Name	ospemifene
Code Name	FC-1271a
Chemical Name	Z-2-[4-(4-chloro-1,2-diphenylbut-1-enyl)phenoxy]ethanol
Molecular Formula/Molecular Weight	C ₂₄ H ₂₃ ClO ₂ ,/378.90

Structure



Pharmacologic Class mixed estrogen receptor agonist/antagonist (SERM)

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 67216 (Ospemifene for postmenopausal VVA)

NDA 16-131 (Clomiphene citrate, for ovulation induction)

NDA 17-970 (Tamoxifen, for adjuvant therapy to ER+ breast tumors)

NDA 20-815 (Raloxifene, for prevent/treat postmenopausal osteoporosis)

NDA 20-497 (Toremifene, for adjuvant therapy to ER+ breast tumors)

NDA 21-757 (Lasofloxifene, for prevent/treat postmenopausal osteoporosis)

NDA 21-843 (Lasofloxifene, for postmenopausal VVA)

NDA 21-962 (Bazedoxifene, for prevent/treat postmenopausal osteoporosis)

DMF (b) (4) (b) (4) Letter of Authorization provided

2.3 Drug Formulation

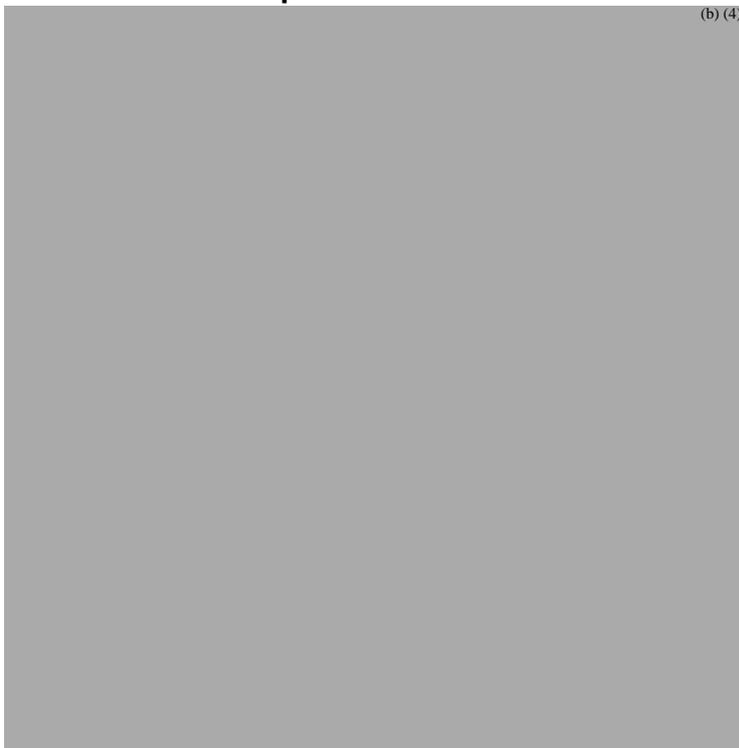
Ospemifene Tablets 60 mg, is a white to off-white, oval, film-coated, biconvex tablet, with one side engraved "60" and contains 60 mg of ospemifene.

2.5 Comments on Impurities/Degradants of Concern

There were 4 synthetic process impurities identified in the drug substance that are also potential degradants. A number of potential impurities were also identified (Applicant's Table 37). The sponsor states, "*The potential impurities/degradation products are highly related to the drug substance, ospemifene. They do not include any new functional group or other structural alerts to produce genotoxicity with relation to the drug substance*", in Module 3.2.P.5.5, page 1. These impurities have qualified by use in the toxicological studies.

Table 1 List of Impurities

(b) (4)

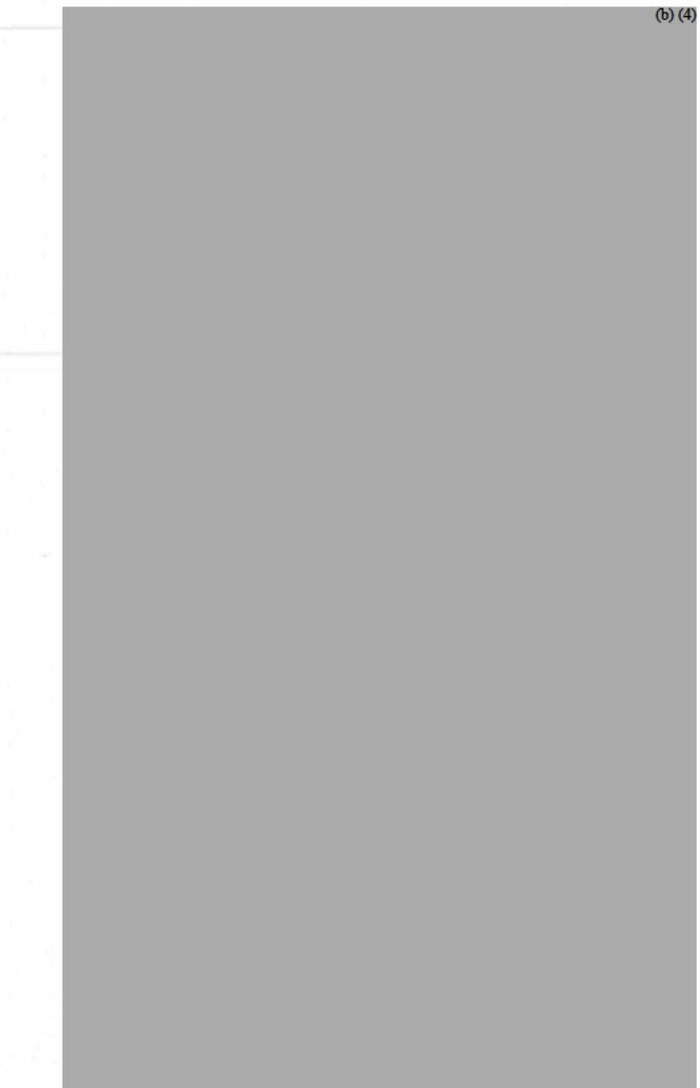


(Excerpted from Applicant's package)

Table 2 Potential Impurities in Ospemifene (FC-1271a) Drug Substance

FC-1271a and its potential organic impurities (structure, chemical name and abbreviation

(b) (4)



(Excerpted from DMF (b) (4))

Table 3 Batches Used for Toxicological and Clinical Studies; Analysis Results and the Uses of Batches

(b) (4)

(Excerpted from Applicant's package)

Degradants generally were detected at low levels in stability testing and well below the specifications. The impurity (b) (4) was the only degradant measured at (b) (4) in the stability batches. This occurred in the accelerated stability (40°C/75%RH) at 6 months at (b) (4) the maximum level at 25°C/75%RH was (b) (4).

Table 4 Summary of Supportive Stability Results in HDPE Bottles and Blisters

Attribute	Room Temperature Storage Conditions (up to 60 months)	Accelerated Storage Conditions (up to 6 months)
Appearance ¹	All comply	All comply
Assay	(b) (4)	(b) (4)
Purity	(b) (4)	(b) (4)
Microbiological Quality	All comply when tested	Not applicable

¹ Appearance was an attribute at the time of testing, and is the same attribute as "Description".

² Less than level of quantitation < 0.05%

(Excerpted from Applicant's package)

2.6 Proposed Clinical Population and Dosing Regimen

Oral once daily to postmenopausal women with moderate to severe vulvar and vaginal atrophy

3 Studies Submitted

3.1 Studies Reviewed

Study #	Study Title
15-44101	Acute oral toxicity of FC-1271a in rats
15-44210	Maximum tolerated dose in cynomolgus monkeys by oral administration
15-44204	4 week oral gavage toxicity study in female rats
15-44205	4 week oral gavage toxicity study in female Cynomolgus monkey
15-44208	28-day oral gavage toxicity study in the female rat
15-44214	A 4 week oral dose study in Cynomolgus monkeys with a 4-week recovery period
15-44403	Ospemifene: a 13-Week Oral (Gavage) Administration toxicity study in the rat
15-44203	13 Week oral (capsule) toxicity study in the dog
15-44402	Ospemifene: a 13-Week oral toxicity study in mice
15-44202	FC-1271a: Toxicity study by oral (gavage) administration to Sprague Dawley rats for 13 weeks
15-44206	FC-1271a: 26-Week oral (gavage administration) toxicity study in the female rat
15-44211	13-Week oral (capsule) toxicity study in the dog with a 4-week recovery period
15-44207	FC-1271a: 39-Week oral (gavage) administration toxicity study in the Cynomolgus monkey
15-44302	FC-1271a: Reverse mutation in five histidine-requiring strains of Salmonella typhimurium
15-04002	FC-1271a: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique
15-44304	FC-1271a: Induction of micronuclei in the bone marrow of treated mice
15-44301	Determination of DNA adducts in the liver of rats treated with antiestrogens
15-04011	FC-1271a: Embryo-fetal development study of Fc-1271a in rats by oral (gavage) administration
15-44503	Study for the effects on embryo-fetal development in the Himalayan rabbit
15-44502	Dose Range-Finding Study for Effects on Embryo-fetal Development after Oral Administration (Gavage) in the Himalayan Rabbit
15-44404	Ospemifene: 104 Week Oral (Gavage) Administration Oncogenicity Study in the Mouse
15-44405	Ospemifene: 104 Week Oral (Gavage) Administration Oncogenicity Study in the Rat
15-44505	Study for Effects on Pre- and Postnatal Development including Maternal Function in the Han Wistar Rat
15-44506	Dose Range-Finding Study for Effects on Pre- and Postnatal Development including Maternal Function in the Han Wistar Rat
15-44407	8 Week Oral (Gavage) Administration Age Comparison Toxicity Study in the Mouse
15-4201	Cardiovascular effects of FC-1271a after oral (capsule) administration in conscious dogs
15-4202	FC-1271a does not have any sedative effect in the hexobarbital sleeping time test in rats
15-4203	Spontaneous motor activity of mice after single oral dose of FC-1271a. Comparison with diazepam and amphetamine
15-4204	FC-1271a has no effect on motor coordination in the rotarod test in mice
15-4205	The effect of FC-1271a on pentylenetetrazol-induced convulsion in mice
15-4206	FC-1271a does not have any central or peripheral anticholinergic effects in tremorine induced tremor and salivation in mice
15-4207	No change in the body temperature after oral administration of FC-1271a in rat
15-4208	The effects of FC-1271a on kidney function in conscious rats
15-4209	The effects of FC-1271a on small intestine motility in conscious mice
15-4210	FC-1271a does not cause muscle relaxation in the traction test in mice
15-4231	Ospemifene and 4-Hydroxyospemifene: effect on HERG-1 Tail currents recorded from stably transfected HEK293 cells
15-4235	Cardiac safety study in cynomolgus monkeys
15-4237	Cardiovascular effects of Ospemifene in the cynomolgus monkey
15-4316	Bi-directional Caco-2 permeability
15-4317	P-GP substrate assessment in MDR-MDCK cell monolayers
15-4305	(³ H)-Ospemifene: A study of absorption, distribution, metabolism and excretion following oral

	and intravenous administration to the rat
15-4303	LC/MS analysis of FC-1271a in vitro metabolites from mouse hepatocyte and human liver microsomes
15-4304	Identification of Cytochrome P450 (CYP) enzymes catalyzing Ospemifene oxidation in human liver microsomes
15-4306	(³ H)-Ospemifene: investigation of metabolite profiles in serum, plasma, urine, faeces and bile following oral and intravenous administration to the female rat
15-4308	(³ H)-Ospemifene: investigation of metabolite profiles in serum, plasma, urine, faeces and bile following oral and intravenous administration to the female cynomolgus monkey
15-4324	In vitro metabolism and metabolizing enzyme identification of 4-hydroxy Ospemifene in human hepatic CYP enzymes
15-4330	Human in vivo metabolic screening of Ospemifene
15-4336	In vitro metabolism and metabolizing enzyme identification of 4-hydroxyOspemifene in human liver microsomes
15-4302	FC-1271a: Inhibition of Cytochrome P450 (CYP)-associated drug metabolism in human liver microsomes: Implications to metabolic fate and potential drug interactions
15-4313	The potential of Ospemifene to Induce CYP-activities in monkey and rat liver
15-4318	Inhibition of CYP model activities by Ospemifene in human liver
15-4320	Quantitative Inhibition of CYP model activities by Ospemifene in human liver preparations
15-4321	Inhibition potential of 4-hydroxyOspemifene towards human hepatic CYP enzymes
15-4325	Ospemifene and Fispemifene: Evaluation of the potential to affect cytochrome P450 enzyme activities in human hepatocytes in vitro
15-4332	Inhibition potential of 4'-hydroxyOspemifene (HM-136) towards cytochrome P450 enzymes

3.2 Studies Not Reviewed

- No primary (25) or secondary (35) pharmacology studies, method validation studies (25) were evaluated by this reviewer.
- 46 literature references or labels of FDA-approved SERMs were submitted, but most were not essential for evaluation of safety, being mechanistic in nature.

Study #	Study Title
15-4314	Kinetic study in the female Syrian hamster after oral administration
15-4312p	FC-1271a: Pharmacokinetic study in minipigs
15-4311	FC-1271a: 14-day oral (gavage) Kinetic study in the female Syrian hamster
15-44213	Ospemifene: tolerability and toxicokinetics in CD-1 mice
15-44701	3-week palatability (feeding) study in the female rat
15-44406	Ospemifene: 21 Day Oral (Gavage) Administration Range-finding Study in the Rat
15-44201	4-weeks oral dose-range study of toremifene alcohol, deaminohydroxytoremifene and 4-hydroxytoremifene in female rat
15-44209	Dose-range finding oral (capsule) toxicity study in the dog
15-4310	Effect of test formulation on exposure of ospemifene after multiple oral dosing in the rat
15-4314	Ospemifene: Kinetic study in the female Syrian hamster after oral administration
15-4322	Comparative bioavailability of ospemifene from two different dosing vehicles in rats
15-4323	Comparative bioavailability of ospemifene from two different dosing vehicles in cynomolgus monkeys
15-4326	Quantitative Inhibition of anastrozole, letrozole and exemastane metabolism by Ospemifene and 4-hydroxyOspemifene in human liver microsome preparations and fresh human hepatocyte cells
15-4327	Contributions of CYP3A4, CYP2C9, CYP2C19, CYP2B6 in the metabolic substrate loss and metabolite formation of Ospemifene
15-4328	Contributions of CYP3A4, CYP2C9, CYP2C19, CYP2B6 in the metabolic substrate loss and metabolite formation of Ospemifene (part 2)

3.3 Previous Reviews Referenced

All reviews were submitted to IND 67216

Review Type	Date in DARRTS	Reviewer	Subject
30-day safety	DFS- 6/30/2002	Alex Jordan, PhD	Pharmacology, safety pharm, supporting tox
General	9/28/2005	Wafa Harrouk, PhD	4 wk rat&monkey, 13 wk, rat&dog, 26 wk rat, 39 wk monkey, repeat dose tox, all gene tox, rat&rabbit EFT
SPA	10/23/2006	Alex Jordan, PhD	exCAC cover sheet
SPA	10/23/2006	Alex Jordan, PhD	mouse review
SPA	10/23/2006	Alex Jordan, PhD	Rat review
Memo	8/25/2008	Alex Jordan, PhD	Male mouse mortality in carci memo
Memo	3/8/2007	Alex Jordan, PhD	Male mouse mortality in carci memo
General	5/7/2007	Alex Jordan, PhD	4 wk monkey

4 Pharmacology

4.1 Primary Pharmacology

Ospemifene is a mixed estrogen agonist/antagonist that has tissue selective activities and species differences. Ospemifene binds to ERs α and β with comparable affinity and cellular potency.

Reviewed by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Ospemifene exerts estrogenic activity on the bone (differentiation of bone cells, similar to raloxifene), estrogen-like activity on the liver (increase of SHBG similar to tamoxifen) and antiestrogenic activity on the breast (inhibiting proliferation similar to raloxifene). In the endometrium, ospemifene has a weaker estrogenic activity than estradiol or tamoxifen and weaker antiestrogenic activity than raloxifene. An estrogenic effect of ospemifene was seen in the vagina (mucification & increase in maturation index) which has not been documented for other SERMs.

4.3 Safety Pharmacology

Studies in *italics* were reviewed by Dr. Wafa Harrouk and directly quoted (DARRTS, 9/28/2005), except study numbers were added by this reviewer:

Neurological effects: *Ospemifene (1, 3, 10, 30 & 100 mg/kg) did not influence hexobarbital-induced sleeping time in rats (#15-4202). A slight increase in spontaneous locomotor activity was seen in mice administered ospemifene at 1000 mg/kg p.o. (#15-4203) A slight proconvulsant effect was seen in mice administered ospemifene at 30 & 100 mg/kg (#15-4205). No effect on rotarod test was seen in mice dosed with 1-100 mg/kg (#15-4204), tremorine-induced tremor & salivation (1-100 mg/kg p.o. 2 hrs before ospemifene in rats) (#15-4206) or body temperature (1-100 mg/kg in rats) (#15-4207) indicative of the lack of effect on motor coordination, central/peripheral anticholinergic effects or interference with neurotransmitter systems, respectively.*

Cardiovascular effects: In conscious, chronically aorta cannulated dog, a slight hypertensive reaction was noted at 30 mg/kg without a change in ECG, blood gases or electrolytes (#15-4201). In a telemetered conscious dog study, ospemifene did not affect blood pressure, heart rate, respiratory parameters or locomotor activity up to 100 mg/kg. No effects on ECG parameters were noted (P-wave amplitude & duration, P-Q interval, QSR or QT intervals) (#15-4211).

Pulmonary effects: See cardiovascular effects.

Renal effects: No effect on urine volume, osmolality or electrolyte excretion (Na & K) was seen in the conscious rat when dosed with ospemifene at 1-100 mg/kg (p.o.) (#15-4208).

Gastrointestinal effects: No effect on the motility of small intestine of conscious mice was noted up to 2 hrs post dose when dosed with 1-100 mg/kg sc in the charcoal propulsion test (#15-4209).

Abuse liability: Not performed.

Other: Ospemifene (1-100 mg/kg/day dosed 2 hrs before test) did not have any effect on skeletal muscle tone when studied in the traction test in mice (#15-4210). No PD interaction with other drugs was noted.

Study title: Ospemifene and 4-Hydroxyospemifene: Effect on HERG-1 tail currents recorded from stably transfected HEK293 cells

Study no.:	15-4231 (848392)
Study report location:	eCTD 4.2.1.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 15, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ospemifene, 01E9, 98.2% 4-Hydroxyospemifene, mv 3+4, 98.6%

Key Study Findings

- Ospemifene and 4-Hydroxy ospemifene inhibited hERG-1 tail currents at $\geq 1 \mu\text{M}$ with a maximal inhibition of 35% for ospemifene and 86% for 4-Hydroxy ospemifene (IC_{50} value= $5.82 \mu\text{M}$).

Methods

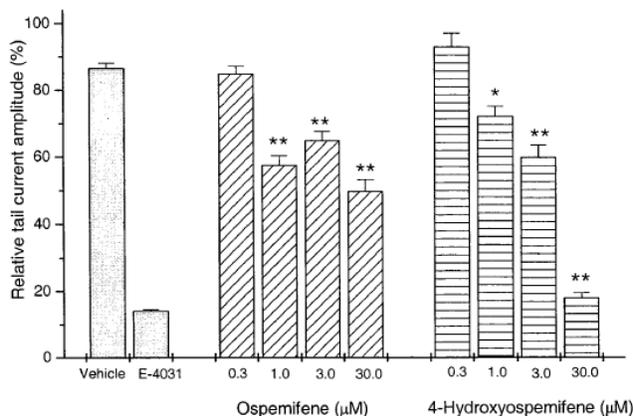
Cell line:	HEK-293
Concentrations in definitive study:	0.3, 1, 3, 30 μM
Basis of concentration selection:	Solubility limit at 40 μM
Negative control:	0.1% DMSO

Positive control: 0.1 μM E-4031
 Formulation/Vehicle: DMSO

Results: Ospemifene and 4-Hydroxy ospemifene significantly inhibited hERG-1 tail currents at ≥1 μM and ≥3 μM in a concentration-related manner, respectively, compared to vehicle control (n=4 cells/concentration). Vehicle control was 86.6% and E-4031 was 14.0%. Relative normalized hERG-1 tail currents were 98.0%, 66.5%, 75.0%, and 75.0% for ospemifene at 0.3, 1, 3, and 30 μM, respectively. No IC₅₀ could be calculated for ospemifene since 50% inhibition was not achieved, and a 35% maximal inhibition was calculated from the logistic dose-response. Relative normalized hERG-1 tail currents were 107.6%, 83.5%, 69.3%, and 20.8% for ospemifene at 0.3, 1, 3, and 30 μM, respectively. The IC₅₀ value for 4-Hydroxy ospemifene was calculated to be 5.82 μM with 86% maximal inhibition.

When compared to maximal human peak concentrations of Ospemifene (1.05 ng/mL, 2.8 μM) and 4-Hydroxy ospemifene (0.25 ng/mL, 0.66 μM), there were **0.1x** and **0.5x** multiples of exposure, respectively, at the NoEC of 0.3 μM. When using free concentrations (based on 98% protein bound), the multiples were **5x** and **23x**.

Figure 1 Effect of Ospemifene and 4-Hydroxy ospemifene on hERG-1 Relative Tail Current in HEK293 Cells



*, P<0.05; **, P<0.01

(Excerpted from Applicant's package)

Study title: Cardiac safety study in cynomolgus monkeys Test item: Ospemifene

Study no.: 15-4235 (CD04/9089FD)
Study report location: eCTD 4.2.1.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: February 20, 2004
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Ospemifene, 01E72, 99.9%

Key Study Findings

- Ospemifene at 500 mg/kg did not affect ECG parameters in a single restrained monkey of each sex.

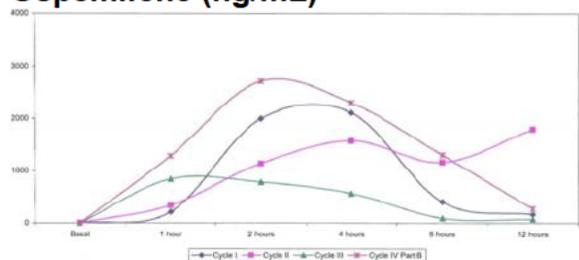
Methods

Doses: 500 mg/kg
Frequency of dosing: Single dose
Route of administration: Oral gavage
Dose volume: 4 mL/kg
Formulation/Vehicle: 15% Labrasol, 18.5% Tween-80 in corn oil
Viomarinilini fatty mixture
18.5% Tween-80 in corn oil (male)
18.5% Tween-80 in corn oil (female)
Species/Strain: Cynomolgus monkey
Number/Sex/Group: 1/sex
Age: ~ 4 years
Weight: 3.9♂, 2.9♀
Satellite groups: none
Unique study design: 3 different vehicles were used to test in one male with 2 weeks between each dose. No vehicle control was used in males.

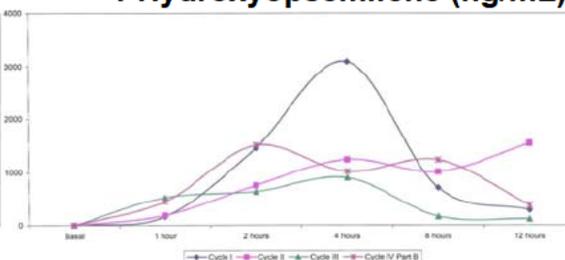
Results:

Ospemifene and 4-Hydroxyospemifene were detected at quantifiable amounts in serum at 1, 2, 4, 8, and 12 hrs post-dose with T_{max} at 2-4 hrs. No significant clinical signs were noted. No significant test article-related effects were noted on ECG parameters, particularly at T_{max} .

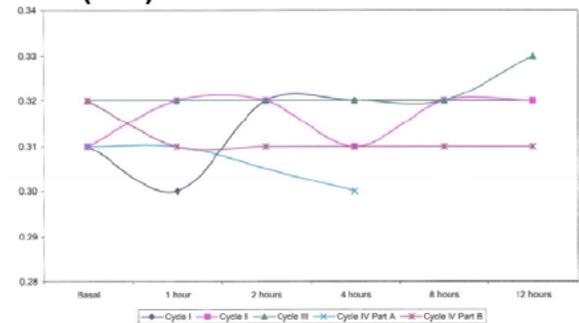
Ospemifene (ng/mL)



4-Hydroxyospemifene (ng/mL)

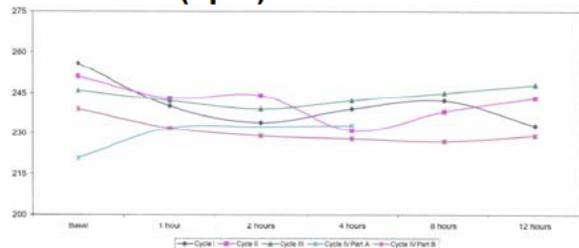


QTc (sec)



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Heart Rate (bpm)



(Excerpted from Applicant's package)

Study title: Cardiovascular effects of Ospemifene in the cynomolgus monkey

Study no.: 15-4237 (1181-010)
 Study report location: eCTD 4.2.1.3.1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: September 23, 2005
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Ospemifene, 01E72, 99.9%

Key Study Findings

- Ospemifene up to 1000 mg/kg had no effect on ECG parameters including heart rate, blood pressure, and corrected QT (QTc) interval.

Methods

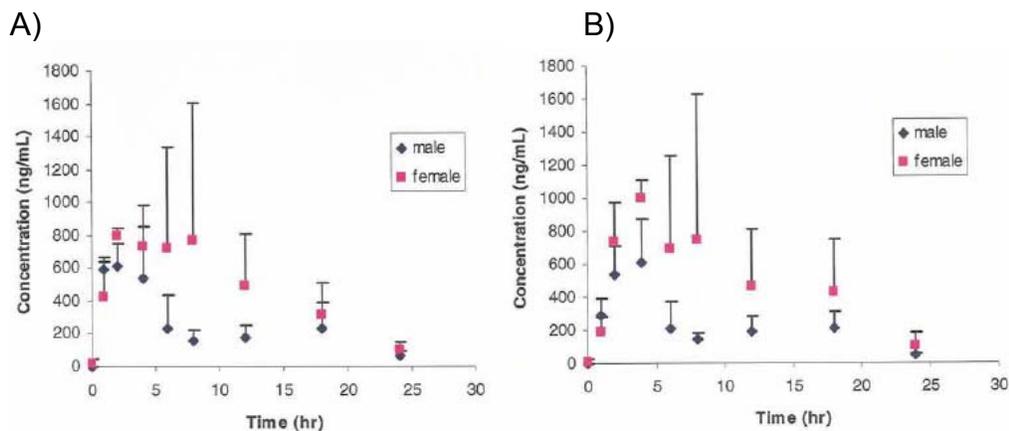
Doses: ♂: 0, 100, 500, 1000 mg/kg
 Frequency of dosing: Single dose
 Route of administration: Oral gavage
 Dose volume: 5 mL/kg
 Formulation/Vehicle: Corn oil
 Species/Strain: Cynomolgus monkey
 Number/Sex/Group: 4/sex
 Age: ~ 4 years
 Weight: 2.28-2.57♂, 2.38-2.97♀
 Satellite groups: none
 Unique study design: Same female received all 4 dose levels, with a 7 day washout between doses

Results:

There were test article-related clinical signs of discolored (white), soft and watery stool at 1, 4, and/or 24 hrs following treatment. There was no mortality and no effect was noted on body weight, emesis, or body temperature in treated animals. There was no effect noted at any dose on ECG parameters such as heart rate, mean, diastolic, and systolic blood pressure, RR interval, PR interval, QRS duration, QT interval, and corrected QT (QTc) interval

Troponins -I and -T were not detected in the serum of treated animals.

Figure 2 Mean ± SD Serum Concentrations of Ospemifene (A) and 4-Hydroxy ospemifene (B) in Monkeys Given 1000 mg/kg (n=4/sex)



(Excerpted from Applicant's package)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Reviewed by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Following oral administration to rats, ospemifene (30 mg/kg) was slowly absorbed, had good initial bioavailability and a significant first-pass metabolism as suggested by low levels of unchanged parent drug and the presence of secondary peaks of drug (both parent drug and metabolite) 24hrs post dose. In the monkey, absorption was rapid but the apparent bioavailability was low indicative of incomplete absorption. Drug was widely distributed, excreted mainly in feces and seemed to accumulate.

Absorption

Following oral administration, the systemic exposure of ospemifene was poor with very low bioavailability of unchanged parent drug (2.6% in female rats) suggestive of first pass metabolism. A concentration peak was seen at 8 hrs after dosing (#15-4305). Following intravenous administration of 5 mg/kg body weight (BW), the volume of distribution and clearance of total drug was high. In the monkey, absorption was rapid (1-4 hrs post dose) after oral administration of 30 mg/kg BW followed by a secondary peak ~ 18-24 hrs post dose (a possible indication of entero-hepatic recirculation) and a slow terminal half-life of 76 hrs (serum) and 149 hrs (whole blood). After i.v. administration, levels of ospemifene fell rapidly (within 5 minutes of dosing) followed by a secondary peak 18-24 hrs after dosing which declined slowly thereafter (#15-4307).

Distribution: *In the rat, tissue distribution was high in the GI, liver, pancreas and ovary, peaked between 2-8 hrs post dose and was not detectable after 24 hrs following acute i.v. administration of ³H-ospemifene (5 mg/kg). No evidence of ospemifene binding to melanin was seen in pigmented animals (#15-4305). In the monkey, the volume of distribution was high compared to total body volume, suggesting extensive distribution. Radioactively labeled ospemifene was found predominantly in the GI, followed by the liver and bile and in the trachea, and was detected 72 hrs post dose (#15-4307).*

Metabolism: *In the mouse hepatocyte culture, 10 metabolites were found and consisted of glucuronidated and hydroxylated products (#15-4303). However, no metabolites were identified in the rat even when dosed up to 2000 mg/kg. In the monkey, a key metabolite, 4-OH-ospemifene, reached a maximum level at 45 minutes post i.v. dosing and was similar to parent drug thereafter (plasma levels, clearance, etc). While no metabolites were identified from human serum, human urine contained 4 metabolites (M1, M3/4, M11 & M12) with M4 being the predominant metabolite (96% of all peaks).*

(#15-4304) Liver microsomes from pooled human livers (n=7) were used to evaluate production of M5 and M11 oxidation metabolites from ospemifene. CYP2C19 was the most active enzyme in M5 formation with CYP2B6 and CYP2C9 having activities in this process. CYP3A4 was the only major enzyme in M11 formation.

Table 5 Inhibition of Ospemifene Oxidations to M4 and M5 Metabolites in Human Liver Microsomes using CYP Enzyme Selective Inhibitors

CYP-selective inhibitor	M4 IC ₅₀ (μM) (percent inhibition at 100 μM in parenthesis)	M5 IC ₅₀ (μM) (percent inhibition at 100 μM in parenthesis)	Remarks ¹
Furafylline (CYP1A2)	>100 (20 %)	>100 (7.3 %)	
Tranlycypromine (CYP2A6)	22.4	>100	CYP2A6-activity IC ₅₀ about 0.4 μM
Ticlopidine (CYP2B6)	33.1	>100	CYP2B6-activity IC ₅₀ about 0.1 μM
Quercetin (CYP2C8)	~100 (50.9 %)	~95 (55.5 %)	
Sulphaphenazole (CYP2C9)	0.43	>100	CYP2C9-activity IC ₅₀ about 0.4 μM
Fluvoxamine (CYP2C19)	5.54	27.3	CYP2C19-activity IC ₅₀ about 0.4 μM
Quinidine (CYP2D6)	>100 (19.8 %)	~100 (46.8 %)	
Pyridine (CYP2E1)	>100 (27.6 %)	>100 (39.0 %)	
Ketoconazole (CYP3A4)	20.4	0.12	CYP3A4-activity IC ₅₀ about 0.1 μM

(Excerpted from Applicant's package)

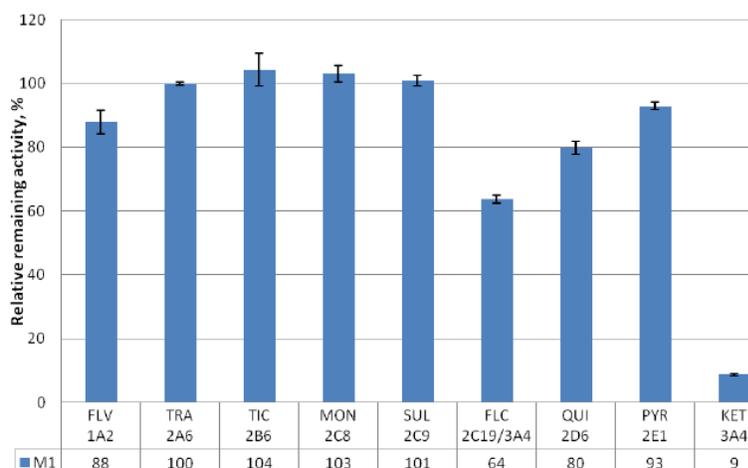
(#15-4306) In the rat, ospemifene and 12 metabolites were identified in plasma, feces, urine, and bile following both a single oral or intravenous dose, including 4-hydroxy ospemifene (designated M1) and carboxylic metabolites.

(#15-4308) In the monkey following oral dosing, ospemifene and M1 were the predominant species detected; whereas unchanged ospemifene was the predominant species detected after intravenous dosing.

(#15-4327) Using human liver microsomes, in vitro metabolism of ospemifene to hydroxylation metabolites M1 and M3 involved CYP3A4, CYP2C9, CYP2C19, and CYP2B6 to some extent each. CYP3A4 and CYP2B6 appeared to be more important for M2 and M4 production.

(#15-4328) CYP3A4 is the predominant contributor to the production of human hydroxylation metabolites in vitro, with CYP2C9 and CYP2C19 having significant contributions.

(#15-4336) CYP3A4 is the predominant contributor to the production of human 4'-hydroxy ospemifene (M2) in vitro.

Table 6 Formation of 4'-hydroxy ospemifene with CYP Specific Inhibitors

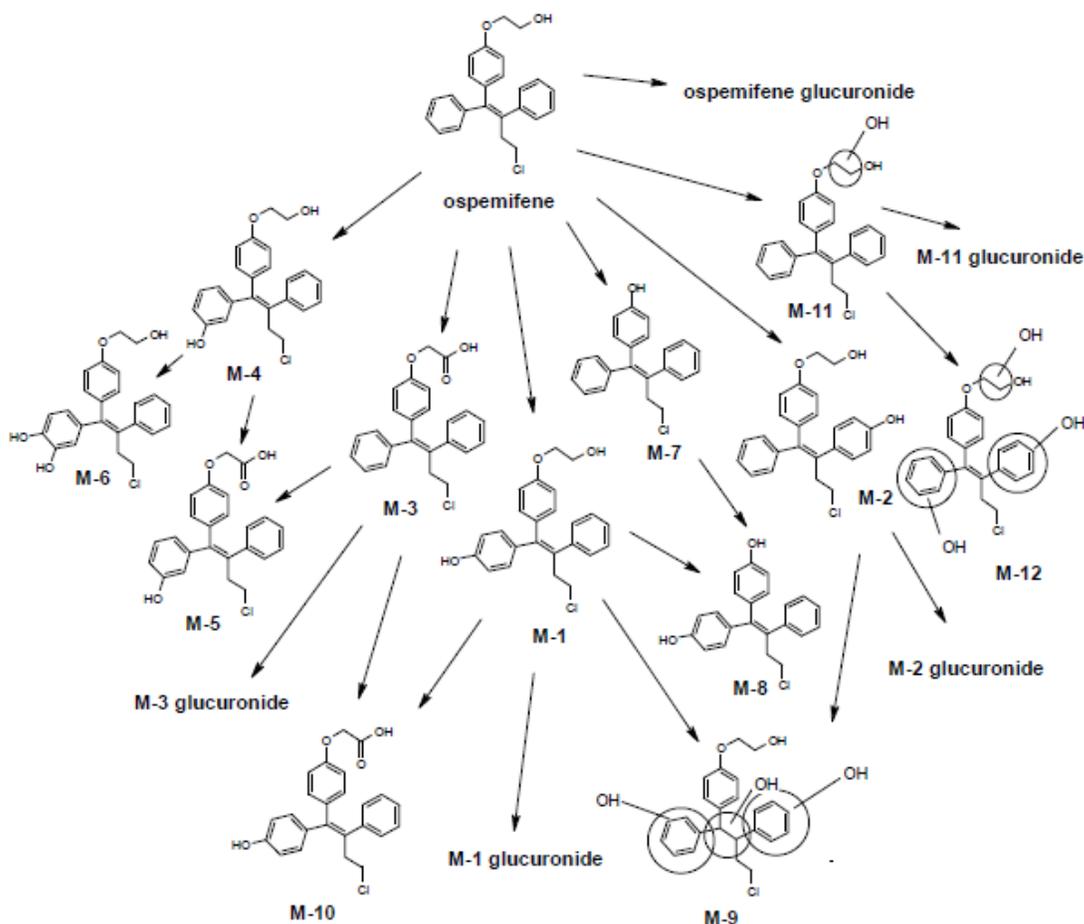
(#15-4330) Further investigation of human serum (n=6 subjects, plus one pooled sample) identified five human metabolites: M1, M3, M4, M5, and M7.

	Pooled	001	003	008	009	010	015
OSP	100%	100%	100%	100%	100%	100%	100%
M1	46 %	95%	103%	30%	80%	80%	48%
M3	11 %	16%	14%	7%	13%	16%	9%
M4	6 %	9%	7%	5%	9%	10%	4%
M5	14 %	22%	9%	7%	18%	23%	31%
M7	4 %	6%	7%	3%	4%	6%	6%

(Excerpted from Applicant's package)

Excretion: The *in vivo* metabolism of ospemifene in the rat and monkey showed extensive metabolism after oral & iv administration with the principal metabolite being 4-hydroxyospemifene which was present in the systemic circulation and the feces.

In the rat, radioactivity was eliminated mainly in the feces in intact animals and in bile duct cannulated animals (74%) after oral administration. Renal elimination was poor (<2%) where most urinary excretion occurred within 24 hrs of dosing (#15-4305). In the monkey, the overall recovery of radioactivity was low with the majority of excreted material found in the feces (oral: 77%; i.v.: 69%). Fecal excretion was rapid where 94% & 77% of radioactive material was eliminated by 48 hrs post dosing when administered via oral & i.v. routes, respectively. Renal elimination was <5% for both routes of administration (#15-4307).

Figure 3 Sponsor's Proposed Metabolic Pathways for Ospemifene

- M-1:** 4-hydroxyospemifene, formed in humans, mouse, rat, hamster, cynomolgus monkey
M-2: 4'-hydroxyospemifene, formed in humans, mouse, rat, hamster, dog, cynomolgus monkey, minipig
M-3: carboxylic acid metabolite, formed in humans, mouse, rat, hamster, cynomolgus monkey, minipig
M-4: 3-hydroxyospemifene, formed in rat, cynomolgus monkey
M-5: 3-hydroxy-side chain carboxylic acid metabolite, formed in humans, mouse (site of hydroxylation unknown), rat, cynomolgus monkey
M-6: 3,4-dihydroxyospemifene, formed in rat
M-7: ospemifene without side chain ethanol, formed in rat, cynomolgus monkey
M-8: 4-hydroxyospemifene without side chain ethanol, formed in humans, cynomolgus monkey
M-9: 4-hydroxy-dihydro-ospemifene, formed in humans
M-10: 4-hydroxy-carboxylic acid metabolite, formed in humans, mouse (site of hydroxylation unknown), rat, cynomolgus monkey
M-11: side chain hydroxylated metabolite, formed in humans, mouse, rat, hamster, dog, cynomolgus monkey, minipig
M-12: dihydroxymetabolite, with one side chain and one aromatic hydroxygroup, formed in humans, mouse, rat, hamster, dog, cynomolgus monkey, minipig

(Excerpted from Applicant's package)

The metabolites 4-hydroxy ospemifene (M1) and 4'-hydroxy ospemifene (designated M2) are the predominant metabolites by exposure in humans and also detected in mouse, rat, and cynomolgus monkey. The pharmacological profile of these metabolites is similar to the parent. Exposure of M1 exceeds that of the parent in rats, dogs and monkeys, but is only 25% of parent exposure in humans. M2 exposure is comparable across these species, ranging from 4-8%.

Table 7 Comparison of M1 and M2 Metabolite Exposure to the Parent Ospemifene

Species	Dose (mg/kg/day)	Ospemifene AUC _{0-24hr} (ng·hr/mL)	M-1 AUC _{0-24hr} (ng·hr/mL)	M-1 (% of osp AUC _{0-24hr})	M-2 AUC _{0-24hr} (ng·hr/mL)	M-2 (% of osp AUC _{0-24hr})
Mouse ^a	1500	25400 (4.7) ^f	18600 (13.7) ^f	73	1090 (2.7) ^f	4
Rat ^b	300	6470 (1.2) ^f	13900 (10.2) ^f	215	379 (0.95) ^f	6
Cynomolgus monkey ^c	100 ^e	29430 (5.4) ^f	29560 (21.8) ^f	100	2395 (6.0) ^f	8
Human ^d	60 mg	5448 (1.0)	1435 (1.0)	25	400 (1.0)	7

(Excerpted from Applicant's package)

Protein binding: In the rat, radiolabeled ospemifene was highly bound to plasma proteins (>93%) both *in vitro* & *ex vivo*. *Ex vivo* binding decreased over time indicative of the transformation of the parent drug into drug related materials (metabolites) which could have a lower binding ability to plasma than the parent drug. Plasma protein binding was not inhibited by warfarin (#15-4305). In the female monkey, plasma protein binding was also high both *in vitro* (>99% over the concentration range of 12-1200 ng/mL) and *ex vivo* (90% at 2hrs & 89% at 72 hrs post dose). After oral administration, binding was lower and declined over time (83% at 2 hrs to 35% at 72 hrs post dose) suggesting the presence of drug-related metabolites (#15-4307). Similar to the rat and monkey, ospemifene was extensively bound to human plasma proteins (>93%) after a single dose *ex vivo* (#15-50206).

Pharmacokinetic drug interactions: Using human liver microsomes *in vitro*, ospemifene inhibited the activity associated with CYP2C9 (IC₅₀=32.2µM) & CYP2C19 (IC₅₀=17 µM). No inhibition of CYP 1A2, 2A6, 2B6, 2D6, 2E1 or 3A4 was noted. While no inhibition was noted in monkeys, a minor induction of CYP1A & CYP2B activities was noted in liver microsomes (#15-4302).

(#15-4313) The potential of ospemifene to induce CYP enzymes in rats and monkeys treated with 3, 30, and 300 mg/kg for 4 weeks and 15, 50, and 150 mg/kg for 39 weeks was evaluated *ex vivo*. In rats, except for CYP2C, CYP2D, and CYP2E, there was a significant effect observed at the high dose of 300 mg/kg. There appeared to be an increase in CYP1A and CYP2A at the high dose. There was no significant dose relationship observed in enzyme activity in monkeys with the few statistically significant changes being modest in magnitude.

Table 8 CYP-associated Activities in vitro in Liver Microsomes from Female Rats

Enzyme	Enzyme activity (pmol/min x mg protein; percentages in parenthesis)				Significance*
	Vehicle group	Low dose group 3 mg/kg	Middle dose group 30 mg/kg	High dose group 300 mg/kg	
ECOD - 2 min	40.8 ± 7.4 (100)	33.0 ± 6.7 (81)	36.9 ± 15.8 (84)	64.0 ± 21.2 (146)	V vs H
ECOD - 15 min	27.6 ± 7.0 (100)	21.8 ± 5.0 (89)	25.2 ± 8.4 (84)	42.4 ± 15.4 (188)	V vs H
EROD - CYP1A	42.5 ± 7.5 (100)	33.9 ± 5.5 (80)	37.2 ± 11.1 (87)	69.3 ± 23.7 (163)	V vs H
PROD - CYP2B	1.56 ± 0.45 (100)	1.21 ± 7.7 (71)	1.88 ± 8.9 (108)	11.0 ± 10.8 (635)	V vs H
TOLB - CYP2C	41.3 ± 11.9 (100)	35.0 ± 5.5 (85)	34.7 ± 5.8 (84)	46.4 ± 21.5 (112)	NS
MEPH - CYP2C	58.7 ± 20.3 (100)	45.2 ± 6.0 (77)	38.7 ± 7.8 (66)	27.6 ± 9.2 (47)	V vs H
DEX - CYP2D	206 ± 49 (100)	131 ± 22 (64)	142 ± 24 (69)	160 ± 29 (78)	V vs L & M
CHLO - CYP2E	253 ± 67 (100)	189 ± 26 (75)	156 ± 25 (62)	252 ± 56 (100)	NS
MID - CYP3A	236 ± 58 (100)	270 ± 69 (115)	215 ± 70 (91)	173 ± 60 (64)	V vs H

*Enzyme activities between treated groups were compared using a two-tailed Student's *t* test. A *p* value of <0.05 was indication of statistically significant difference. ECOD, 7-ethoxycoumarin O-deethylase, presumably an all-round substrate; EROD, ethoxyresorufin O-deethylase, CYP1A1/2; PROD, pentoxyresorufin O-depentylase, CYP2B; TOLB, Tolbutamide (methyl)hydroxylase (CYP2C); MEPH, Mephenytoin 4'-hydroxylase (CYP2C); DEXT, Dextromethorphan O-demethylase (CYP2D); CHLO, Chlorzoxazone 6-hydroxylase (CYP2E1); MID, midazolam hydroxylase (CYP3A).

(Excerpted from Applicant's package)

Table 9 CYP-associated Activities in vitro in Liver Microsomes from Female Cynomolgus Monkeys

Enzyme	Enzyme activity (pmol/min x mg protein; percentages in parenthesis)				Significance*
	Vehicle group	Low dose group 15 mg/kg	Middle dose group 50 mg/kg	High dose group 150 mg/kg	
ECOD - 2 min	291 ± 47 (100)	269 ± 73 (93)	333 ± 65 (109)	301 ± 67 (99)	NS
ECOD - 15 min	223 ± 40 (100)	217 ± 68 (97)	265 ± 49 (118)	246 ± 57 (110)	NS
EROD - CYP1A	212 ± 37 (100)	90.3 ± 45 (43)	157 ± 54 (74)	62 ± 42 (30)	V vs L & H
COH - CYP2A	300 ± 17 (100)	235 ± 43 (78)	184 ± 115 (61)	200 ± 69 (67)	V vs M & H
BUH - CYP2B	676 ± 175 (100)	694 ± 214 (103)	870 ± 176 (129)	723 ± 130 (107)	NS
TOLB - CYP2C	71.6 ± 12.2 (100)	46.6 ± 17.8 (65)	74.9 ± 10.8 (105)	78.1 ± 16.0 (109)	NS
MEPH - CYP2C	75.0 ± 11.9 (100)	65.2 ± 24.5 (87)	62.6 ± 12.3 (83)	71.2 ± 20.4 (95)	NS
DEX - CYP2D	490 ± 39 (100)	384 ± 136 (78)	262 ± 71 (54)	349 ± 70 (71)	V vs M & H
CHLO - CYP2E	465 ± 155 (100)	464 ± 79 (100)	387 ± 118 (83)	356 ± 43 (77)	NS
MID - CYP3A	1645 ± 410 (100)	1679 ± 91 (102)	1736 ± 51 (106)	1561 ± 137 (93)	NS

*Enzyme activities between treated groups were compared using a two-tailed Student's *t* test. A *p* value of <0.05 was indication of statistically significant difference. ECOD, 7-ethoxycoumarin O-deethylase, presumably an all-round substrate; EROD, ethoxyresorufin O-deethylase, CYP1A1/2; COH, coumarin 7-hydroxylase, CYP2A; BUH, Bupropion hydroxylase, presumably CYP2B; TOLB, Tolbutamide (methyl)hydroxylase (CYP2C); MEPH, Mephenytoin 4'-hydroxylase (CYP2C); DEXT, Dextromethorphan O-demethylase (CYP2D); CHLO, Chlorzoxazone 6-hydroxylase (CYP2E1); MID, midazolam hydroxylase (CYP3A).

(Excerpted from Applicant's package)

(#15-4318) Using human liver microsomes, ospemifene most potently inhibits the in vitro activity of CYP2B6 with an IC₅₀ value of 7.8 μM. CYP2C9, CYP2C19, CYP2C8 and CYP2D6 were less inhibited in order of decreasing potency with IC₅₀ values ranging from 10.0 to 48.7 μM. Only one CYP3A4 substrate was inhibited by ospemifene.

Table 10 Inhibition of CYP Enzymes by Ospemifene

	IC ₅₀ (μ M) MsSir	IC ₅₀ (μ M) Single
Melatonin 6-hydroxylase (CYP1A2)		
Ospemifene	> 100	n.d.
Fluvoxamine	0.08	0.07
Coumarin 7-hydroxylase (CYP2A6)		
Ospemifene	> 100	> 100
Tranlycypromine	1.0	0.38
Bupropion hydroxylase (CYP2B6)		
Ospemifene	7.8	> 100 ¹
Ticlopidine	0.1	0.3
Amodiaquine de-ethylase (CYP2C8)		
Ospemifene	36.4	n.d.
Quercetin	57.8	100
Tolbutamide (methyl)hydroxylase (CYP2C9)		
Ospemifene	10	32
Sulphaphenazole	0.2	0.35
Omeprazole 5-hydroxylase (CYP2C19)		
Ospemifene	22.5	17 ²
Fluconazole	6.4	n.d.
Omeprazole demethylase (CYP2C19)		
Ospemifene	47.2	17 ²
Fluconazole	5.7	n.d.
Dextromethorphan O-demethylase (CYP2D6)		
Ospemifene	48.7	> 100
Quimidine	0.035	0.08
Chlorzoxazone 6-hydroxylase (CYP2E1)		
Ospemifene	> 100	> 100
Pyridine	36.6	2.75
Midazolam 1'-hydroxylase (CYP3A4)		
Ospemifene	> 100	n.d.
Ketoconazole	1.8	0.065
Testosterone 6β-hydroxylase (CYP3A4)		
Ospemifene	> 100	> 100
Ketoconazole	0.07	0.32
Omeprazole sulphoxidase (CYP3A4)		
Ospemifene	> 100	n.d.
Ketoconazole	0.13	n.d.
Omeprazole 3-hydroxylase (CYP3A4)		
Ospemifene	37.9	n.d.
Ketoconazole	0.08	n.d.

¹substrate and reaction 7-ethoxy-4-(trifluoromethyl)coumarin O-deethylase²substrate and reaction mephenytoin 4'-hydroxylase*(Excerpted from Applicant's package)*

(#15-4324) Using human liver microsomes, the primary metabolite 4-hydroxy ospemifene (M1) most potently inhibits the in vitro activity of CYP2C9 with an IC₅₀ value of 1.1 μ M. CYP2C19, CYP2D6, CYP2B6, and CYP2C8 were less inhibited in order of decreasing potency with IC₅₀ values ranging from 15.3 to 27.7 μ M. CYP3A4 inhibition was \geq 35.9 μ M, depending on substrate.

Table 11 Inhibition of CYP Enzymes by 4-hydroxy ospemifene

	IC ₅₀ (μ M)	Calculate IC ₅₀ (μ M)
Melatonin 6-hydroxylase (CYP1A2)		
4-hydroxyospemifene	> 100	NA
Fluvoxamine	0.08	0.06
Coumarin 7-hydroxylase (CYP2A6)		
4-hydroxyospemifene	> 100	NA
Tranlycypromine	1.0	0.5
Bupropion hydroxylase (CYP2B6)		
4-hydroxyospemifene	26.5	26.2
Ticlopidine	0.1	0.3
Amodiaquine de-ethylase (CYP2C8)		
4-hydroxyospemifene	27.7	9.2
Quercetin	57.8	19.3
Tolbutamide (methyl)hydroxylase (CYP2C9)		
4-hydroxyospemifene	1.1	1.1
Sulphaphenazole	0.2	0.2
Omeprazole 5-hydroxylase (CYP2C19)		
4-hydroxyospemifene	15.3	13.9
Fluconazole	6.4	5.8
Omeprazole demethylase (CYP2C19)		
4-hydroxyospemifene	19.7	n.d.
Fluconazole	5.7	n.d.
Dextromethorphan O-demethylase (CYP2D6)		
4-hydroxyospemifene	25.5	24.5
Quinidine	0.035	0.08
Chlorzoxazone 6-hydroxylase (CYP2E1)		
4-hydroxyospemifene	> 100	NA
Pyridine	36.6	2.75
Midazolam 1'-hydroxylase (CYP3A4)		
4-hydroxyospemifene	> 100	NA
Ketoconazole	1.8	1.7
Testosterone 6β-hydroxylase (CYP3A4)		
4-hydroxyospemifene	> 100	NA
Ketoconazole	0.07	0.07
Omeprazole sulphoxidase (CYP3A4)		
4-hydroxyospemifene	35.9	35.2
Ketoconazole	0.13	0.12
Omeprazole 3-hydroxylase (CYP3A4)		
4-hydroxyospemifene	50.0	n.d.
Ketoconazole	0.08	n.d.

(Excerpted from Applicant's package)

(#15-4326). Ospemifene did not inhibit the metabolism of the aromatase (CYP17) inhibitor exemestane in both human liver microsomes and human hepatocytes; there was slight inhibition (>50%) by 4-hydroxy ospemifene.

(#15-4332) The M2 metabolite, 4'-hydroxy ospemifene, weakly inhibited CYP enzymes in vitro, with the most potent inhibition occurring for CYP2C8 at an IC₅₀ value= 7 μ M.

Table 12 Inhibitory potency of 4'-hydroxy ospemifene (HM-136) towards CYP activities in the incubations with a pool of human liver microsomes

Enzyme	Substrate	Reaction	IC ₅₀ ¹ (μM)	K _i ² (μM)	[I]/K _i ³	FDA Class ⁴
CYP1A2	Melatonin	6-hydroxylation	>100	>100	<0.01	Not likely
CYP2A6	Coumarin	7-hydroxylation	>100	>100	<0.01	Not likely
CYP2B6	Bupropion	Hydroxylation	15	15	<0.01	Not likely
CYP2C8	Amodiaquine	Desethylation	7	2	0.05	Not likely
CYP2C9	Tolbutamide	Methylhydroxylation	12	11	<0.01	Not likely
CYP2C19	Omeprazole	Demethylation	50	33	<0.01	Not likely
		5-hydroxylation	50	33	<0.01	Not likely
CYP2D6	Dextrometorphan	O-demethylation	86	71	<0.01	Not likely
CYP2E1	Chlorzoxazone	6-hydroxylation	>100	>100	<0.01	Not likely
CYP3A4	Midazolam	1-hydroxylation	78	63	<0.01	Not likely
		Testosterone	6β-hydroxylation	>100	>100	<0.01
	Omeprazole	Sulfoxidation	85	57	<0.01	Not likely
		3-hydroxylation	54	36	<0.01	Not likely

¹ Calculated with equation 1

² Calculated based on equation 2

³ Calculation based on the observed peak concentration in humans 40 ng/mL, at steady state following administration of ospemifene at the highest proposed clinical dose, and the molecular weight 395 g/mol.

⁴ Based on FDA classification of drug-drug interactions likely ($I/K_i > 1$), possible ($0.1 < I/K_i < 1$) or not likely ($I/K_i < 0.1$) (FDA 2006)

(Excepted from Applicant's package)

(#15-4320) Using human liver microsomes and an "N-in-1" cocktail (all substrates in one tube) ospemifene weakly inhibited in vitro activities of CYP2B6, CYP2C9, CYP2C19, CYP2C8, CYP3A4, and CYP2D6 (in order of decreasing potency) with IC₅₀ values of 7.8 μM - 48.7 μM. The K_i values were fairly consistent with and generally higher than the IC₅₀ values.

	K_i (μM)	IC₅₀ (μM)
	Current study	MsSir
Bupropion hydroxylase (CYP2B6)		
Ospemifene	15 (comp)	7.8
Ticlopidine	nm	0.1
Amodiaquine de-ethylase (CYP2C8)		
Ospemifene	~40 (comp)	36.4
Quercetin	nm	57.8
Tolbutamide (methyl)hydroxylase (CYP2C9)		
Ospemifene	17 (comp at low)	10
Sulphaphenazole	nm	0.2
Omeprazole 5-hydroxylase (CYP2C19)		
Ospemifene	~35 (non-comp)	22.5
Fluconazole	nm	6.4
Dextromethorphan O-demethylase (CYP2D6)		
Ospemifene	78 (comp)	48.7
Quinidine	nm	0.035
Omeprazole 3-hydroxylase (CYP3A4)		
Ospemifene	>100	37.9
Ketoconazole	nm	0.08

¹ substrate and reaction 7-ethoxy-4-(trifluoromethyl)coumarin O-deethylase

² substrate and reaction mephenytoin 4'-hydroxylase

(Excerpted from Applicant's package)

(#15-4321) Using human liver microsomes, and an N-in-1 cocktail, 4-hydroxy ospemifene inhibited in vitro CYP2C9, CYP2C19, CYP2D6, CYP2B6, CYP2C8, and CYP3A4 activities (in order of decreasing potency) with IC₅₀ values of 1.1 μM - 35.9 μM. Only two of 4 CYP3A4 assays using different substrates produced an IC₅₀ value. CYP1A2, CYP2A6, and CYP2E1 were not inhibited by the metabolite.

	IC ₅₀ (µM)	Calculated IC ₅₀ (µM)
Melatonin 6-hydroxylase (CYP1A2)		
4-hydroxyospemifene	> 100	NA
Fluvoxamine	0.08	0.06
Coumarin 7-hydroxylase (CYP2A6)		
4-hydroxyospemifene	> 100	NA
Tranlycypromine	1.0	0.5
Bupropion hydroxylase (CYP2B6)		
4-hydroxyospemifene	26.5	26.2
Ticlopidine	0.1	0.3
Amodiaquine de-ethylase (CYP2C8)		
4-hydroxyospemifene	27.7	9.2
Quercetin	57.8	19.3
Tolbutamide (methyl)hydroxylase (CYP2C9)		
4-hydroxyospemifene	1.1	1.1
Sulphaphenazole	0.2	0.2
Omeprazole 5-hydroxylase (CYP2C19)		
4-hydroxyospemifene	15.3	13.9
Fluconazole	6.4	5.8
Omeprazole demethylase (CYP2C19)		
4-hydroxyospemifene	19.7	n.d.
Fluconazole	5.7	n.d.
Dextromethorphan O-demethylase (CYP2D6)		
4-hydroxyospemifene	25.5	24.5
Quimidine	0.035	0.08
Chlorzoxazone 6-hydroxylase (CYP2E1)		
4-hydroxyospemifene	> 100	NA
Pyridine	36.6	2.75
Midazolam 1'-hydroxylase (CYP3A4)		
4-hydroxyospemifene	> 100	NA
Ketoconazole	1.8	1.7
Testosterone 6β-hydroxylase (CYP3A4)		
4-hydroxyospemifene	> 100	NA
Ketoconazole	0.07	0.07
Omeprazole sulphoxidase (CYP3A4)		
4-hydroxyospemifene	35.9	35.2
Ketoconazole	0.13	0.12
Omeprazole 3-hydroxylase (CYP3A4)		
4-hydroxyospemifene	50.0	n.d.
Ketoconazole	0.08	n.d.

(Excepted from Applicant's package)

(#15-4325) In isolated human hepatocytes (n=4, not pooled), ospemifene at 20 µM induced CYP1A2, CYP2B6 and CYP3A4 activities by 52.4-fold (1/4 donors), ~2.0-fold (2/4 donors) and 2.4-fold (1/3 donors), respectively. No induction by ospemifene was noted for CYP2C9 and CYP2C19.

Study title: Bi-directional CACO-2 Permeability

Study no.: 15-4316 (03-HORM.P01)
 Study report location: eCTD 4.2.2.7.1
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: June 3, 2003
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: Ospemifene, Not specified

Key Study Findings

- Ospemifene is highly permeable into Caco-2 cells, and had no measurable efflux in this system.

Methods

Cell line: Caco-2
 Concentrations in definitive study: 10 µM
 Negative control: DMSO
 Positive control: 100 µM digoxin
 Formulation/Vehicle: DMSO

Results: There was no difference in A to B or B to A directional movement of Ospemifene, suggesting high permeability and no active efflux mechanism (n=2).

Test Article	Percent Recovery ^(C)			Blank Papp ^(D)	Papp, A-to-B			Papp, B-to-A			Papp ^{B-A} / Papp ^{A-B} Ratio ^(E)	Absorption Potential ^(A)	Significant Efflux ^(B)
	Blank	A-to-B	B-to-A		Rep. 1	Rep. 2	Avg	Rep. 1	Rep. 2	Avg			
Ospemifene	85	61	68	6.66	3.25	5.51	4.38	3.50	3.42	3.46	0.8	High	No

(Excerpted from Applicant's Package)

Study title: P-GP substrate assessment in MDR-MDCK cell monolayers

Study no.: 15-4317 (03-HORM.P02)
 Study report location: eCTD 4.2.2.7.1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: September 8, 2003
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: Ospemifene, not provided

Key Study Findings

- Ospemifene was freely permeable, and has no measurable efflux in this system (n=2).

Methods

Cell line: MDR-MDCK
 Concentrations in definitive study: 10 µM ± 10 µM cyclosporine A
 Negative control: DMSO
 Positive control: 100 µM digoxin
 Formulation/Vehicle: DMSO ± 10 µM cyclosporine A

Results: Neither A to B or B to A directional transport were inhibited by the P-GP inhibitor cyclosporine A, while the P-GP dependent digoxin was.

Test Article & Condition	Recovery (%)		Papp, A-to-B				Papp, B-to-A				Papp ^{B-A} Papp ^{A-B} Ratio
	A-to-B	B-to-A	Rep. 1	Rep. 2	Rep. 3	AVG.	Rep. 1	Rep. 2	Rep. 3	AVG.	
Ospemifene	75	61	5.75	5.46	3.76	4.99	4.58	3.61	3.85	4.02	0.8
Ospemifene + CSA	81	91	2.96	4.06	3.34	3.45	3.59	3.42	3.79	3.60	1.0
Digoxin	95	100	0.12	0.29	0.30	0.23	9.39	8.85	9.76	9.33	39.8
Digoxin + CSA	102	110	0.09	0.37	0.40	0.29	0.73	1.00	0.51	0.74	2.6

5.2 Toxicokinetics

Table 4.6 Exposure to ospemifene and 4-hydroxyospemifene during repeated dose 4-week rat and 39-week monkey and single dose minipig studies. Steady state mean serum C_{max} and AUC_{0-24h} values are presented. Comparison with human data is included. The oral vehicle in the animal studies was 0.5% CMC.

Species (Ref.) Dose (mg/kg/day)	C_{max}		ΔAUC_{0-24h}		Total ΔAUC_{0-24h} Osp + 4-OH-Osp
	Osp	4-OH-Osp	Osp	4-OH-Osp	
Rat (6)					
3	16	56	62	403	465
30	86	324	712	3441	4153
300	274	772	3142	10196	13338
1000 ^a	360	918	4209	12170	16379
Monkey (16)					
15	95	138	604	714	1318
50	220	213	1427	1559	2986
150	342	893	3512	6990	10502
1000 ^b (12)	420	241	4234	3148	7382
Minipig ^c (2)					
15	112	nd	696	nd	696
50	185	nd	2138	nd	2138
150	277	nd	3163	nd	3163
500	487	nd	6323	nd	6323
Human (27,28)					
60 mg/day ^c	612	139	4452	2870	7322
50 mg/day ^d	796	-	6488	-	-
100 mg/day ^d	708	-	7488	-	-

nd = not detected; a = Cremophor EL vehicle; b = dosing daily for 9 days; c = single dose study; d = 12-week study (steady state); - = no data available

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Table 2. The exposure to ospemifene, M-1, and M-2 at steady state in different species at the highest dose level used in toxicity studies

Species	Dose (mg/kg/day)	Ospemifene AUC _{0-24hr} (ng·hr/mL)	M-1 AUC _{0-24hr} (ng·hr/mL)	M-1 (% of osp AUC _{0-24hr})	M-2 AUC _{0-24hr} (ng·hr/mL)	M-2 (% of osp AUC _{0-24hr})
Mouse ^a	1500	25400 (4.7) ^f	18600 (13.7) ^f	73	1090 (2.7) ^f	4
Rat ^b	300	6470 (1.2) ^f	13900 (10.2) ^f	215	379 (0.95) ^f	6
Cynomolgus monkey ^c	100 ^e	29430 (5.4) ^f	29560 (21.8) ^f	100	2395 (6.0) ^f	8
Human ^d	60 mg	5448 (1.0)	1435 (1.0)	25	400 (1.0)	7

Comparison to the exposure observed in humans after repeated administration of 60 mg ospemifene daily has been shown in parenthesis below the AUC value.

^a Study 15-44404, female mice, week 52.

^b Study 15-44405, female rats, week 52.

^c Study 15-44214 and 15-44214C.

^d Study 15-50927.

^e Lowest dose level used for calculations as exposure decreased with increasing dose.

^f Calculated as AUC_{0-24hr} in the animal species / AUC_{0-24hr} observed in humans.

6 General Toxicology

In the rat, decreases in body weight and food consumption were noted. Other findings were limited to the exaggerated pharmacological effect of ospemifene on reproductive organs (ovarian cysts, thin uterus with endometrial stromal atrophy and epithelial hypertrophy/hyperplasia, vaginal mucification, mammary ductal hypertrophy) and pituitary vacuolation and were in line with known SERM effects. NOEL for the rat was 300 mg/kg/day. In the dog, no effect on BW, food consumption, ECG, blood pressure. Findings were limited to exaggerated pharmacological effects of ospemifene on reproductive organs (testis, epididymis & prostate atrophy, dilated endometrial glands). NOEL was 8 mg/kg/day. No target organs were identified in the dog. However, due to the potential effect of SERMs on the dog model (e.g., uterine pathology), sponsor has replaced the dog model with the monkey. In the monkey, an increase in liver and ovarian weight and presence of ovarian cysts were noted. Histopathological changes in the ovaries, uterus, vagina and mammary glands were also noted in the monkey, all of which are considered exaggerated pharmacological effects. NOEL was 150 mg/kg/day.

6.1 Single-Dose Toxicity

Compiled by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Study	Dose	Findings
Single dose; SD Rat (n=5/sex) (Study # 15-44101)	Oral gavage: 2000 mg/kg	1 male had a mild dilation in renal pelvis (slight) with no histopathological findings. NOEL >2000 mg/kg.
Single ascending dose, Cynomolgus monkeys (n=2 females) (Study # 15-44210)	Oral gavage: single ascending dose of 150, 300, 600 & 1000 mg/kg/day on days 1, 3, 5 & 8-16, respectively	Both females: Decreased erythrocyte, hemoglobin & hematocrit and increased reticulocyte counts. Increased ALT/GPT serum levels. No histopathological findings. AUC: Drug exposure increased less than dose-proportionally with increasing dose.

Single dose; Gottingen minipigs (n=2 females /group) (RR250-01863): A PK study	Oral gavage: 15, 50, 150 or 500 mg/kg in 0.5% carboxymethyl-cellulose (CMC)	Tmax=2-4 hrs; Cmax & AUC increased with dose but were less than dose proportional. No evidence of 4-hydroxyospemifene was found in the minipig sera.
Dose range-finding, female dogs (n=2/group)	Group 1: increasing doses of 16, 50, 150, 500 or 1000 mg/kg (1/day for 3 days) Group 2: 1000 mg/day for 7 days	≥500 mg/kg: Initial transient reduction in food consumption/body weight gain. Both 500 & 1000 mg/kg doses showed a saturation of bioavailability and similar plasma exposure. Highest dose chosen was 500 mg/kg

6.2 Repeat-Dose Toxicity

Compiled by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Study	Dose	Findings
4-week; SD female rats (n=5 /group & n=10/group except control for TK analysis) (Hormos Study # 1504004)	Oral gavage: 0, 3, 40, 80, 160 or 320 mg/kg in 0.5% CMC	All doses: Decreased food consumption & correlated decreased body weight (BW) gain was seen especially on week 1. A general dose-related trend towards a decrease in ovarian, uterine & adrenal weight was seen. Vaginal mucification of the epithelium & metestrus/diestrus epithelium was seen in most treated females. An increased number of luteal cysts & some cases of uterine atrophy & hydrometra. 3, 80 & 320 mg/kg: A significant reduction in ovarian weights. 40, 80 & 60 mg/kg: Ovarian cysts were noted with a correlated decrease in the number of corpora lutea & secondary follicles. TK: All animals were exposed to drug. Exposure increased with dose but plateaued at 160 mg/kg dose. NOAEL was not established.
4-week, SD female rats (n= 9/group) (Hormos Study # 844408)	Oral gavage: 0, 3, 30, 300 or 1000 mg/kg in 0.5% CMC or Cremophor EL	All doses: A trend towards a decrease in BW was seen from day 8 onwards. Ovarian findings: ↓ ovarian weight at 3 & 30 mg/kg but ↑ weights at 300 & 1000 mg/kg possibly due to presence of cysts (follicular & partially filled with blood). Uterine findings: ↓ uterine weight at 300 & 1000 mg/kg in cremophor vehicle & in 3, 30 & 300 mg/kg in 0.5% CMC along with squamous cell metaplasia and in HD endometrial hyperplasia (minimal). 300 mg/kg: ↓ hematopoiesis & glycogen depletion (both vehicles). 1000 mg/kg: ↑ liver weight, ↓ hematopoiesis (cremophor EL vehicle). TK: All animals were exposed to drug & its 4-hydroxymetabolite. Exposure increased with dose but plateaued at 300 mg/kg dose. NOAEL was not established.
13-week, beagle dogs (n=4/sex/group) (study # 15-44203)	Oral, capsule: 0, 0.5, 2 or 8 mg/kg/day	2 mg/kg/day: decrease in epididymidi weight 8 mg/kg/day: slight increases in thrombin, mean lymphocyte counts, cholesterol, creatinine kinase & alkaline phosphatase. ≥ 2 mg/kg/day: Decreases in testes, epididymidi, and uteri weights correlating with histopathology findings (absence of mature spermatids & reduced diameter of seminiferous tubules in males & adenomyosis and dilated endometrial glands <u>in all treated females</u>). Other findings included acinar atrophy of the prostate. NOAEL = 0.5 mg/kg/day
4-week, Cynomolgus monkeys (n=4 females)/ (study # 15-44205)	Oral gavage: 0, 8, 25 & 75 mg/kg/day	All doses: Slight increase in ALT levels correlating with increased liver weights. A decrease in corpora lutea numbers and follicular cysts were seen in all treated groups. Increased ovarian weight and presence of ovarian cysts in 1 MD & 1 HD female. 75 mg/kg/day: Increased uterine weight. NOAEL =75 mg/kg/day

Reviewed by Dr. Alex Jordan (DARRTS, 5/7/2007):

Study title: 4 week oral (gavage) administration toxicity study in the cynomolgus monkey

Key study findings: NoAEL of 75 mg/kg

Study no.: (b) (4) no. 1716-008

Volume #, and page #: vol. 1

Conducting laboratory and location: (b) (4)

Date of study initiation: Oct. 1999

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: FC-1271a (ospemifene), batch 99E27, purity not stated

Methods

Doses: 8, 25, 75 mg/kg

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 4 females/gp

Route, formulation, volume, and infusion rate: oral gavage, 0.5% CMC, 4 ml/kg

Satellite groups used for toxicokinetics or recovery: none

Age: 3-4 yrs

Weight: 2.5-3.5 kg

Results:

Mortality: none

Clinical signs: none

Body weights: no effects

Food consumption: no effects

Ophthalmoscopy: no effects

EKG: no evidence of cardiotoxicity, no effects on BP

Hematology: no treatment related changes

Clinical Chemistry: Increase in ALT. 30.1, 31.5, 39.5, 34.4 U/L in control, LD, MD, HD. No liver histopath.

Urinalysis: no effects

Gross Pathology: Ovarian cyst in one MD and one HD female.

Organ Weight:

Ovary wts slightly to moderately increased in all treated gps. Uterine wts increased in HD. Liver wts increased dose-related in gps 2 to 4. None of the organ wt changes were statistically significant.

Histopathology: Adequate Battery: yes

Peer review: yes (), no ()

Decrease in number of corpora lutea in treated gps compared to control. Ovarian cysts in 2 LD, 1 MD and 3 HD females.

Reviewed by Dr. Alex Jordan (DARRTS, 5/7/2007):

Study title: A 4 week oral dose toxicity study of ospemifene in cynomolgus monkeys with a 4 week recovery

Key study findings: NoAEL of 1250 mg/kg

Study no.: SR05308

Volume #, and page #: Vol 1

Conducting laboratory and location: (b) (4)

Date of study initiation: 12/2005

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Ospemifene, batch 01E72, 99.9%

Methods

Doses: 100, 500, 1250 mg/kg

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 4 females/gp ; 2 extra control and HD for recovery

Route, formulation, volume, and infusion rate: oral gavage, corn oil, 5 ml/kg

Satellite groups used for toxicokinetics or recovery: none

Age: 2 yrs 9 mo. – 3 yrs 7 mo

Weight: 1.85-2.8 kg

Results:

Mortality: none

Clinical signs:

Signs of diarrhea (loose, watery or muddy stool) in monkeys given ospemifene in corn oil

dose (mg/kg)	week 1	week 2	week 3	week 4
control	4.67	3.00	0.83	0.75
100	5.00	5.25	2.75	1.25
500	5.75	5.50	6.00	2.75
1250	9.00	9.67	7.17	5.00

Body weights: no drug related effects

Food consumption: no effects

Ophthalmoscopy: no effects

EKG: no effects on heart rate, PR, QRS and QT intervals and QTc.

Clinical Chemistry: Increased ALT in 2 MD and 3 HD females. Normal at recovery and no liver histopath.

Hematology: no treatment related changes

Urinalysis: no effects

Gross Pathology: no drug related lesions

Organ Weight:

Liver wts significantly increased dose-related in gps 2 to 4.

Histopathology: Adequate Battery: yes

Peer review: yes (), no ()

Slight increase in ovarian cysts in all treated gps.

species/dose mg/kg	duration	vehicle	AUC _{0-24h} (ug.h/ml)		AUC _{0-24h} (ug.h/ml)
			Osp	OHosp	Osp + OHosp
monkey 8	4 weeks	CMC			
8			0.89	-	-
25			2.52	-	-
75			2.80	-	-
monkey	39 weeks	CMC			
15			0.60	0.71	1.32
50			1.43	1.56	2.99
150			3.51	6.99	10.5
monkey	4 weeks	corn oil			
100			29.4	29.6	59.0
500			20.3	19.8	40.1
1250			12.0	10.4	22.4
human (mg/day)	12 weeks	capsule			
25			1.94	-	-
50			6.44	-	-
100			7.34	-	-
200			11.6	-	-
60 ^a			4.45	2.87	7.32

^a single dose study
 - no data available

Reviewed by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Study Title: “FC-1271a: Toxicity study by oral (gavage) administration to Sprague Dawley rats for 13 weeks’

Key study findings:

- A decrease in body weight was seen in all treated females and in males treated at >8 mg/kg/day and correlated with decreased food consumption.
- A decrease in organ weight relative to body weight was seen in prostate and seminal vesicles (males) and ovary, uterus & pituitary (females) compared to vehicle controls.
- Histopathology results included atrophy in prostate at ≥MD (n=1 MD and n=6, all minimal to slight) along with atrophy in seminal vesicles at HD (n=2, minimal) with reduced epithelial thickness, reduced secretion and increased amount of interstitial tissue. HD-treated animals showed slight chronic vasculitis surrounding the small interstitial blood vessels. Mild vasculitis was also seen around the epididymis. In females, a reduced number of luteal and endometrial glands & increased number of cystic follicles was seen in all treated groups. Females treated with ≥MD showed focal vacuolization (minimal) in oviductal epithelial cells and a a dose-related myometrial atrophy.
- Based on the exaggerated pharmacological nature of the findings, the NOAEL for this study was 32 mg/kg/day.

Study no: (b) (4) study # 15-44202/ (b) (4) . # TOX 95002
Volume # and page #: Vol. 9, 004909

Conducting laboratory and location:

(b) (4)

(b) (4)

Date of study initiation: February 02, 1995 (Start of dosing)**GLP compliance:** Yes**QA Report:** Yes**Drug, lot # and % purity:** Ospemifene or FC-1271a, batches A (d3rr1 ½) and B (d3m1v14 or 10046V), 99.45%.**Methods****Doses:** Animals were administered an oral gavage dose of 0, 0.5, 2.0, 8.0 or 32 mg/kg/day in 0.5% CMC for 13 weeks. Animals were housed in groups of 5/cage.**Species/Strain:** SD rats (b) (4) were acclimatized for ~ 11 days prior to the start of the study.**Number/sex/group or time point (main study):** 10/sex/group.**Route, formulation, volume and infusion rate:** Oral gavage, compound dissolved in 0.5% CMC, volume 5 ml/kg. Doses were adjusted according to the most recent body weight.**Satellite groups used for toxicokinetics or recovery:** None**Age:** Animals were 41 days old at the start of treatment.**Weight:** Males weighed 90-149g and females 90-136g at the start of dosing.**Sampling times:** See individual endpoints.**OBSERVATIONS AND TIMES****Clinical signs:** Observations were made once daily starting from their arrival day and throughout the study period (prior to dosing, immediately after at the end of the day).**Body weights:** Animals were weighed pretest, prior to day 1 and once weekly thereafter.**Food consumption:** Food consumption/cage was recorded weekly up to the last treatment day before necropsy.**Ophthalmoscopy:** Ophthalmologic examinations were made pretest (Week -2) and during week 13 using an indirect and a direct ophthalmoscope.**EKG:** Not performed.**Hematology:** Blood samples were obtained after an overnight fast at necropsy.**Clinical Chemistry:** Same as in "Hematology" above.**Urinalysis:** Samples were collected overnight from 5 rats/sex/group predose and on weeks 6 & 12 using metabolic cages.**Toxicokinetics:** Not performed**Gross Pathology:** All animals were necropsied at the end of the study following an overnight fast. Necropsies were performed on all moribund and dead animals.**Organ Weight:** Adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, seminal vesicles, testes and thymus were weighed after an overnight fast at scheduled necropsy.**Histopathology:** Tissues listed (page 31; adequate battery) were stored for all animals.

The following tissues were examined from all control and high dose groups:

Submandibular salivary glands, brain, thyroid & parathyroids, trachea, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, urinary bladder, aorta, sciatic nerve, tongue, skeletal muscle, eyes, optic nerves & mammary gland with skin. In addition the following tissues were examined from all animals: All

gross lesions, pituitary, heart, thymus, lungs, adrenals, liver, kidneys, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, vagina, spleen, submandibular & mesenteric lymph nodes and sternum. Bone marrow smears were taken from all animals at necropsy and examined from control & HD-treated animals.

RESULTS

Mortality: A male treated at LD (0.5 mg/kg) showed signs of lethargy and circling behavior on week 4 and was terminated. Upon necropsy, animal showed complete lymphoid depletion in the spleen & cortical region of the submandibular and mesenteric lymph nodes. Sponsor explained the death as possibly linked to inheritable immunosuppressive condition. No other treatment-related deaths were noted.

Clinical signs: None.

Body weights: A decrease in body weight was seen in all treated females and in males treated at >8 mg/kg/day and correlated with decreased food consumption.

Food consumption: A decrease in food consumption was seen in both sexes treated at >8 mg/kg/day correlating with the decrease in body weights.

Ophthalmology: No treatment related findings were noted.

Hematology: Decreases in white blood cells (WBC) and platelets and increases in monocytes and SPA were seen in HD-treated compared to controls.

Week 13	control		0.5 mg/kg/day		2.0 mg/kg/day		8.0 mg/kg/day		32.0 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
Albumin	38.2±2	37.2±1	37.2±1.5	35.6±1.7	37.8±1.3	35±1.7	37.3±1.5	35±1.4	37.4±1**	35.4±0.9**
Cholesterol	2.8±0.3	3.5±0.3	2.4±0.3	2.7±0.4	2±0.4	2.3±0.3	1.8±0.3	1.7±0.4	1.3±0.3**	1.5±0.2**
AFOS	287.5±57	226±38	298±25	220±59	286±34	245±49	234±27	297±54	269±44	314±80**
ALT	60.5±21	58±9.6	63±11	63.4±18	60.4±9.5	68.3±10	75±14	61±11	73±20**	73.5±15**

** , Statistical analysis was done on combined sexes (p<0.001) for albumin, cholesterol & ALT compared to controls.

AFOS was significantly increased in females treated at ≥8 mg/kg/day compared to controls.

Bone marrow: No treatment-related changes were noted.

Clinical chemistry: Decreases in albumin, cholesterol and increases in ALT were noted in animals treated with 32 mg/kg/day compared to vehicle controls. AFOS was increased in females treated at >8 mg/kg/day compared to vehicle controls.

Week 13	control		0.5 mkd		2.0 mkd		8.0 mkd		32.0 mkd	
	M	F	M	F	M	F	M	F	M	F
WBC (10 ³ /μl)	7.2±2.7	5.8±2	7.9±2.75	5.3±0.9	8.2±2	5±1.6	5.3±2.6	3.8±0.9	6.5±2**	4.9±1.5**
Platelets	977±109	975±127	106±113	863±95	973±125	846±80	877±102	855±66	907±69**	823±79**
Monocytes	1.9±1	1.7±1.8	2.3±1.4	2.3±1	3±1.8	3±1.4	2.6±2	3.2±2.3	3.5±2**	4.5±2**
SPA	28.4±2.3	27.2±1.3	29.5±2	29±1.8	31±2	30±2	31.4±1	31±1	32.2±3**	29.7±2**

** , Statistical analysis was done on combined sexes (p<0.001) compared to controls

Urinalysis: No treatment-related changes were noted.

Organ weight: An increase in organ weight relative to body weight was seen in the kidney (females only) compared to vehicle controls (significant at >2 mg/kg/day). In addition, decreased absolute weights of the adrenals, heart, spleen, pituitary, prostate, seminal vesicles, uterus & ovary were seen compared to vehicle controls. When compared relative to body weight, decreased weight in prostate and seminal vesicles (males) and ovary, uterus & pituitary (females) were found to be statistically significant compared to vehicle controls.

Absolute weight	Control		0.5 mg/kg/day		2.0 mg/kg/day		8.0 mg/kg/day		32.0 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
Adrenal (mg)	60±1	60±4	60±5	60±0.1	50±6	50±4**	50±6	50±9**	50±5	50±10**
Kidney	2±0.2	1.3±0.08	2±0.1	1.2±0.13	2.1±0.3	1.1±0.06***	1.7±0.1***	1.1±0.2***	1.7±0.3***	1.2±0.1
Heart	1.2±0.1	0.8±0.06	1.2±0.1	0.76±0.06	1.2±0.1	0.8±0.3	1±0.07***	0.7±0.07***	1±0.1***	0.7±0.08***
Spleen	0.7±0.08	0.6±0.07	0.7±0.05	0.5±0.04	0.7±0.08	0.4±0.05***	0.6±0.07	0.4±0.05***	0.6±0.1	0.45±0.05***
Pituitary (mg)	10±1.3	12±10	10±12	9.0±0.9** *	10.9±11	7.0±0.7***	8.7±0.8***	7.0±0.9***	8.4±1.8***	7.6±1.4***
Prostate	0.68±0.19	-	0.66±0.14	-	0.63±0.19	-	0.63±0.19* *	-	0.32±0.12**	-
Seminal vesicles	1.44±0.2	-	1.44±0.2	-	1.32±0.3**	-	1.05±0.26* *	-	0.66±0.35**	-
Uterus	-	0.4±0.07	-	0.36±0.1	-	0.2±0.02**	-	0.17±0.02**	-	0.16±0.03**
Ovary	-	0.1±0.02	-	0.09±0.02	-	0.07±0.01**	-	0.07±0.01**	-	0.08±0.01**

* all weights were in grams unless noted otherwise

Gross pathology: Treatment-related gross changes seen were limited to reduced size of the prostate & seminal vesicles (32 mg/kg/day group).

Histopathology:

Prostate: Atrophy was seen at 8 (n=3, minimal/slight) & 32 (n=6, minimum/slight) mg/kg/day with reduced number of acini & increased interstitial tissue (thickness & number were not specified).

Seminal vesicles: Atrophy was seen at 32 mg/kg/day (n=3, minimal) with reduced epithelial thickness, reduced secretion and increased interstitial tissue.

Ovary: A decreased number of luteal glands & increased number of cystic follicles were seen in all treated groups. Animals treated with 8 & 32 mg/kg/day showed focal vacuolization (minimal) in oviductal epithelial cells.

Finding	Control	Fc-1271a dose (mg/kg/day)				
		0.5	2.0	8.0	32	
Number of animals studied	10	10	9	10	10	
Number of luteal glands,	decrease of	0	0	2	2	2
	absence of	0	3	7	6	8
Increase of large cystic follicles,	minimal	0	4	1	5	3
	slight	0	0	2	0	3
	moderate	0	0	0	0	3
Vacuolisation in oviductal epithelium, minimal	0	0	0	3	6	

Uterus: Hypertrophy of luminal columnar epithelium was seen in all treated females. Histologically, the epithelium consisted of large epithelial cells with a large round nucleus arranged in a multilayer with minimal superficial stratification. A decrease in the number of endometrial glands was seen at all doses and a dose-related myometrial atrophy seen at the 2 highest doses.

Finding	Control	Fc-1271a dose (mg/kg/day)			
		0.5	2.0	8.0	32
Number of animals studied	10	10	10	10	10
Hypertrophy of columnar luminal epithelium	0	4	7	8	8
Presence of hypertrophic multilayer epithelium, lumen,					
minimal amount	0	0	1	4	1
slight amount	0	0	3	2	4
moderate amount	0	0	0	0	2
only epithelium type	0	0	0	0	1
Decrease in number of endometrial glands,					
minimal	0	2	0	0	0
slight	0	1	4	1	0
moderate	0	0	6	8	9
absence of	0	0	0	1	1
Myometrial atrophy	0	0	1	9	10

Vagina: An increased incidence in metestrus-like infiltration of polymorphonuclear cells in the vaginal epithelium was seen at the 2 highest doses.

Testis: A HD-treated animal showed slight chronic vasculitis surrounding the small interstitial blood vessels. Mild vasculitis was also seen around the epididymis.

Finding	Control	Fc-1271a dose (mg/kg/day)			
		0.5	2.0	8.0	32
Number of animals studied	10	10	10	10	10
Epithelial keratohyalinisation	2	5	8	0	2
Increased infiltration of polymorphonuclear cells,					
minimal	0	0	0	8	6

Reviewed by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Study Title: “FC-1271a: 26-Week oral (gavage administration) toxicity study in the female rat”

Key study findings:

- A decrease in body weight gain was seen in all treated females compared to controls starting from week 1 of the study and correlated with decreased food consumption.
- 2) An increase in liver weight relative to overall mean body weight was seen in animals treated with >3 mg/kg/day and correlated with an increase in hepatocellular hypertrophy. A decrease in uterus and ovary weights (both adjusted and as a ratio of body weight) were also seen. Histopathology findings included the ovaries (follicular cysts, cystopapillary granulomas cell hyperplasia), uterus (stromal atrophy), vagina (mucification indicative of persistent estrus at >3 mg/kg/day), pituitary (vacuolation at >3 mg/kg/day) and mammary glands (ductal hypertrophy at >3 mg/kg/day).
- 3) Based on the exaggerated pharmacological nature of the findings, the NOAEL for this study was 300 mg/kg/day.

•
Study no: (b) (4) study # 15-44206 (b) (4) #1716/9

Volume # and page #: Vol. 10, 1 (005219)

Conducting laboratory and location: (b) (4)

Date of study initiation: February 02, 1995 (Start of dosing)

GLP compliance: Yes

QA Report: Yes

Drug, lot # and % purity: Ospemifene or FC-1271a, batch # 99E (27 rr1 ½), 99.45%.

Methods

Doses: Animals were administered an oral gavage dose of 0, 3, 30 or 300 mg/kg/day in 0.5% CMC for 13 weeks. Animals were housed in groups of 5/cage.

Species/Strain: Crl:CD (SD)IGSBR strain rats (b) (4) Margate) were acclimatized for 2 weeks prior to the start of the study.

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

Route, formulation, volume and infusion rate: Oral gavage, compound was dissolved in 0.5% CMC, 10 ml/kg. Doses were adjusted according to the most recent body weight.

Satellite groups used for toxicokinetics or recovery: None

Age: Animals were 10 weeks old at the start of treatment.

Weight: Animals weighed 209.3-249.7g at the start of dosing.

Sampling times: See individual endpoints.

OBSERVATIONS AND TIMES

Clinical signs: Observations were made once daily starting from their arrival day and throughout the study period (prior to dosing, immediately after dosing, at 0.5, 1, 2 & 4 hrs after dosing during the 1st month of the study and weekly thereafter).

Body weights: Animals were weighed pretest, prior to day 1 and once weekly thereafter.

Food consumption: Food consumption/cage was recorded weekly and recorded as g/animal/week.

Ophthalmoscopy: Ophthalmologic examinations were made for all animals pretest and on control & HD group in week 25.

EKG: Not performed.

Hematology: Blood samples were obtained from 10 animals/group on weeks 13 & 26 after an overnight fast.

Clinical Chemistry: Same as in "Hematology" above.

Urinalysis: Samples were collected overnight from 10 rats/group on weeks 12 & 25 in the absence of food & water.

Toxicokinetics: Blood was collected from all animals at the time of necropsy (~ 24 hrs after dosing).

Gross Pathology: All animals were necropsied at the end of the study following an overnight fast. Necropsies were performed on all moribund and dead animals.

Organ Weight: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, thyroid & parathyroids & uterus were weighed after an overnight fast at scheduled necropsy.

Histopathology: Tissues listed (page 31; adequate battery) were stored for all animals.

The following tissues were examined from all control and high dose groups: Adrenals, aorta, brain, cecum, colon, duodenum, eyes, femur, gross lesions, heart, ileum,

jejunum, kidneys, liver, lungs, mammary gland, mandibular & mesenteric lymph nodes, esophagus, optic nerve, ovaries, pancreas, pituitary, salivary glands, sciatic nerve, skin, spinal cord, spleen, sternum with bone marrow, stomach, thymus, thyroid & parathyroids, trachea, urinary bladder, uterus and vagina. In addition the following tissues were examined from all animals: Adrenals, uterus, ovaries, vagina, pituitary and mammary glands. Bone marrow swears were taken from all animals at necropsy but were not examined.

Others: Bone ash density was conducted on the right tibia from 5 animals/group by weighing the dehydrated bones & measuring their volumes. The mineral content of the bone was also evaluated (ash residue). Bone ash density was determined based on the ratio of ash residue to the bone volume.

RESULTS

Mortality: None.

Clinical signs: Lesions in the tail, head or body, staining to the body or head and hair thinning was seen in all groups including vehicle controls. Thin or hunched appearance increased in incidence in animals treated with FC-1271a.

Body weights: A decrease in body weight gain was seen in all treated females compared to controls starting from week 1 of the study (Table 1.2).

Table 2.2
Group mean food consumption over selected intervals

Test article Group	Control		Fc-1271a		Statistics	
	1	2	3	4		
Level (mg/kg/day)	0	3	30	300		
Week to Week of study	Mean food consumption (g/animal/week) for Group:					
	1F	2F	3F	4F		
1 to 4	Mean	137.9	123.8**	115.8***	121.8***	A
	SD	2.40	4.84	5.79	3.56	
1 to 26	Mean	131.0	122.4	119.7	121.2	DR* A
	SD	4.88	5.44	7.32	6.34	
5 to 13	Mean	131.3	121.1	118.6*	120.2*	A
	SD	5.16	4.84	6.51	6.16	
14 to 26	Mean	128.8	122.8	121.6	121.8	A
	SD	5.71	6.25	8.97	7.52	

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test
A = ANOVA, regression and Dunnett's

Ophthalmology: No treatment related findings were noted.

Hematology: On week 13, an increase in red blood cells (RBC) and thromboplastin time (PT) was seen in all treated groups along with a decrease in MCV in HD-treated rats. On week 26, PT levels were still increased in all treated rats (Table 3).

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Table 3
Group mean hematology
Occasion: Week 13

Test article Group	Control		Fc-1271a		MCH pg	
	1	2	3	4		
Level (mg/kg/day)	0	3	30	300		
Group/ Sex	Hb g/dL	RBC mil/cmm	PCV %	MCV fL		
1F Mean	15.3	8.08	44.7	55.3	29.0	
SD	0.8	0.37	2.4	1.1	0.5	
2F Mean	35.9	8.47*	46.0	54.4	18.7	
SD	0.7	0.35	2.0	1.4	0.6	
3F Mean	35.8	8.58**	46.2	53.8	18.5	
SD	0.5	0.32	1.5	1.7	0.6	
4F Mean	36.6	8.52**	45.8	53.7*	18.4	
SD	0.5	0.16	1.2	1.1	0.5	DR*
Statistics	A	A	A	A	A	

Table 3
Group mean hematology
Occasion: Week 26

Test article Group	Control		Fc-1271a		MCH pg	
	1	2	3	4		
Level (mg/kg/day)	0	3	30	300		
Group/ Sex	Hb g/dL	RBC mil/cmm	PCV %	MCV fL		
1F Mean	15.2	8.26	45.8	46.1	18.4	
SD	0.6	0.40	1.3	2.1	0.5	
2F Mean	35.5	8.51	47.0	47.0	18.2	
SD	0.6	0.38	1.4	1.6	0.6	
3F Mean	35.4	8.56	46.6	46.6	18.1	
SD	0.3	0.26	1.7	1.1	0.7	
4F Mean	35.2	8.47	46.6	46.6	18.0	
SD	0.5	0.25	1.5	1.5	0.5	
Statistics	A	A	A	A	A	

Group/ Sex	MCHC g/dL	PLAT 1000/cmm	PT s	APTT s
1F Mean	34.3	942	18.2	16.4
SD	0.6	97	1.3	2.1
2F Mean	34.5	1095	19.7*	16.9
SD	0.4	157	1.1	3.0
3F Mean	34.3	1060	20.7***	17.3
SD	0.3	92	1.1	2.0
4F Mean	34.2	979	20.6***	16.0
SD	0.4	190	0.8	3.4
Statistics	A	A	A	A

Group/ Sex	MCHC g/dL	PLAT 1000/cmm	PT s	APTT s
1F Mean	33.0	1036	18.4	19.6
SD	0.8	139	0.9	4.0
2F Mean	33.1	1077	20.5**	19.6
SD	0.4	64	0.9	2.4
3F Mean	33.2	1060	21.7***	18.7
SD	0.5	74	1.7	3.2
4F Mean	32.7	1079	22.2***	19.4
SD	0.5	143	1.6	1.6
Statistics	A	A	A	A

* P<0.05
** P<0.01
*** P<0.001

* P<0.05
** P<0.01
*** P<0.001

DR = significant dose response test
A = ANOVA, regression and Dunnett's

A = ANOVA, regression and Dunnett's

Bone marrow: No treatment-related changes were noted.

Clinical chemistry: On week 13, increases in alkaline phosphatase (ALK PHOS) and a decrease in total protein (T Prot), albumin (ALB) & total cholesterol (Tot Chol) were seen. On week 26, the same trends (e.g., increased ALK PHOS and decreased T Prot, albumin & total cholesterol) were significantly different for all treatment groups compared to controls.

Week 13				
	Control	3 mg/kg	30 mg/kg	300 mg/kg
Alk Phos (IU/l)	122±41	197***±32	198***±27	193***±54
T protein (g/l)	75±5	71±3	70*±3	67***±2
Albumin (g/l)	51±7	44**±2	43***±1	41***±2
Cholesterol (T) (mmol/l)	2.3±0.5	1.6**±0.3	1.3***±0.4	1.4**±0.6

Week 26				
	Control	3 mg/kg	30 mg/kg	300 mg/kg
Alk Phos (IU/l)	92±28	178***±44	164***±26	148**±34
T protein (g/l)	81±5	73***±3	72***±3	69***±2
Albumin (g/l)	40±3	34***±1	32***±2	32***±1
Cholesterol (T) (mmol/l)	2.6±0.6	1.3***±0.4	1.1***±0.2	1.4***±0.6

Urinalysis: Urine volume was significantly decreased in all treated groups on week 13 but was only significant for LD-treated rats on week 26.

Urine Volume (ml)	Control	3 mg/kg	30 mg/kg	300 mg/kg
week 13	4.9±1.5	2.9**±0.7	3.5*±1	2.6***±1
Week 26	5.0±1.2	3.7*±1	5.1±1.2	4.0±1.2

TK: On week 26 of the study, serum concentrations of ospemifene and its metabolite, 4-hydroxy- FC-1271a (TOR VI) were dose-dependent and linear. Plasma levels of the metabolite were much higher than the parent drug. Calculations of TK parameters (AUC, Tmax, Cmax) were not provided (Table 2).

Table 2: Average (±SD) treatment group serum concentrations of FC-1271a and metabolite TOR VI

Group	Dose (mg/kg/day)	Average Concentration (ng/ml)	
		FC-1271a	TOR VI
1	0	—	—
2	3	42.4*	89.2 ± 11.7
3	30	56.5 ± 22.1	335.0 ± 248.3
4	300	149.8 ± 99.0	872.3 ± 482.0

— indicates no detection

*N=1

Organ weight: An increase in liver weight relative to overall mean body weight was seen in animals treated with >3 mg/kg/day. A decrease in uterus and ovary weights (both adjusted and as a ratio of body weight) were also seen

Organ weight (g)	Control	3 mg/kg	30 mg/kg	300 mg/kg
liver (Adjusted to BW)	6.5	6.3	6.9*	7.15***
Uterus (unadjusted to BW)	0.079	0.05***	0.076	0.118*
Ovaries (unadjusted to BW)	0.82	0.45***	0.38***	0.334***

Gross pathology:

Ovaries: A dose-related increase in cysts was noted (0 control, 2 LD, 5 MD & 11 HD).

Uterus: A dose-related increase in thinning of uterus was seen (0 control, 1 LD, 5 MD, 5 HD)

Lung: An increase in pale foci was seen on the lungs (0 control, 2 LD, 2 MD, 4 HD)

Histopathology: Gross changes seen at necropsy in the ovary correlated with histopathological changes (follicular cysts, cystopapillary granulosa cell hyperplasia) and uterus (stromal atrophy). Other changes seen included the vagina (mucification indicative of persistent estrus at >3 mg/kg/day), pituitary (vacuolation at >3 mg/kg/day) and mammary glands (ductal hypertrophy at >3 mg/kg/day). In the liver, an increase in

hepatocellular hypertrophy (0 controls & 9 HD), an increase in pigmentation of the spleen (minimal in 8 control & mild in 19 HD) and foamy histiocytes of the lungs (6 control & 11 HD) were seen in HD-treated rats.

Incidence of selected microscopic findings					
Tissue and finding	mg/kg/day	1F	2F	3F	4F
		0	3	30	300
Ovary	No examined:	20	20	20	20
		4	20	14	8
		1	0	6	12
		0	0	0	4
Uterus	No examined:	20	20	20	20
		1	20	20	20
		3	20	20	20
Vagina	No examined:	20	20	20	20
		4	18	4	1
		0	1	15	19
Pituitary	No examined:	20	19	20	19
		0	1	7	10
Mammary gland	No examined:	20	20	20	19
hypertrophy – ductal		0	0	4	12

Bone ash density: No treatment-related change was seen.

Reviewed by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Study Title: “13-Week oral (capsule) toxicity study in the dog with a 4-week recovery period”

Key study findings:

- An increase in organ weight relative to body weight in the liver and uterus and a decrease in ovarian weight was seen in all treated dogs.
- 2) Histopathology findings were limited to the liver (centrilobular hepatocyte hypertrophy in dogs treated with \geq MD), uterus and vagina (cystic endometrial hyperplasia, squamous metaplasia and endometrial inflammation in all treatment groups). All treated dogs lacked antral follicles or corpora lutea, suggesting an inhibition of ovulation due to treatment.
- 3) Based on the exaggerated pharmacological nature of the findings, the NOAEL for this study was 500 mg/kg/day (250x the human equivalent dose).

Study no: (b) (4) study # 15-44211/ (b) (4) #845925

Volume # and page #: Vol. 2, 1 (SN 005, YY, June 20, 2005)

Conducting laboratory and location: (b) (4)

Date of study initiation: October 21, 2002

GLP compliance: Yes (study conducted according to Swiss GLP practices)

QA Report: Yes

Drug, lot # and % purity: Ospemifene batch # 01E9, 98.2%.

Methods

Doses: Animals were administered oral doses (capsules) containing of 0, 80, 200 or 500 mg/kg/day in 0.5% CMC for 13 weeks. Animals (n= 2 control & n=2 HD) were allowed a treatment-free period of 4 weeks after the end of the treatment period.

Species/Strain: Pure bred Beagle dogs (b)(4) were acclimatized for 2 weeks prior to the start of the study.

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

Dose (mg/kg/day)	0	80	200	500
Number of animals (main)	6	4	4	6
Number of animals (recovery)	2			2

Route, formulation, volume and infusion rate: The appropriate amount of the compound was weighed and placed directly into gelatin capsules (size 11). Doses were adjusted by body weights and generally split into 4 capsules/dose. Control animals were given similar size and equal number of empty capsules.

Satellite groups used for toxicokinetics or recovery: 2 for control & 2 for HD-treated groups

Age: Animals were 10-11 weeks old at the start of treatment.

Weight: Animals weighed 6.4-9.3Kg at the start of dosing.

Sampling times: See individual endpoints.

OBSERVATIONS AND TIMES

Clinical signs: Observations were made twice daily starting from their arrival day and twice daily during the treatment period.

Body weights: Animals were weighed pretest on day 1 and once weekly thereafter.

Food consumption: Food consumption was recorded daily by weighing food before and after feeding.

Ophthalmoscopy: Ophthalmologic examinations were made for all animals pretest, on week 13 and on week 4 of the recovery period

EKG: Electrocardiograms of each animal was recorded pretest, on weeks 1, 13 of the study and on week 4 after recovery. During the study period, recordings were made prior to and at 1.5hrs post dose. HR, p wave duration, amplitude of P-Q, QRS, Q-T intervals were measured and QTc was calculated.

Hematology: Blood samples were obtained from all animals in the morning pre-test, on weeks 6, 13 & week 4 of the recovery period. Animals were fasted overnight but allowed free access to water.

Clinical Chemistry: Same as in "Hematology" above.

Urinalysis: Samples were collected in the morning under the same conditions as in hematology.

Toxicokinetics: Blood samples were collected from all animals at pre-test, week 4, 13 and week 4 of the recovery period. Samples were stored for possible hormone analysis for estradiol, progesterone, LH, FSH and sex-hormone binding globulin. Serum levels of ospemifene and its major metabolite 4- hydroxy ospemifene were determined.

Gross Pathology: All animals were necropsied at the end of the study following an overnight fast. Necropsies were performed on all moribund and dead animals.

Organ Weight: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, thymus, thyroid & parathyroids & uterus were weighed after an overnight fast at scheduled necropsy.

Histopathology: Tissues listed (page 23; adequate battery) were examined for all animals. Additional sections from the mammary gland, uterus, vagina and liver were collected for possible immunohistochemistry analysis.

RESULTS

Mortality: None.

Clinical signs: Occasional vomiting, watery/loose stool were observed occasionally in all groups including controls. In 1 HD-treated dog, decreased activity and lateral recumbancy were noted once on day 10 of the study.

Body weights: No treatment-related findings were noted.

Food consumption: No consistent treatment-related changes were noted. On week 1, HD-treated animals had a reduced food intake which was reversed in weeks 3-9 to a higher food consumption which abated for the remaining of the study compared to controls.

Ophthalmology: No treatment related findings were noted.

EKG: No treatment related findings were noted.

Hematology: A decrease in red blood cells (RBC), hemoglobin (Hb), hematocrit and platelets was seen in HD-treated animals possibly indicative of bone marrow suppression or increased blood loss. An increased production of neutrophils and monocytes correlate with possible immunosuppression. Following the recovery period, no difference in hematology parameters was noted between control and treated dogs.

Week 13	Control	80 mg/kg/day	200 mg/kg/day	500 mg/kg/day
N	6	4	4	6
RBC	6.77±0.5	6.2±0.32	6.08±0.55	6.06*±0.26
Hb (mmol/l)	9.9±0.8	8.9±0.45	9±0.7	8.75*±0.3
Hct	0.44±0.03	0.39±0.02	0.39±0.03	0.38*±0.02
Neutrophils	7.4±1.05	11.8±4.2	10.8±1.2	11.8**±2.3
Basophils	0.1±0.02	0.08±0.02	0.075±0.006	0.058*±0.025
Monocytes	0.442±0.14	0.75±0.2	0.8*±0.2	0.75*±0.2
Platelets	366±56	450±60	490±63	526*±135

Clinical chemistry: On week 13, a decrease in creatinine was noted in all treated dogs. An increase in cholesterol (not significant), triglycerides (not significant) and alkaline phosphatase were seen in all treated dogs. These changes might be due to stress the animals were undergoing or a general ill health. No other consistent changes from controls were noted among treated dogs.

Week 13	Control	80 mg/kg/day	200 mg/kg/day	500 mg/kg/day
Creatinine	61.8±3.5	47.3**±4	48.9**±5	49.9**±4.2
cholesterol	3±0.4	4.4±0.4	4.3±1.4	4.9**±1
Triglyc	0.3±0.06	0.47±0.1	0.4±0.1	0.46±0.09
Alk Phos	38.06±14	129.3**±34	159**±63	168**±42

TK: A dose-related (but not dose-proportional) was seen with increasing doses of ospemifene. A time-dependent decrease in plasma levels was seen after 13 weeks of dosing, suggesting metabolic induction (Table 15). Plasma levels of 4-hydroxy-ospemifene were only detected in some samples (LD & HD groups) and also decreased over time in the HD-treated group (Table 16).

Table 15 Comparison of mean toxicokinetic parameters of ospemifene throughout the different groups after single (1x) and repeated (9 and 13 weeks) oral (capsule) administration.

Dose dependency						
Group/ Occasion	Dose (mg/kg)	Factor	AUC _{0-24h} (ng·h/ml)	Factor	C _{max} (ng/ml)	Factor
2/ 1x	80	-	43106	-	4478	-
3/ 1x	200	2.5	79674	1.9	7732	1.7
4/ 1x	500	2.5	118167	1.6	9285	1.2
2/ week 9	80	-	31590	-	3357	-
3/ week 9	200	2.5	47279	1.5	4430	1.3
4/ week 9	500	2.5	66104	1.4	4527	1.0
2/ week 13	80	-	22624	-	2547	-
3/ week 13	200	2.5	42237	1.9	4442	1.7
4/ week 13	500	2.5	46253	1.1	3831	0.9
Time dependency						
2/ 1x	80	-	43106	-	4478	-
2/ week 9	80	1.0	31590	0.7	3357	0.8
2/ week 13	80	1.0	22624	0.7	2547	0.8
3/ 1x	200	-	79674	-	7732	-
3/ week 9	200	1.0	47279	0.6	4430	0.6
3/ week 13	200	1.0	42237	0.9	4442	1.0
4/ 1x	500	-	118167	-	9285	-
4/ week 9	500	1.0	66104	0.6	4527	0.5
4/ week 13	500	1.0	46253	0.7	3831	0.9

2, 3 and 4 = female groups at the dose level of 80, 200 and 500 mg/kg
1x = single administration
week 9, week 13 = repeated administration during 9 and 13 weeks

Table 16 Comparison of mean toxicokinetic parameters of 4-hydroxy-ospemifene throughout the different groups after single (1x) and repeated (9 and 13 weeks) oral (capsule) administration of ospemifene.

Dose dependency						
Group/ Occasion	Dose (mg/kg)	Factor	AUC _{0-24h} (ng·h/ml)	Factor	C _{max} (ng/ml)	Factor
2/ 1x	80	-	488	-	40	-
3/ 1x	200	2.5	-	-	-	-
4/ 1x	500	6.3*	1597	3.3*	138	3.5*
2/ week 9	80	-	615	-	58	-
3/ week 9	200	2.5	-	-	0	-
4/ week 9	500	6.3*	1431	2.3*	108	1.9*
2/ week 13	80	-	-	-	-	-
3/ week 13	200	2.5	-	-	-	-
4/ week 13	500	6.3*	997	-	81	-
Time dependency						
2/ 1x	80	-	488	-	40	-
2/ week 9	80	1.0	615	1.3	58	1.5
2/ week 13	80	1.0	-	-	-	-
3/ 1x	200	-	-	-	-	-
3/ week 9	200	1.0	-	-	-	-
3/ week 13	200	1.0	-	-	-	-
4/ 1x	500	-	1597	-	138	-
4/ week 9	500	1.0	1431	0.9	108	0.8
4/ week 13	500	1.0	997	0.7	81	0.8

2, 3 and 4 = female groups at the dose level of 80, 200 and 500 mg/kg
1x = single administration
week 9, week 13 = repeated administration during 9 and 13 weeks
*: 4 versus 2

Urinalysis: No treatment-related changes were noted.

Organ weight: An increase in organ weight relative to body weight was seen in the liver and uterus in all treated dogs compared to vehicle controls. In addition, a decrease in ovarian weight was seen in all treated dogs. A similar pattern was seen for liver and

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uterine weight when organ weight was measured as a ratio of body weight but not for ovarian weight. No difference between control and treated dogs was seen after the 4-week recovery period.

Absolute weight (g)	Control	80 mg/kg/day	200 mg/kg/day	500 mg/kg/day
Liver	247±36	316*±43	322*±33	371**±28
Uterus	25.3±21	60.9*±11	56.4±23	66.9*±13.9
Ovaries	0.93±0.18	0.55**±0.08	0.51**±0.045	0.8**±0.08

Gross pathology: Treatment-related gross changes seen were limited to thickening of the cervical and vaginal mucosa.

Histopathology:

Liver: Centrilobular hepatocyte hypertrophy (minimal to moderate) was seen in dogs treated with ≥200 mg/kg/day (n= 3 MD & all HD-treated). In 1 HD-treated dog, multilamellar inclusions in hepatocytes were also seen.

Uterus & vagina: All treated dogs had minimal to moderate cystic endometrial hyperplasia. In the uterus/cervix mucosa, minimal/slight squamous metaplasia was also seen (n= 3 in LD & MD and all HD-treated). All treated groups had minimal/slight endometrial inflammation (n=1-2 in all groups).

Ovary: None of the treated dogs had antral follicles or corpora lutea, suggesting an inhibition of ovulation due to treatment.

DOSE GROUP:	01		02		03		04	
SEX :	M	F	M	F	M	F	M	F
NO. ANIMALS:	-	4	-	4	-	4	-	4
LIVER	-	4	-	4	-	4	-	4
- Hepatocell.hypertr.								
GRADE 1 :	-	-	-	-	-	1	-	-
GRADE 2 :	-	-	-	-	-	2	-	3
GRADE 3 :	-	-	-	-	-	-	-	1
TOTAL AFFECTED :	-	-	-	-	-	3	-	4
MEAN SEVERITY :	-	-	-	-	-	1.7	-	2.3
UTERUS	-	4	-	4	-	4	-	4
- Squamous metaplasia								
GRADE 1 :	-	-	-	-	-	-	-	1
GRADE 2 :	-	-	-	3	-	3	-	3
TOTAL AFFECTED :	-	-	-	3	-	3	-	4
MEAN SEVERITY :	-	-	-	2.0	-	2.0	-	1.8
- Cystic hyperplasia								
GRADE 1 :	-	-	-	1	-	-	-	1
GRADE 2 :	-	-	-	1	-	1	-	1
GRADE 3 :	-	-	-	2	-	3	-	2
TOTAL AFFECTED :	-	-	-	4	-	4	-	4
MEAN SEVERITY :	-	-	-	2.3	-	2.8	-	2.3
- Endometrial inflamm.								
GRADE 1 :	-	-	-	-	-	2	-	2
GRADE 2 :	-	-	-	1	-	1	-	-
TOTAL AFFECTED :	-	-	-	1	-	3	-	2
MEAN SEVERITY :	-	-	-	2.0	-	1.3	-	1.0

DOSE GROUP:	01		02		03		04	
SEX :	M	F	M	F	M	F	M	F
NO. ANIMALS:	-	4	-	4	-	4	-	4
VAGINA	-	4	-	4	-	4	-	4
- Mononuclear foci								
GRADE 1 :	-	1	-	1	-	-	-	-
GRADE 2 :	-	1	-	-	-	-	-	-
TOTAL AFFECTED :	-	2	-	1	-	-	-	-
MEAN SEVERITY :	-	1.5	-	1.0	-	-	-	-
ADRENAL CORTICES	-	4	-	4	-	4	-	4
- Vacuolation: fasc.								
GRADE 1 :	-	1	-	2	-	4	-	4
TOTAL AFFECTED :	-	1	-	2	-	4	-	4
MEAN SEVERITY :	-	1.0	-	1.0	-	1.0	-	1.0

Reviewed by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

Study Title: "FC-1271a: 39-Week oral (gavage) administration toxicity study in the Cynomolgus monkey"

Key study findings:

- Treatment-related changes included the liver (centrilobular hypertrophy, HD), ovaries (follicular and paraovarian cysts, endometrial hyperplasia with cystic glands and increased stromal connective tissues in all treated animals), uterus (cystic glands, endometrial hyperplasia and adenomyosis in all treated animals), vagina (epithelial atrophy and atrophy) and mammary glands (increase in inflammatory cell foci, glandular vacuolation and glandular atrophy in all treated groups). Based on the exaggerated pharmacological nature of the findings, the NOAEL for this study was 150 mg/kg/day

Study no: (b) (4) study # 15-44207/(b) (4) #1716/10

Volume # and page #: Vol. 11, 1 (00528)

Conducting laboratory and location: (b) (4)

Date of study initiation: April 12, 2000 (Start of dosing)

GLP compliance: Yes

QA Report: Yes

Drug, lot # and % purity: Ospemifene, batch # 99E27 (b) (4) purity not provided

Methods

Doses: Animals were administered a daily oral gavage dose of ospemifene (0, 15, 50 or 150 mg/kg/day in 0.5% CMC) for 39 weeks. Animals were housed individually.

Species/Strain: Cynomolgus monkeys were acclimatized for 6 weeks prior to the start of the study.

Number/sex/group or time point (main study): 4 females/group.

Route, formulation, volume and infusion rate: Oral gavage, compound was dissolved in 0.5% CMC, volume 4 ml/kg. Doses were adjusted according to the most recent body weight.

Satellite groups used for toxicokinetics or recovery: none

Age: Animals were 4-9 years old at the start of treatment.

Weight: Animals weighed 2.4-4.3 Kg at the start of dosing.

Sampling times: See individual endpoints.

OBSERVATIONS AND TIMES

Clinical signs: Observations were made twice daily starting from their arrival day and throughout the study period for morbidity, mortality, behavior, appearance and feces.

Body weights: Animals were weighed once weekly throughout the study period, 1 day before and on the day of necropsy.

Food consumption: Individual food consumption was recorded once weekly, 1 day before and on the day of necropsy.

Ophthalmoscopy: Ophthalmologic examinations were made for all animals pretest and during weeks 13, 26 & 39 of treatment.

EKG: HR, RP, P, PR, QRS, QT intervals, QTc & QT dispersion in seconds, BP, systolic & diastolic pressure and MAP were recorded pre-dose, and during weeks 1, 13, 26 & 39 of treatment.

Hematology: Blood samples were obtained from all animals once pre-dose and during weeks 13, 26 & 39 of the study.

Clinical Chemistry: Same as in "Hematology" above. Serum hormone levels for FSH, progesterone, LH, prolactin, E2, SGBG and GHI were measured whenever sufficient blood were obtained.

Urinalysis: Samples were collected overnight from all animals (fasting, no food or water) once predose and during weeks 13, 26 & 39 of the study.

Toxicokinetics: Blood was collected from all animals on day 1 and during week 39 at 0, 1, 2, 4, 8, 12 & 24hrs after dosing.

Gross Pathology: All animals were necropsied at the end of the study following an overnight fast. Necropsies were performed on all moribund and dead animals.

Organ Weight: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, thymus, thyroid & parathyroids & uterus were weighed after an overnight fast at scheduled necropsy.

Histopathology: Tissues listed (page 23; adequate battery) were stored for all animals. Bone marrow swears were prepared from all animals at necropsy but were not examined.

RESULTS

Mortality: None.

Clinical signs: Soft feces, emesis, wounds, and abrasions were seen on few occasions in all groups of treated monkeys.

Body weights: A trend towards a decrease in group mean body weight was seen for MD-treated monkeys. No consistent treatment-related effect was seen in other treatment groups.

BW change (Kg)	0 mg/kg/day	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Week 1-39	0.5±0.2	0.1±0.6	-0.1±0.3	0.2±0.3

Food consumption: A decrease in food consumption was seen among treated females and was especially different for MD-treated monkeys, correlating with the decrease in this group's overall body weight.

Food consumed (g/week)	0 mg/kg/day	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Week 1-39	612±69	549±59	536±46	572±57

Ophthalmology: No treatment related findings were noted.

EKG: No treatment related findings were noted.

Hematology: No treatment related findings were noted.

Bone Marrow: No treatment related findings were noted.

Clinical chemistry: On week 39, increases in creatinine, alanine aminotransferase and gamma glutamyl transferase were noted for most treatment groups. Sex hormone binding globulin levels decreased in all treatment groups.

Week 39	Control	15 mg/kg	50 mg/kg	150 mg/kg
Creatinine (µmol/l)	67.8±7.5	76±5	65.4±17	100.4*±21.5
ALT (U/l)	26±6.6	200.35*±126	262.2*±167	233*±157
GGT (U/l)	35.5±11	51.3±12	84.6*±30	80.5*±54
SHBG (nmol/l)	38±6	18.5*±4	21*±7.5	20*±7.5

GGT, gamma glutamyl transferase; SHGH= sex hormone binding globulin

Urinalysis: No treatment-related changes were noted.

TK: On week 39 of the study, serum concentrations of ospemifene and its metabolite, 4-hydroxy- FC-1271a (TOR VI) were dose-dependent and linear. Plasma levels of the metabolite were similar to that of the parent drug.

Day 1: Ospemifene	15 mg/kg	50 mg/kg	150 mg/kg
Cmax (ng/ml)	94.5±42	147±74.5	279.5±102
Tmax (hrs)	1.8±1.5	3.5±1	3.8±3
AUC (ng/ml.hr)	849±267	1389±433	3184±697

Day 1 : 4-Hydroxy Ospemifene	15 mg/kg	50 mg/kg	150 mg/kg
Cmax (ng/ml)	61±23	126.5±57	506±403
Tmax (hrs)	8.3±11	5.3±4.7	19±10
AUC (ng/ml.hr)	8147±219	1407±406	5218±3171

Week 39: Ospemifene	15 mg/kg	50 mg/kg	150 mg/kg
Cmax (ng/ml)	128±89	220±93	342.3±145
Tmax (hrs)	1.0	1.3±0.5	2.8±1.5
AUC (ng/ml.hr)	604±248	1427±1035	3512±1300

Week 39: 4-hydroxy-Ospemifene	15 mg/kg	50 mg/kg	150 mg/kg
Cmax (ng/ml)	138±75.5	213.4±45	893.3±502
Tmax (hrs)	1.0	1.3±0.5	3.5±1
AUC (ng/ml.hr)	714±373	1559±748	6990±3801

Organ weight: A trend toward an increase in liver and ovaries weight was seen in all treated monkeys. A decrease in spleen weight was also noted for all groups.

Mean organ weight (g)	Control	15 mg/kg	50 mg/kg	150 mg/kg
Liver	72.8±11	77.6±13	82±22	84.8±14
Spleen	3.8±1.4	3±2.5	1.9±0.5	2.5±0.65
Ovaries (total)	0.46±0.14	0.54±0.06	0.5	0.67

Values for ovaries were for 2 samples and did not have standard of deviation

Gross pathology:

Ovaries: A dose-related increase in cysts was noted (0 control, 1 LD, 2 MD & 2 HD).

Uterus: Enlargement of the uterus was seen in 1 LD, 2 MD and 2 HD-treated females.

Histopathology: Gross changes seen at necropsy included:

Liver: Increased glycogen storage (1 LD, 3 MD & 2 HD) and centrilobular hypertrophy (n=2 HD)

Ovaries: Follicular cysts (0, 2, 4, 3) and paraovarian cysts (0, 1, 1, 0) in LD and HD-treated monkeys, endometrial hyperplasia with cystic glands and increased stromal connective tissues was seen in all treated animals. While no corpora lutea were noted in the LD and MD groups, an increase in corpora lutea was noted in HD-treated groups (n=3 compared to n=4 in controls).

Uterus: Cystic glands (0, 1, 3, 3) endometrial hyperplasia (0, 2, 3, 3) and adenomyosis (0, 0, 0, 2).

Vagina: Epithelial atrophy was seen in HD-treated animals (n=3 compared to none in all other groups). Epithelial vacuolation was seen in LD & MD treated groups (0, 2, 1, 0).

Mammary glands: An increase in inflammatory cell foci (control 0, LD 2, MD 2, HD 1), glandular vacuolation (2, 4, 4, 4) and glandular atrophy (0, 2, 0, 1) were seen in of all treated groups.

Reviewed by Dr. Alex Jordan (DARRTS, 10/23/2006):

Study title: 13-week oral toxicity study in rats

Key study findings: minimal toxicity

Study no.: (b) (4) # 1716-051

Volume #, and page #: vol. 7, pg 1

Conducting laboratory and location: (b) (4)

Date of study initiation: January, 2006

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Ospemifene, lot # 1052557, 99.6% pure.

Methods

Doses: 50, 300, 2000 mg/kg/day

Species/strain: Crl:Wistar(Han) rats

Number/sex/group or time point (main study): 10

Route, formulation, volume, and infusion rate: oral gavage, suspension in corn oil, vol. 6.67 mL/kg

Satellite groups used for toxicokinetics or recovery: 9/sex/dosed gp ; 3/sex/gp controls

Age: ~ 6 wks

Weight: 130.2 – 188.6 g

Results:

Mortality: One HD female sacrificed moribund in week 3 with thinness, hunched posture, sluggishness, rapid respiration, raised hair and semi-closed eyes.

Clinical signs: Slight increase in thinning fur in treated females. Immediately after dosing, higher incidence of salivation in males and females from all treatment gps starting from day 14 and raised tail noted in both sexes in all gps from day 37 and persisted until end of study.

Body weights: Marked suppression of BW gains in males at all dose levels but not dose proportional. Final body wts were 31.2, 33.9 and 29.8% lower than controls for gps 2, 3 and 4, respectively.

All treated females showed an initial deficit in BW gain which was significant until wk 4 but thereafter there was recovery and wts were similar to controls at end of study although MD and HD females weighed 5.9 and 6.2% less than controls.

Food consumption: Reduced in all dosed males throughout the study and in females for the first 6 wks.

Ophthalmoscopy: No effects

EKG: Not done

Hematology: HD females showed slight but significant decrease in Hb and reticulocyte concentrations. There was a significant reduction in white blood cells and neutrophils in all dosed males and a more variable reduction in lymphocytes. No WBC changes in females.

Clinical chemistry: Variable changes in liver enzymes without dose response. Males had lower testosterone levels with over half the animals with undetectable (below 0.7 mmol/L) levels (controls 1.4-20.9 mmol/L) with no apparent change in LH, prolactin or FSH. Dose related increase in LH in all treated females.

Urinalysis: Specific gravity slightly higher in HD females.

Gross pathology: Small seminal vesicle and prostates for treated males. Thin uterus and ovarian cysts in all treated females.

Organ weights

All treated males had higher relative adrenal (up to 100%), liver (up to 48%) and lower rel prostate wts (up to 45%) than controls.

Treated females had higher rel liver wts (up to 34%) and lower uterus (absolute and rel with a 44% reduction in rel wt at HD)

Histopathology: Adequate Battery: yes

Peer review: yes (), no ()

Mammary gland: Tubulo-alveolar differentiation was seen in all treated males that was characterized by formation of small tubules and ducts.

Seminal vesicle/prostate: Diffuse glandular atrophy in all treated males.

Kidney: reduction in level of hyaline droplets in all treated males.

Ovary: increased incidence of ovarian cysts in all dosed females. Cysts composed of spaces containing proteinaceous fluid, associated with both follicular structures and CL. Cysts were 0/10 in control and 10/10 for all treated females.

Treated females were either in metestrus or had an abnormal diestrus morphology.

Toxicokinetics: Rats

Dose	Day	Ospemifene				4-hydroxyospemifene			
		AUC _{0-24h} (ug.h/ml)		Cmax (ug/ml)		AUC _{0-24h} (ug.h/ml)		Cmax (ug/ml)	
		M	F	M	F	M	F	M	F
50	1	5.5	3.2	0.8	0.4	3.5	7.9	0.5	1.0
	91	6.3	4.9	0.8	0.7	6.6	9.2	1.2	1.2
300	1	27.4	21.4	2.3	1.8	9.3	31.8	0.8	2.3
	91	12.6	18.0	1.4	1.8	9.0	18.2	1.1	1.8
2000	1	35.4	27.2	2.1	2.0	11.2	37.4	0.7	3.7
	91	13.9	16.7	1.4	1.1	9.6	12.0	1.3	1.0

Human: Postmenopausal women given 60 mg of ospemifene orally for 12 wks had AUC's_{0-24h} of Ospemifene + 4-OHospemifene of 10 ug.h/ml. AUC's_{0-24h} of Ospemifene + 4-OHospemifene of 10 ug.h/ml.

Plasma protein binding was approximately 98% at 2 hrs decreasing to 95% at 24 hrs in humans. In rats, protein binding was 95% at 2 hrs decreasing to 64% at 24 hrs.

T_{1/2} = 3.5-6.8 hrs in rats

Reviewed by Dr. Alex Jordan (DARRTS, 10/23/2006):

Study title: 13-week oral toxicity study in mice

Key study findings: minimal toxicity

Study no.: 1181-008

Volume #, and page #: vol. 2, pg 1

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: June, 2005

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Ospemifene, lot # and purity not stated.

Methods

Doses: 50, 300, 2000 mg/kg/day

Species/strain: Crl:CD1 mice

Number/sex/group or time point (main study): 10

Route, formulation, volume, and infusion rate: oral gavage, suspension in corn oil, vols of 0.5, 1.0 and 6.67 mL/kg

Satellite groups used for toxicokinetics or recovery: 38/sex/dosed gp

Age: ~ 6 wks

Weight: 21.6 – 34.1 g

Results:

Mortality: One HD female found dead on day 83 due to renal necrosis secondary to thrombosis/infarction. An ovarian tumor was found in the animal and may have contributed to the renal infarct.

Clinical signs: no drug related findings.

Body weights: Small degree of weight increase in MD and HD females. No changes in males.

Food consumption: No changes

Ophthalmoscopy: No effects

EKG: Not done

Hematology: No drug related changes were noted. No effects on bone marrow

Clinical chemistry: One HD male had increased AST and ALT but group means were not different from controls. Total protein and albumin values were statistically higher in HD males than controls. Two HD females had higher AST and ALT values compared to controls.

Urinalysis: Not done

Gross pathology: Dose dependent increase in number of ovarian cysts were seen in females of all treated gps.

Organ weights:

There were increased relative liver wt percentages in males (9.9%, 13.2%, 22.8% for LD, MD, HD, respectively). Females at MD and HD had increased mean liver wts (14.4% and 36.7%, respectively).

There was decreased relative pituitary wts in MD and HD females and decreased actual pituitary wt in MD females.

Decreased uterus with cervix rel wt in MD and HD females (30.3% and 31.7%).

Reduced mean kidney wts in HD males (17.4%).

Relative ovarian wts were increased in HD females by 187.7% probably reflecting the increased numbers of cysts.

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

Peer review: yes (), no ()

In the liver, centrilobular hepatocellular hypertrophy was seen most consistently in HD males and females.

In the testes, there were dose-related changes in the interstitial (leydig) cells characterized by cellular hypertrophy, increased cytoplasmic vacuolation and minimal hyperplasia.

Prostate atrophy was seen at the HD.

Ovarian cysts and cystic dilation of endometrial glands were increased in a dose related manner.

Toxicokinetics: Mice

Dose	Day	Ospemifene				4-hydroxyospemifene			
		AUC _{0-24h} (ug.h/ml)		Cmax (ug/ml)		AUC _{0-24h} (ug.h/ml)		Cmax (ug/ml)	
		M	F	M	F	M	F	M	F
50	1	3.8	5.1	1.0	1.0	1.7	2.8	0.2	0.5
	91	2.7	2.6	0.8	0.7	1.2	1.4	0.3	0.2
300	1	6.6	11.3	1.2	2.3	3.7	7.0	0.5	0.8
	91	8.8	8.1	1.3	1.5	4.3	5.3	0.5	0.8
2000	1	28.6	50.9	4.3	7.9	11.3	23.1	1.4	3.0
	91	21.2	44.6	3.9	8.9	8.6	21.5	1.2	2.2

Human: Postmenopausal women given 60 mg of ospemifene orally for 12 wks had AUC's_{0-24h} of Ospemifene + 4-OHospemifene of 10 ug.h/ml.

Total exposure (parent + main metabolite) at the high dose of 2000 mg/kg was about 3 (males) to 7 (females) higher than humans taking 60 mg. Sponsor claims that the expected clinical dose is 60 mg.

Plasma protein binding was approximately 98% at 2 hrs decreasing to 95% at 24 hrs in humans. In rats, protein binding was 95% at 2 hrs decreasing to 64% at 24 hrs. No binding studies were conducted with mice.

7 Genetic Toxicology

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

Study Title: “FC-1271a: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*”

Key study findings:

- Ospemifene did not induce mutation in 5 salmonella strains under conditions of the study that caused precipitation at doses ≥ 200 $\mu\text{g}/\text{plate}$ in the presence or absence of metabolic activation.

Study no: 15-44302 (formerly study # 1504001); (b) (4) Study # 1716/6

Volume # and page #: Vol. 12, section 17 (page # 07112)

Conducting laboratory and location: (b) (4)

Date of study initiation: July 27, 1999

GLP compliance: Yes

QA Report: Yes

Drug, lot # and % purity: C-1271a, batch # 99E27, 101.4%

Methods

Strains/species/cell line: Salmonella strains TA98, 100, 1535, 1537 and 102 were used both in the absence and presence of metabolic activation.

Doses used in the definitive study: Doses of 1.6, 8, 40, 200, 1000 or 5000 $\mu\text{g}/\text{plate}$ were used in the dose range (DR) finding study and in 1 definitive study. In a second definitive experiment, doses of 12.5, 25, 50, 100, 200 & 400 $\mu\text{g}/\text{plate}$ were used.

Basis of dose selection: Due to precipitation at doses ≥ 200 $\mu\text{g}/\text{plate}$ in the DR and the 1st definitive experiment, a second definitive experiment was used with a maximum dose of 400 $\mu\text{g}/\text{plate}$.

Negative controls: DMSO (vehicle control).

Positive controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, glutaraldehyde, 2-aminoanthracene and benzo[a]pyrene.

Incubation and sampling times: Plates were incubated at 37°C for 3 days in the dark

Results

Study Validity: Dose selection was limited by the precipitation at doses ≥ 200 $\mu\text{g}/\text{plate}$ in the presence or absence of S9. Acceptance and evaluation criteria were acceptable.

Study outcome: Ospemifene did not cause toxicity in either the presence or absence of metabolic activation. Precipitation was noted at ≥ 200 $\mu\text{g}/\text{plate}$ in all strains used as seen in the DR finding and the 1st definitive study (see Table 9). A similar pattern was seen when doses were limited to 400 $\mu\text{g}/\text{plate}$ in the presence or absence of S9. Both positive and negative controls fell within acceptable ranges.

Table 9

Test strain: TA102 -S-9

Treatment (µg/plate)	Reversion numbers/plate				
Solvent	475	465	434	459	457
1.6	516	480	481		
8	428	479	453		
40	533	440	432		
200	396 M-Ppn	408 M-Ppn	436 M-Ppn		
1000	428 M+Ppn	414 M+Ppn	420 M+Ppn		
5000	376 M+Ppn	348 M-Ppn	436 M+Ppn		
Positive	889	980	971		

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Treatment (µg/plate)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnnett's t value
Solvent	458.00	5		15.13			
1.6	492.33	3	1.07*	20.50	0.75 *	21.46	1.52 NS
8	453.33	3	0.99	25.50	0.21 NS	-1.45	-0.22 NS
40	468.33	3	1.02	56.15	0.00 NS	0.01	0.42 NS
200	413.33	3	0.90	20.53	0.58 NS	-0.27	-2.07 NS
1000	420.67	3	0.92	7.02	0.48 NS	-0.05	-1.72 NS
5000	386.67	3	0.84	44.96	0.60 NS	-0.01	-3.39 NS
Positive	946.67	3	2.07	50.14			
M Statistic = 2.048							

Key to significance:

* p ≤ 0.05 ** p ≤ 0.01 *** p ≤ 0.005 NS non significant

Key to postfixes:

Ppn : Precipitation of test article observed
M : Plate counted manually
* : Maximum increase above control

FC-1271a: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 µl	33 ± 13	135 ± 19	23 ± 5	9 ± 4	381 ± 37
FC-1271a	12.5	36 ± 6	123 ± 7	20 ± 4	6 ± 2	380 ± 42
	25	24 ± 5	117 ± 16	19 ± 3	7 ± 4	448 ± 30
	50	29 ± 3	122 ± 4	17 ± 2	9 ± 1	391 ± 52
	100	31 ± 9	138 ± 7	19 ± 5	10 ± 2	406 ± 68
	200	28 ± 2	122 ± 18	22 ± 1	7 ± 5	353 ± 24
	400	38 ± 5 (M+Ppn)	100 ± 18 (M+Ppn)	20 ± 4 (M+Ppn)	7 ± 6 (M+Ppn)	390 ± 111 (M+Ppn)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	118 ± 19	1559 ± 193	121 ± 12	58 ± 4	1419 ± 113

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SD : Standard deviation

B[a]P : Benzo[a]pyrene
AAN : 2-Aminoanthracene

Ppn : Precipitation of test article observed
M : Plate counted manually

7.2 In Vitro Assays in Mammalian Cells

Study Title: "FC-1271a: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique"

Key study findings:

- Ospemifene did not induce mutation at the tk locus of L5178Y mouse lymphoma cells in 2 experiments in the absence or presence of S9 in 5 salmonella strains under conditions of the study.

Study no: 1504002; (b)(4) **Study #** 1716/5-D6183

Volume # and page #: Vol. 12, 12, section 18 (page # 07185)

Conducting laboratory and location: (b)(4)

Date of study initiation: July 27, 1999

GLP compliance: Yes

QA Report: Yes

Drug, lot # and % purity: C-1271a, batch # 99E27, 101.4%

Methods

Strains/species/cell line: Ospemifene was assayed for its ability to induce mutations at the tk locus in mouse lymphoma cells.

Doses used in the definitive study: In the 3hr treatment group using 8 doses ranging from 10-60 µg/ml (3hrs), the top 3 doses caused extreme cytotoxicity in the absence of S9. The top doses selected (40 & 60 µg/ml) resulted in ~22% and 24% relative survival, respectively. In the 24hrs experiment, the top dose of 60 µg/ml caused cytotoxicity. The 2 top doses of 50 & 60 µg/ml yielded relative survival of 16.5 & 3.6%, respectively.

Cultures selected for mutation assessment

Experiment 1 (µg/mL)		Experiment 2 (µg/mL)	
- S-9	+ S-9	- S-9	+ S-9
0	0	0	0
10	10	10	20
20	20	20	30
30	30	30	35
35	35	35	40
40	40	40	45
	45	45	50
	50	50	60*
	60		
NQO 0.05	BP 2	NQO 0.02	BP 2
NQO 0.1	BP 3	NQO 0.04	BP 3

* One replicate discarded on Day 2 due to excessive toxicity

Basis of dose selection: A cytotoxicity DR experiment was conducted where 6 doses ranging from 7.81-250 µg/ml were incubated for a 3hrs period in the absence or presence of 9. Doses were limited by precipitation. The 2 highest doses caused cytotoxicity. A 24hrs DR finding experiment where 9 doses ranging from 0.98-250 µg/ml were tested in the absence of S9 caused extreme cytotoxicity at the top 2 doses.

Negative controls: DMSO (vehicle control).

Positive controls: 4-nitroquinolone and benzo[a]pyrene.

Incubation and sampling times: Plates were incubated at either 3hrs or 24hrs.

Results

Study Validity: Dose selection was limited by precipitation at doses ≥50 µg/plate in the presence or absence of S9. Acceptance and evaluation criteria were acceptable.

Study outcome: Ospemifene did not cause an increase in mutant frequency in a dose-dependent manner when tested up to the limit of precipitation (~60 µg/ml) in the absence or presence of S9 in experiment 1 and in the experiment 2 in the absence of S9. An increase in mutant frequency was seen at 35 µg/ml and 45 µg/ml in the absence of S9 in experiment 2. Due to the small increase and absence of a significant trend in the highest dose tested, these findings are not considered biologically significant.

FC-1271a: summary of results

Experiment 1 (3 hour treatment +/- S-9)

Treatment (µg/mL)	-S-9			Treatment (µg/mL)	+S-9		
	%RS	RTG	MF5		%RS	RTG	MF5
0	100.00	1.00	161.90	0	100.00	1.00	158.17
10	110.23	1.26	118.91 NS	10	114.40	1.08	121.05 NS
20	78.44	1.15	139.31 NS	20	101.22	0.82	154.52 NS
30	88.38	1.03	132.25 NS	30	82.43	0.71	177.81 NS
35	42.29	0.64	123.91 NS	35	76.55	0.74	145.89 NS
40	21.89	0.39	128.18 NS	40	87.86	0.90	123.42 NS
45 \$	2.09			45	59.28	0.59	143.04 NS
50 \$	0.05			50	45.40	0.45	146.65 NS
60 \$	0.00			60	24.06	0.38	133.57 NS
Linear trend			NS	Linear trend			NS
NQO				BP			
0.05	113.17	1.16	304.94	2	70.14	0.81	524.67
0.1	77.53	0.99	427.80	3	43.65	0.52	777.56

\$ 5-TFT resistant mutants/10⁶ viable cells 2 days after treatment
 %RS Percent relative survival adjusted by post treatment cell counts
 \$ Not plated for viability / 5-TFT resistance
 NS Not significant
 *, **, *** Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively

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Experiment 2 (24 hour treatment - S-9, 3 hour treatment + S-9)

Treatment (µg/mL)	-S-9			Treatment (µg/mL)	+S-9		
	%RS	RTG	MF5		%RS	RTG	MF5
0	100.00	1.00	71.83	0	100.00	1.00	82.29
10	89.50	1.13	93.14 NS	20	99.85	1.17	89.15 NS
20	98.16	1.05	102.64 NS	30	72.47	1.11	86.26 NS
30	69.97	0.99	93.93 NS	35	79.45	1.08	89.24 NS
35	63.81	0.81	128.19 *	40	72.22	0.90	107.65 NS
40	44.49	0.64	101.67 NS	45	53.94	0.71	117.64 NS
45	40.33	0.57	109.89 *	50	58.19	0.79	107.00 NS
50	16.08	0.27	105.00 NS	60	3.59	0.12	112.38 NS
60 \$	0.15			70 \$	0.39		
Linear trend			**	Linear trend			**
NQO				BP			
0.02	116.09	0.82	265.30	2	61.28	1.35	417.36
0.04	88.00	0.88	412.17	3	32.12	0.93	392.83

\$ 5-TFT resistant mutants/10⁶ viable cells 1 days after treatment
 %RS Percent relative survival adjusted by post treatment cell counts
 \$ Not plated for viability / 5-TFT resistance
 ! Based on one replicate only
 NS Not significant
 * Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level
 *, **, *** Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study Title: "FC-1271a: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique"

Key study findings:

- Ospemifene did not induce mutation at the tk locus of L5178Y mouse lymphoma cells in 2 experiments in the absence or presence of S9 in 5 salmonella strains under conditions of the study.

Study no: 1504002; ^{(b) (4)} **Study #** 1716/5-D6183

Volume # and page #: Vol. 12, section 18 (page # 07185)

Conducting laboratory and location:

(b) (4)

Date of study initiation: July 27, 1999**GLP compliance:** Yes**QA Report:** Yes**Drug, lot # and % purity:** C-1271a, batch # 99E27, 101.4%**Methods**Strains/species/cell line: CD-1 mice.Doses used in the definitive study: Concentrations of 500, 1000 and 2000 mg/kg were used in groups of males and females. Due to the absence of finding among males, only females were used in the definitive study.Basis of dose selection: A DR finding study where 3 animals/sex were dosed with 500, 1000 and 2000 mg/kg once daily for 2 consecutive days did not cause any toxicity.Negative controls: 0.5% (w/v) carboxy methylcellulose (vehicle control) and DMSO (negative control).Positive controls: Cyclophosphamide (CPA)Incubation and sampling times: Bone marrow samples were collected 24hrs after the last dose.**Results**Study Validity: Dose selection was acceptable. Acceptance and evaluation criteria were acceptable.Study outcome: Ospemifene did not cause an increase in mean ratio of PCE/NCE or in the mean frequency of micronucleated PCEs.

Data for FC-1271a

Treatment group (mg/kg/day)	Kill time (hours)	Sex	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000 cells)
				per treatment group (= sd)
Vehicle control	24	♀	1.11	0.49 (± 0.75)
500	24	♀	1.01	0.37 (± 0.23)
1000	24	♀	1.12	0.37 (± 0.51)
2000	24	♀	1.23	0.25 (± 0.27)
CPA, 40-	24	♀	0.85	5.14 (± 3.91)

- administered as a single dose
sd standard deviation

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7.4 Other Genetic Toxicity Studies**Study Title:** "Determination of DNA adducts in the liver of rats treated with antiestrogens"**Key study findings:**

- Ospemifene did not form DNA adducts in liver of treated rats when tested at 45 mg/kg/day for 2 weeks under conditions of the study.

Study no: 15-44301**Volume # and page #:** Vol. 12, section 20 (page # 07323)

Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: March 23, 1995

GLP compliance: No, [redacted] (b) (4) SOPs were followed

QA Report: Yes

Drug, lot # and % purity: FC-1271a, batch # d3rr1 1/2

Methods

Strains/species/cell line: SD rats (n=10 animals/group), 4 months of age

Doses used in the definitive study: Oral gavage, 45 mg/kg/day for 2 weeks with either ospemifene or tamoxifen.

Basis of dose selection: Not provided

Negative controls: 0.5% (w/v) carboxy methylcellulose (vehicle control) and DMSO (negative control).

Positive controls: Tamoxifen (TAM)

Incubation and sampling times: Liver samples were collected at necropsy, processed for ³²P-postlabelling method and analyzed by HPLC. Detection limit was 2 adducts/108 nucleotides.

Results

Study outcome: Two (2) rats treated with tamoxifen died during the dosing period, possibly due to gavaging errors. Only tamoxifen treated rats produced hepatic DNA adducts (14.5-15.4 adducts/ 10⁶ nucleotides). Control and Ospemifne treated livers did not show any adduct formation.

8 Carcinogenicity

Study title: Ospemifene: 104 Week Oral (Gavage) Administration Oncogenicity Study in the Mouse

Study no.:	15-44404
Study report location:	eCTD #0000, 4.2.3.4.1.1.15-44404
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 24, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Three lots of Ospemifene were used: Lot 4-Batch #1052557, 99.6% Lot 5-Batch #1052557, 99.6% Lot 6-Batch #1195107, 100.2%
CAC concurrence:	Yes

Key Study Findings

Adequacy of Carcinogenicity Study

- There was no evaluation of carcinogenicity in male mice. Dosing was halted in Week 14 and all males were sacrificed by Week 27. Applicant received FDA concurrence for decision to terminate all male groups. The indication is for females only, so male mouse data is not essential for risk assessment.
- Doses were based on MFD of 1500 mg/kg as the high dose, and the low dose exposures were comparable to clinical exposure were used with exCAC concurrence. There was sufficient survival to conduct a statistical evaluation. Neoplastic findings were consistent with known estrogen/anti-estrogen pharmacology in mice. The exposure based on AUC at termination did not achieve sufficient multiples of the clinical exposure at the proposed dose (2x, 4x, and 5x). However, this appears to be caused by a time-dependent decrease in exposure at all doses from 13 to 52 weeks.

Appropriateness of Test Models

Species and strain were same as used in repeat dose toxicity studies, and the oral route is the proposed clinical route. Both neoplastic and non-neoplastic treatment-related effects were related to pharmacology in estrogen target organs, including ovary, uterus, mammary gland, bone, liver, adrenal, and pituitary.

Evaluation of Tumor Findings

Oral ospemifene was tumorigenic in female CD-1 mice treated for two years. Female mice had significant treatment-related increases in adrenal and ovary neoplasms without a dose relationship. The incidences of adrenal and ovarian neoplasms were above maximum historical control rates for female CD-1 mice. Liver and pituitary neoplasm showed a statistical increase compared to concurrent control, but were within historical control incident rates. Additionally, there were significant decreases in mammary gland and uterus neoplasms. All findings are consistent with the established pharmacology/toxicology of ospemifene, and the carcinogenicity of other mixed estrogen agonist/antagonists.

Evaluation of carcinogenicity in male mice was not possible. Male mice did not tolerate ospemifene with unexpected morbidity due to urogenital and abdominal swelling in all treated groups beginning Week 3.

Methods

Doses: 0, 100, 400, 1500, 0
 Frequency of dosing: daily
 Dose volume: 5 mL/kg (Initiation to Week3/Day 5)
 6 mL/kg (Week3/Day 5 to Termination)
 Route of administration: Oral gavage
 Formulation/Vehicle: Corn oil
 Basis of dose selection: MFD (volume of dose) based on 13 week daily
 oral 100, 400, 1500 mg/kg DRF
 Species/Strain: Mouse/Crl:CD1 (ICR)
 Number/Sex/Group: 51/sex/group
 Age: Approx. 7 weeks
 Weight: ♀: 20.3 - 30.5g; ♂: 26.6 - 41.2g
 Animal housing: 2 exclusive rooms, ♂: 1/cage, ♀: 3/cage
 Paradigm for dietary restriction: None, *ad libitum* to SQC Rat and Mouse
 Maintenance Diet No. 1, Expanded (b) (4)
 Dual control employed: Yes
 Interim sacrifice: No
 Satellite groups: Control 1: 12/sex/group,
 Treated Groups: 42/sex/group
 Deviation from study protocol: Early termination of all male groups by Week
 27 due to unexpected test article intolerance.
 Treatment to males was halted during Week
 15, then 1 male/group was sacrificed until
 Week 27, when all remaining males were
 sacrificed.

Observations and Results

Table 13 Animals Replaced During First 4 Weeks of Treatment

Group and Sex	Animal number	Week (Day)	Reason for replacement
4M	164	1 (6)	Poor condition
2F	337	4 (22)	Not stated in log
	664	4 (24)	Found dead
3F	712-714	1 (3)	Replaced by animals 787-789, due to a male animal being returned to the female cage after the Day 1, 8 hour toxicokinetic bleed
4F	452	2 (12)	Found dead
	453	1 (1)	Not stated in log
	758	1 (4)	Found dead

(Table excerpted from Applicant's package)

Mortality

Males: Twenty seven males were sacrificed before the male study was terminated early with 1, 7, 9, and 10 of Control 1, Control 2, LD, MD, and HD groups, respectively

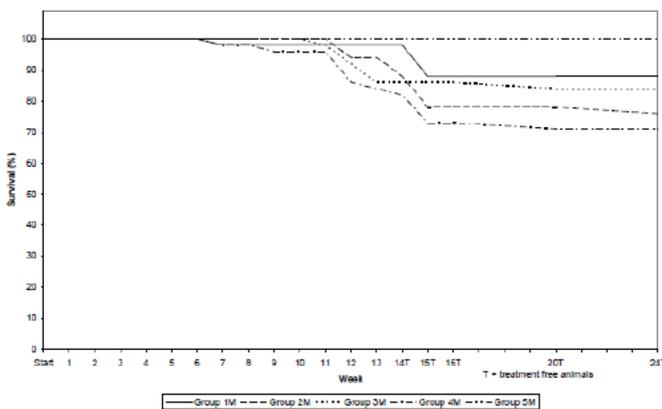
(n=51/group). There were 0, 0, 6, 7, and 8 animals sacrificed due to severe urogenital and/or abdominal swelling with 0, 0, 0, 0, and 2 killed for other reasons but with the swelling.

Table 14 Male Mice Sacrificed Early during 2-Year Oral Ospemifene Carcinogenicity Study Due to Urogenital Swelling

	Reason Sacrificed	mg/kg				
		0 Control 1	100	400	1500	0 Control 2
Main (n=51)	Urogenital Swelling (US)	0	6	7	8	0
	Other, but with US	0	0	0	2	0
	Other	1	1	2	0	0
Satellite (n=42)	Urogenital Swelling (US)	0*	6	6	7	--
	Other, but with US	0*	1	0	3	--
	Other	0*	1	0	0	--
Total Sacrificed		1/63	15/93	15/93	20/93	

*, n=12 for Control 1

Figure 4 Group Mean Survival (%) of Male Mice during a 2-Year Oral Ospemifene Carcinogenicity Study up to Week 24



During Week 15 five animals from Groups 1, 2 and 4 were sent to necropsy due to welfare reasons

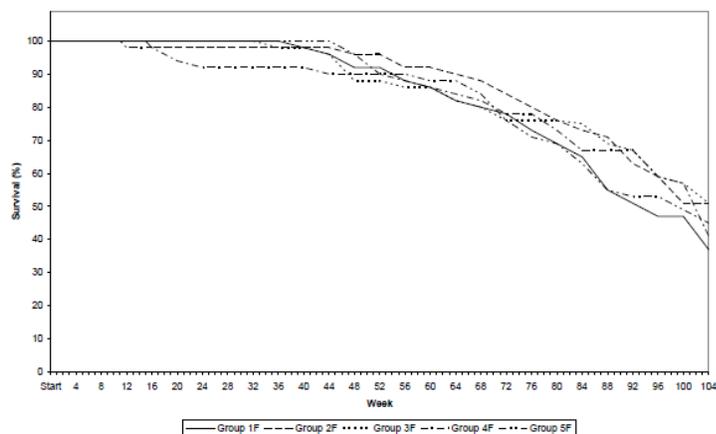
(Figure excerpted from Applicant's package)

Females: There were 140 early deaths in the main study with no statistical significant treatment-related effected noted. In fact, treated animals survived at a slightly better rate than control animals. The causes were considered by the applicant to be consistent for the strain and age.

Table 15 Disposition of Female Mice during a 2-Year Oral Ospemifene Carcinogenicity Study

	Group/Sex/Dose Level (mg/kg/day)				
	1F (0)	2F (100)	3F (400)	4F (1500)	5F (0)
Number animals/group	51	51	51	51	51
Found dead	4	8	7	5	6
Killed early	28	17	18	25	22
Total decedents	32	25	25	30	28
Total survivors	19	26	26	21	23

(Table excerpted from Applicant's package)

Figure 5 Group Mean Survival (%) of Females during a 2-Year Oral Ospemifene Mouse Carcinogenicity Study

(Figure excerpted from Applicant's package)

Clinical Signs- daily, weekly for tumor masses

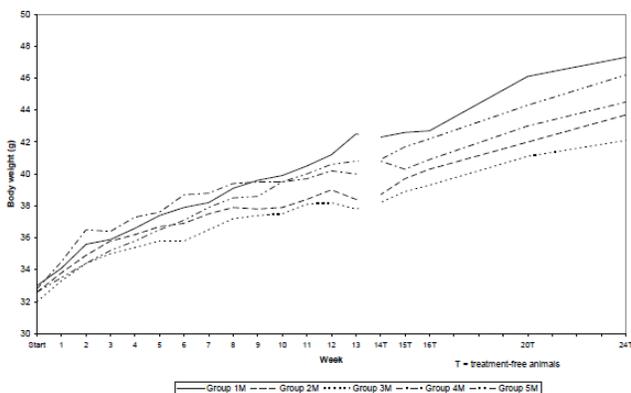
Males: The urogenital, abdominal, and/or scrotal swelling beginning at Week 4 was the predominant treatment-related finding; resulting significant morbidity and early termination of all males by Week 27. Even though treatment was halted in Week 15, the swelling generally did not recede at LD and MD by Week 27, but was slightly reduced in incidence at HD. The % males affected were 0%, 51%, 30%, and 69% in combined controls, LD, MD, and HD, respectively.

Females: There was urogenital swelling noted in 1 or 2 animal in all groups, but did not occur in any group until Week 36. Abdominal swelling was noted in all groups beginning in Week 32, but was higher in ospemifene-treated groups without a strong dose relationship observed. The effect began to appear treatment related around Weeks 47-48. There was a slight dose-dependent increase in head hair loss noted reaching a maximum of 4 HD animals (12%). There was no apparent treatment-related effect on palpable tumor masses.

Body Weights- pre-dose, Day 1, weekly for 16 weeks, then monthly

Males: Body weight gain in the LD, MD, and HD groups was reduced by 35%, 34%, and 19%, respectively, over the total period of dosing in males (Weeks 1-14). After stopping treatment, body weight gain recovered fully at LD, and partially for MD and HD (13% and 18%, respectively).

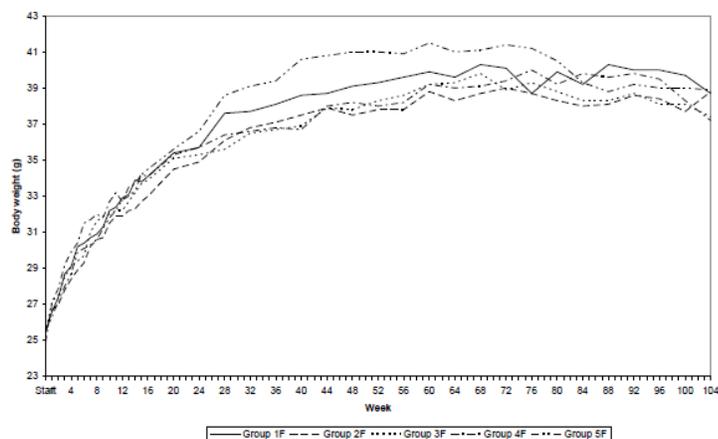
Figure 6 Group Mean Body Weights of Male Mice during a 2-Year Oral Ospemifene Carcinogenicity Study up to Week 24



(Figure excerpted from Applicant's package)

Females: There was an overall decrease in body weight gain in the MD and HD by 13% and 8%, respectively. The MD and LD weighed 3% and 4% less than control at the end of the study. All treated groups had significant body weight gain reductions during Weeks 13 to 28. The LD group reduction was not as dramatic (22% compared to 42% and 45%) during this period, and was similar to control during the remainder of the study.

Figure 7 Group Mean Body Weights of Female Mice during a 2-Year Oral Ospemifene Carcinogenicity Study over Time



(Figure excerpted from Applicant's package)

Table 16 Mean Body Weight Gain (g) for Female Mice during a 2-Year Oral Ospemifene Carcinogenicity Study

Week		1F	2F	3F	4F	5F	Statistics
Start-13	Mean	7.7	6.7	7.2	8.7	7.5	A
	SD	2.79	2.89	2.09	2.64	2.90	
13-28	Mean	4.4	3.9*	2.9***	2.6***	5.6	J
	SD	3.08	2.75	2.56	1.86	3.65	
28-52	Mean	1.5	1.9	2.7	1.7	2.9	A
	SD	2.73	2.31	2.42	2.16	3.55	
52-104	Mean	-1.2	-0.0	-1.9	-1.0	-2.1	A
	SD	3.51	4.20	5.06	3.76	4.56	
Start-104	Mean	13.0	13.6	11.5	12.1	13.4	A
	SD	5.83	4.86	5.68	5.22	5.63	

* P<0.05
 ** P<0.01
 *** P<0.001

A = ANOVA, dose response and Dunnett's
 J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon

(Table excerpted from Applicant's package)

Feed Consumption- weekly for 16 weeks, then monthly

Overall mean food consumption (g/animal/week) was significantly reduced in all treated groups. While no single week showed a significant reduction, the first three 13-week intervals, had significant reduction in mean feed consumption compared to control. The MD group had the greatest magnitude of reduction and there was also a significant reduction in the MD mean consumption from Weeks 56-104.

Ophthalmology- pre-dose, then Weeks 52 and 100 on 20 control and HD animals. Extended to LD and MD during Week 103.

There were 14 (35%) females at HD with incidence of corneal opacities compared to 7 (14%) control animals. No other findings of note.

Gross Pathology

Males: Protrusion of multiple abdominal organs was observed in treated animals sacrificed before Week 13, correlating with observed urogenital swelling without significant microscopic correlates. In LD and HD males shortly sacrificed after Week 14, the attending pathologist confirmed this finding which was determined to be herniation of the inguinal canal into the scrotum. These findings were not observed at necropsy after Week 15. Connective tissue protrusion and large urogenital fat pad was noted at LD and HD correlating with swollen urogenital area.

Table 17 Type and Incidence of Protruding Organs in Male Mice Sacrificed Before Week 13 and After Dosing Cessation Week 14

	Before Week 13					After Dosing Cessation Week 14				
	Group/Sex/Dose level (mg/kg/day)					Group/Sex/Dose level (mg/kg/day)				
	1M (0)	2M (100)	3M (400)	4M (1500)	5M (0)	1M (0)	2M (100)	3M (400)	4M (1500)	5M (0)
Number of animals examined	1	6	7	9	0	5	5	0	5	0
Mesenteric lymph node – protruding	0	1	2	1	0	Ileum – protruding	0	3	0	3
Jejunum – protruding	0	2	3	3	0	Caecum – protruding	0	4	0	3
Ileum – protruding	0	3	4	4	0	Colon – protruding	0	2	0	3
Caecum – protruding	0	3	4	6	0	Seminal vesicle – protruding	0	0	0	1
Colon – protruding	0	3	4	4	0	Urinary bladder – protruding	0	0	0	1
Seminal vesicle – protruding	0	1	0	2	0	Connective tissue				
Urinary bladder – protruding	0	0	1	3	0	- protruding	0	0	0	2
						- thick	1	5	0	5
						- large	1	2	0	4
						- firm	0	1	0	0

(Tables excerpted from Applicant's package)

Females: Overall, microscopic findings were rare, and except for the ovaries, occurred in both decedent and terminal sacrifice animals. Findings in the ovary were confined to termination animals and consisted of large ovaries with both masses and/or multiple masses at all doses with $\leq 10\%$ incidence. Dark liver was observed more often in decedent animals with increased frequency in treated animals at $\geq MD$, while pale liver more commonly occurred in decedent control animals. Dark mesenteric lymph node occurred more often in $\geq MD$. Findings of distended and dark gall bladder and distended and convoluted uterus occurred more frequently in control groups than treated animals. Microscopic findings directly correlated with gall bladder, ovary and uterus findings.

Table 18 Selected Macroscopic Findings of Note in Female Mice at Necropsy during a 2-Year Oral Ospemifene Carcinogenicity Study (n=51 total/group)

Tissue	Finding	0 mg/kg Control 1 (32, 19)*		100 mg/kg (25, 26)*		400 mg/kg (25, 26)*		1500 mg/kg (30, 21)*		0 mg/kg Control 2 (28, 23)*	
		D	T	D	T	D	T	D	T	D	T
Liver	Dark	1	0	0	0	5	0	5	5	2	0
	Pale	4	1	1	0	1	0	0	0	8	2
Mesenteric LN	Dark	0	1	0	0	1	4	4	4	0	2
Gall Bladder	Distended	14	10	0	1	0	1	1	1	7	9
	Dark	4	1	0	0	1	0	0	0	3	0
Uterus	Distended	12	10	2	4	4	4	6	6	13	19
	Convoluted	12	6	0	3	1	0	0	0	11	10
Ovary	Mass	-	0	-	5	-	4	-	4	-	0
	Multiple mass	-	0	-	3	-	1	-	3	-	0
	Large	-	0	-	3	-	3	-	1	-	0

* , first number in parentheses is decedent and second number is termination kill

Histopathology

Peer Review - Not stated

Neoplastic

Males were not evaluated.

While the incidence rates of liver and pituitary tumors were numerically higher in treated groups than controls, they were within the historical control (HC) ranges for female CD-1 mice. There was a decreased incidence of mammary and skin tumors noted in dosed groups compared to controls with 5 to 0 in the mammary gland and 4 to 0 in the skin, respectively. Other neoplastic findings in general were sporadic and consistent with the age, sex, and strain of mice.

Ovary-There was a statistically significant treatment-related increase in neoplastic findings in the ovary. These included benign and malignant tubulostromal tumors, sex-cord stromal tumors, granulosa cell tumors, and luteal tumors, compared to controls. Tubulostromal adenomas occurred in all dose groups, while both tubulostromal carcinomas were noted at the MD. Benign and malignant sex-cord stromal tumors occurred at increased incidence in all dose groups, compared to controls; there were no malignant tumors observed in controls. Benign luteomas showed a treatment-related increase incidence in all dose groups (2-10%) compared to control groups (5%), but the numbers were higher for LD (7) than MD and HD (4 each). Malignant luteomas were present in 5 MD and 1 HD animal compared to 0 control and LD animals. Benign granulosa cell tumors were present in 1 LD and 3 HD animals, and malignant granulosa cell tumors were found in 3 MD and 1 HD animals, compared to none in control animals. Additionally, two thecoma (both at MD), three cystadenoma (1 LD and 2 HD), and two cystadenocarcinoma (1 LD and 1 HD) were noted in treated groups, but not in controls. Except for benign sex chord stromal tumors (2 total, 1 each control group) and benign luteomas (5 total), no tumors were noted in control animals. There was no pattern related to occurrence in decedent or terminal kill animals.

Adrenal- Based on the overall pattern; there was a treatment-related increase in neoplastic findings in the adrenal. No tumors were noted in control and LD animals, and all malignant tumors were noted at HD (3, all in decedent animals). Two pheochromocytoma (1 benign and 1 malignant in decedents), 1 cortical adenoma, and 2 cortical carcinoma were present at HD. There was 1MD and 4 HD benign subcapsular cell adenoma noted.

Table 19 Selected Neoplastic Findings of Note in Female Mice at Necropsy during a 2-Year Oral Ospemifene Carcinogenicity Study (n=51/group)

Tissue	Finding	0 mg/kg Control 1	100 mg/kg	400 mg/kg	1500 mg/kg	0 mg/kg Control 2
Ovary	B- Thecoma			2		
	B- Cystadenoma		1		2	
	M- Cystadenocarcinoma		1		1	
	B- Tubulostromal adenoma		2	6	2	
	M- Tubulostromal carcinoma			2		
	B- Sex chord stromal tumor	1	8	13	13	1
	M- Sex chord stromal tumor		8	1	2	
	B- Luteoma	2	10	4	4	3
	M- Luteoma			5	1	
	B- Granulosa cell tumor		1		3	
	M- Granulosa cell tumor			3	1	
Adrenal	B- Subcapsular cell adenoma			1	4	
	B- Pheochromocytoma				1	
	M- Pheochromocytoma				1	
	B- Cortical adenoma				1	
	M- Cortical carcinoma				2	
Liver	B- Hepatocellular adenoma				2	
	M- Hepatocellular carcinoma				1	
	B- Hemangioma		1			
Pituitary	B- Adenoma	1		1	2	
	M- Adenoma				1	
Skin	M- Sarcoma	1				
	M- Fibrous histiocytoma					2
	M- Hemangiosarcoma					1
Mammary gland	B- Adenoma	1				1
	M- Adenocarcinoma	1				2
Uterus	B- Stromal polyp	9		1	2	3
	B- Hemangioma	2				
	B- Granular cell tumor					1
	M- Stromal sarcoma			1	1	1
	M- Leiomyosarcoma	2		1		1
	M- Histiocytic sarcoma					1

B, Benign; M, Malignant

Table 20 Adrenal and Ovary Neoplastic Findings in Female Mice at Necropsy during a 2-Year Oral Ospemifene Carcinogenicity Study

		Incidence of neoplastic lesions: adrenal gland									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
		0	100	400	1500	0	0	100	400	1500	0
Adrenal gland subcapsular cell tumour	No. examined:	-	-	-	-	-	51	51	51	51	51
	Grade -	-	-	-	-	-	51	51	50	47	51
	+	-	-	-	-	-	0	0	1	4	0
cortical adenoma	Grade -	-	-	-	-	-	51	51	51	50	51
	+	-	-	-	-	-	0	0	0	1	0
cortical carcinoma	Grade -	-	-	-	-	-	51	51	51	49	51
	+	-	-	-	-	-	0	0	0	2	0

Key: "-" = finding not present, "+" = finding present

		Incidence of neoplastic lesions: ovary									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
		0	100	400	1500	0	0	100	400	1500	0
Ovary Benign sex cord stromal tumour	No. examined:	-	-	-	-	-	51	51	51	51	51
	Grade -	-	-	-	-	-	50	43	38	38	50
	+	-	-	-	-	-	1	8	13	13	1
malignant sex cord stromal tumour	Grade -	-	-	-	-	-	51	50	50	49	51
	+	-	-	-	-	-	0	1	1	2	0
tubulostromal adenoma	Grade -	-	-	-	-	-	51	49	45	49	51
	+	-	-	-	-	-	0	2	6	2	0
tubulostromal carcinoma	Grade -	-	-	-	-	-	51	51	49	51	51
	+	-	-	-	-	-	0	0	2	0	0
Benign granulosa cell tumour	Grade -	-	-	-	-	-	51	50	51	48	51
	+	-	-	-	-	-	0	1	0	3	0
malignant granulosa cell Tumour	Grade -	-	-	-	-	-	51	51	48	50	51
	+	-	-	-	-	-	0	0	3	1	0
Benign luteoma	Grade -	-	-	-	-	-	49	41	47	47	48
	+	-	-	-	-	-	2	10	4	4	3
malignant luteoma	Grade -	-	-	-	-	-	51	51	46	50	51
	+	-	-	-	-	-	0	0	5	1	0

Key: "-" = finding not present, + = present

(Tables excerpted from Applicant's package)

The applicant's statistical analysis was conducted on combined neoplasms according to FDA Guidance. All determinations of significance were made comparing to control. Six neoplasms had a significant increasing dose-response and four had a significant decreasing dose-response. The following statistical significant increasing neoplasms were labeled (a)-(f):

- (a) adrenal subcapsular cell adenoma
- (b) adrenal cortical tumor
- (c) liver hepatocellular tumor
- (d) ovary adenoma/carcinoma
- (e) ovary sex cord/stromal tumor

(f) pituitary adenoma/carcinoma

Both adrenal (a) and (b) were significant for non-fatal neoplasms for overall dose-response, and for combined neoplasms at HD. Incidence (%) of adrenal (a) in HD group (7.8% in HD compared to 5.8% maximum HC rate) and (b) in all treated and HD groups are above HC rates for female CD-1 mice (2.0% and 5.9% compared to 0% in HC).

Ovary (d) was significant for overall dose-response of non-fatal neoplasms, and for combined neoplasms when comparing overall dose-response and HD. The 5.9% incidence of was greater compared to 3.9% maximum HC rate. Ovary (e) was significant for non-fatal neoplasms, and for combined neoplasms at all doses and overall dose-response. The incidence in all treated groups was 4 to 6-times higher than the maximal HC incidence (41.5% to 66.7% compared to 10.8%, respectively).

Liver (c) was significant for combined neoplasm overall dose-response. Pituitary (f) was significant for combined treatment -related neoplasm.

Table 21 List of Statistically Significant Increasing Dose-Response Neoplasms with P-Values and % Incidence (Combined Fatal+Non-fatal) from a 2-Year Oral Ospemifene Carcinogenicity Study

Tissue	Finding	Dose Response Trend (% incidence)			Control vs. LD (% incidence)			Control vs. MD (% incidence)			Control vs. HD (% incidence)		
		F	NF	All	F	NF	All	F	NF	All	F	NF	All
Adrenal	(a) subcapsular cell adenoma		0.001										0.007 (7.8)
	(b) cortical adenoma/carcinoma		0.008										0.042 (5.9)
Ovary	(d) adenoma/carcinoma		0.050	0.031			n.s. (3.8)						0.038 (5.9)
	(e) sex chord stromal tumor		<0.001	<0.001		<0.001	<0.001 (41.5)	.042	<0.001	<0.001 (66.7)		<0.001	<0.001 (51.0)
Liver	(c) hepatocellular adenoma/carcinoma			0.007									0.040 (5.9)
Pituitary	(f) adenoma/carcinoma			0.027									

n.s., not significant

The following were statistically significant decreasing combined neoplasms and are labeled (g)-(j):

- (g) mammary gland epithelial tumor:
Overall dose response (P=0.007)
- (h) uterus smooth muscle tumor:
Overall dose response (P=0.037)
- (i) blood vessel tumor:
Overall dose response (P=0.005)
- (j) uterus stromal tumor:
Controls v LD (P=0.002)

Non Neoplastic

Males: With so few males evaluated, there were few notable findings - all in decedent animals. There was interstitial cell hyperplasia of the testis in 1/10 and 2/10 of LD and HD (MD not evaluated), respectively, and benign interstitial cell adenoma in 1 HD male. Cellular debris of the epididymis was noted in 1/10 and 2/10 males at LD and HD, respectively.

Table 22 Selected Microscopic Findings of Note in Male Mice at Necropsy during a 2-Year Oral Ospemifene Carcinogenicity Study

Tissue	Finding	0 mg/kg Control 1 (5,1)*		100 mg/kg (5,5)*		400 mg/kg (0,0)*		1500 mg/kg (5,5)*		0 mg/kg Control 2 (0,4)*	
		D	T	D	T	D	T	D	T	D	T
Decedent/Termination											
Testis	Interstitial cell (IC) hyperplasia	0	0	1	0	--	--	2	0	--	--
	Benign IC adenoma	0	0	0	0	--	--	1	0	--	--
Epididymis	Cellular debris	0	0	1	0	--	--	2	0	--	--

*, first number in parentheses is decedent and second number is termination kill

--, not examined

Females: Findings were generally low and sporadic, except in estrogen target organs such as uterus, ovary, vagina, clitoral gland, and bone. In general, treatment-related effects showed little or no dose-dependency with the effects - or lack thereof - noted at all doses. This is likely due to saturation of exposure with only a 1.5 - 2.5 maximal increase in AUC from LD to MD and/or HD.

Ovary- There were increased incidences of hematopoiesis, tubulostromal hyperplasia (T-SH), sex chord stromal hyperplasia (SCSH), and interstitial cell hyperplasia (I-CH), and decreased incidences of arteritis and hemorrhagic cysts in treated groups compared to control. No dose-relationship was observed for these findings. Minimal to severe hematopoiesis was not present in control animals but was observed in 2, 3, and 6 LD, MD and HD groups, respectively. Minimal to severe T-SH was noted in 7, 6, and 6 in the LD, MD, and HD groups, respectively, and was not observed in control animals. Minimal to severe SCSH was on average about twice as common in treated animals with 23, 33, and 25 in the LD, MD, and HD groups, respectively, and 25/102 in controls. Minimal to moderate I-CH was observed in 40, 27, and 37 in the LD, MD, and HD groups, respectively, while only 24/102 control animals, for an increase by 2-fold. Arteritis was not observed in any treated animal and was noted in 11/102 control animals. There were 5, 2, and 3 incidences of hemorrhagic cysts in LD, MD, and HD, respectively compared to 11 and 7 in control groups.

Uterus- There was a strong treatment-related increase of stromal hyalinization (collagen deposits near and around glandular regions) noted in almost all treated animals (148/153), but only was observed in 2/102 control animals. Squamous hyperplasia occurred in 3 decedents of each treated group, and 2 animals in Control 2 group. Incidence of minimal to moderate adenomyosis was increased by ~2-fold in treated groups with no dose-relationship compared to controls; severity did generally increase with dose. Cystic endometrial hyperplasia was essentially present in all

treated animals (48, 51, and 50 out of 51/group at LD, MD, and HD, respectively), but was present in fewer controls (80%). This finding was highlighted by the applicant, likely due to the reduced uterine neoplasms observed. Like the ovary, arteritis was increased in control animals (17/102), but unlike the ovary, 2 LD and 3 HD animals had this finding.

Vagina- There was an increase of 2-fold in incidence of minimal to severe squamous cell hyperplasia (SCH) in treated groups, compared to control. Additionally, all treated animals with SCH had a finding of vaginal mucification, whereas the incidence in control animals was reduced from ~40% to ~20%.

Clitoral gland- There was a dose-dependent increase in incidence and severity of minimal to severe squamous cell hyperplasia noted. There were 3, 5, and 8 animals at LD, MD, and HD, respectively, with none observed in control animals.

Bone- Minimal to severe hyperostosis of the femur and sternum was increased with incidences of 78-96% compared to 4-6% in controls at all doses. The overall severity increased with dose, and all (12/12) control animal findings were minimal. Fibro-osseous hyperplasia was noted in 2 HD animals at termination.

Liver- There was a slight increase in hematopoiesis in all treated groups that occurred more often in decedents than terminal kills. This appears to correlate with gross pathology findings of dark liver in some treated animals.

Gall bladder- Distention that correlated with gross pathology was decreased in treated groups with 1, 4, and 2 in LD, MD, and HD, respectively, compared to control groups (22 and 15).

Adrenal- There were increases in the presence of cortical foci (eosinic, basophilic, and normochromatic) in all treated groups with 4, 7, and 5 in LD, MD, and HD, respectively, compared to 1 in combined control groups. Cortical atrophy was present in 2, 3, and 3 in LD, MD, and HD, respectively, compared to none in control groups. Corticomedullary pigment incidence increased in all treated groups compared to control.

Mammary gland- There was decreased acinar hyperplasia, cystic hyperplasia, and inflammatory cell infiltration and increased duct ectasia, but incidences were low. There were no incidences of acinar hyperplasia and inflammatory cell infiltration in treated animals compared to 3 and 10 in control groups, respectively. There were 1, 1, and 2 incidences of duct ectasia in LD, MD, and HD, respectively compared to none in combined controls.

Eye- Corneal mineralization was slightly increased at HD compared to controls (12% vs. 5%). This possibly reflects the observed ophthalmologic finding of corneal opacity.

Table 23 Selected Microscopic Findings of Note in Female Mice at Necropsy during a 2-Year Oral Ospemifene Carcinogenicity Study (n=51 total/group)

Tissue	Finding	0 mg/kg Control 1 (32,19)*		100 mg/kg (25,26)*		400 mg/kg (25,26)*		1500 mg/kg (30,21)*		0 mg/kg Control 2 (28,23)*	
		D	T	D	T	D	T	D	T	D	T
Decedent/Termination											
Mammary gland	Acinar hyperplasia	2	-	0	-	0	-	0	-	1	-
	Cystic hyperplasia	1	3	0	2	0	0	0	4	12	2
	Duct ectasia	0	-	1	-	1	-	2	-	0	-
	Inflammatory cell infiltration	2	4	0	0	0	0	0	0	1	3
Femur+marrow	Hyperostosis	0	2	21	19	20	24	28	21	1	2
Sternum+marrow	Hyperostosis	1	2	22	25	21	26	28	21	2	3
Bone	Fibro-osseous hyperplasia	-	0	-	0	-	0	-	2	-	0
Liver	Hematopoiesis	5	5	9	10	7	10	13	12	4	9
Gall bladder	Distension	12	10	0	1	3	1	1	1	6	9
	Cholelithiasis	3	0	0	1	0	0	0	1	0	3
Adrenal	Corticomedullary pigment	10	11	12	19	12	16	16	19	6	6
	Cortical foci(all types)	1	0	0	4	0	7	1	4	0	0
	Cortical atrophy	0	0	1	1	2	1	3	0	0	0
Ovary	Arteritis	4	4	0	0	0	0	0	0	1	2
	Hemorrhagic cysts	8	3	2	3	1	1	2	1	4	3
	Tubulostromal hyperplasia	0	0	0	7	1	5	3	3	0	0
	Sex chord stromal hyperplasia	1	11	7	16	13	20	9	16	4	9
	Interstitial cell hyperplasia	4	9	18	22	10	17	19	18	6	5
Uterus	Arteritis	4	5	1	1	0	0	0	1	2	6
	Stromal hyalinization	1	0	23	26	25	25	28	21	1	0
	Squamous hyperplasia	0	0	3	0	3	0	3	0	1	1
	Adenomyosis	9	6	12	11	11	17	16	16	3	10
	Cystic endometrial hyperplasia	23	18	22	25	23	26	27	21	21	20
Vagina	Squamous cell hyperplasia	14	14	23	25	25	26	29	21	16	5
	Mucification	7	3	16	23	19	20	23	21	8	4
Clitoral gland	Squamous cell hyperplasia	0	0	1	2	1	4	3	5	0	0
Eye	Corneal mineralization	1	2	1	1	0	3	0	6	0	2

*, first number in parentheses is decedent and second number is termination kill

-, not noted in any decedent/terminal kill dose group

Toxicokinetics- Day 1, Months 3, 6, 9, and 12 at 2, 4, 6, 8, 12 and 24 hrs post-dose. Three animals/group were used, with no animal bled twice during any given TK period- usually every other time.

Females: Ospemifene was rapidly absorbed and eliminated at all doses with a t_{max} range of 2 - 6 hr and $t_{1/2}$ range of 3.39 - 8.72 hr, respectively. It appears that t_{max} decreased over time while $t_{1/2}$ increased. Exposure based on AUC and C_{max} decreased over time, suggesting metabolic activation caused the reduction. Exposure did not increase in a consistent, dose-proportional manner; only through Week 13 were there any dose-related increases in exposure. In fact, AUC exposure plateaued from 400 to 1500/1000 mg/kg at all timepoints, except Day 1.

HM-187 showed comparable t_{max} and $t_{1/2}$ ranges to the parent with 2 - 12 hr and 2.31 - 6.77 hr, respectively. Unlike ospemifene, there was not a dramatic decrease in exposure over time, and there was a less than dose-proportional increase in exposure. These data suggests that formation of the HM-187 metabolite was rate-limiting

compared to elimination. The AUC ratios (MR_{AUC}) ranged from 0.2 - 0.8 showing that HM-187 concentration was less than the parent at all times and doses. However, there was a duration-dependent increase in the ratios.

HM-136 showed comparable t_{max} ranging from 2 - 8 hr to parent and $t_{1/2}$ ranging from 2.8 - 7.8 hr, but in most instances $t_{1/2}$ was not calculable, likely due to the generally low levels measured. Like ospemifene and HM-187, exposure increased less than dose-proportional and decreased in a duration-dependent manner. The AUC ratio to parent was much lower ranging from 0.021 to 0.065. Again like for HM-187, there was a duration-dependent increase in the ratios.

Males: There was only data collected on Day 1 and during Week 13, but the findings were generally similar to females showing to sex-based difference.

Table 24 Toxicokinetics of Ospemifene during a 2-Year Oral Ospemifene Mouse Carcinogenicity Study

Occasion	Dose (mg/kg/day)	Sex	AUC _{0-τ} (ng.h/mL)	AUC _{0-τ} (norm)	C _{max} (ng/mL)	C _{max} (norm)	t _{max} (h)	t _{1/2} (h)
Day 1	100	Male	37600	376	6530	65.3	2.00	3.42
		Female	42400	424	6750	67.5	4.00	3.44
	400	Male	96400	241	16500	41.3	2.00	2.65
		Female	57100	143	6920	17.3	2.00	3.94
	1500	Male	82600	55.1	9990	6.66	6.00	NC
		Female	123000	82.3	17700	11.8	6.00	3.55
Week 13	100	Male	19300	193	5170	51.7	2.00	4.16
		Female	18400	184	4310	43.1	2.00	NC
	400	Male	32100	80.1	6670	16.7	2.00	3.52
		Female	34700	86.7	4650	11.6	4.00	3.39
	1500	Male	30300	20.2	4010	2.68	2.00	3.80
		Female	31100	20.8	3600	2.40	2.00	5.69
Week 26	100	Female	16300	163	4770	47.7	2.00	5.37
	400		19300	48.2	2940	7.34	4.00	6.31
	1000		28900	19.3	2740	1.82	4.00	5.74
Week 39	100	Female	20000	200	3770	37.7	2.00	5.94
	400		30300	75.7	4480	11.2	2.00	3.34
	1000		31700	21.1	2830	1.89	2.00	3.91
Week 52	100	Female	11400	114	3190	31.9	2.00	4.51
	400		21700	54.3	4120	10.3	2.00	4.31
	1000		25400	17.0	2270	1.51	2.00	8.72

AUC (norm) = AUC [ng.h/mL] / dose [mg/kg/day]

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]

τ = 24 hours

NA = Not applicable

NC = Not calculable

(Table excerpted from Applicant's package)

Table 25 Toxicokinetics of Metabolite HM-187 during a 2-Year Oral Ospemifene Mouse Carcinogenicity Study

Occasion	Dose (mg/kg/day)	Sex	AUC _{0-τ} (ng.h/mL)	AUC _{0-τ} (norm)	C _{max} (ng/mL)	C _{max} (norm)	t _{max} (h)	t _{1/2} (h)	RA _{AUC}	RA _{Cmax}	MR _{AUC}	MR _{Cmax}
Day 1	100	Male	9960	99.6	1830	18.3	4.00	NC	NA	NA	0.265	0.281
		Female	12400	124	1800	18.0	4.00	2.82	NA	NA	0.293	0.267
	400	Male	21300	53.3	2680	6.69	2.00	2.43	NA	NA	0.221	0.162
		Female	17600	43.9	1940	4.86	6.00	5.34	NA	NA	0.308	0.281
	1500	Male	16500	11.0	2150	1.43	4.00	2.62	NA	NA	0.200	0.215
		Female	25700	17.2	3330	2.22	6.00	4.26	NA	NA	0.209	0.188
Week 13	100	Male	8770	87.7	2090	20.9	2.00	6.33	0.880	1.14	0.454	0.404
		Female	9630	96.3	1890	18.9	2.00	6.77	0.774	1.05	0.524	0.439
	400	Male	11400	28.5	1840	4.60	2.00	2.96	0.535	0.687	0.355	0.276
		Female	18400	46.0	2200	5.51	8.00	NC	1.05	1.13	0.531	0.474
	1500	Male	11200	7.46	1050	0.702	2.00	4.26	0.678	0.490	0.369	0.262
		Female	20600	13.7	1490	0.991	12.0	NC	0.799	0.446	0.661	0.413
Week 26	100	Female	11200	112	2440	24.4	2.00	NC	0.900	1.36	0.686	0.512
	400		13000	32.5	1860	4.66	2.00	2.31	0.740	0.959	0.673	0.635
	1500		23000	15.3	2070	1.38	4.00	4.26	0.894	0.622	0.795	0.758
Week 39	100	Female	11000	110	2110	21.1	2.00	4.66	0.887	1.17	0.552	0.561
	400		16400	40.9	1830	4.58	2.00	4.28	0.933	0.942	0.541	0.408
	1500		20300	13.6	1930	1.29	2.00	NC	0.790	0.579	0.643	0.681
Week 52	100	Female	7910	79.1	1760	17.6	2.00	3.38	0.635	0.976	0.691	0.551
	400		12700	31.7	1950	4.88	2.00	3.20	0.722	1.01	0.584	0.474
	1500		18600	12.4	1250	0.836	2.00	NC	0.721	0.376	0.730	0.281

AUC (norm) = AUC [ng.h/mL] / dose [mg/kg/day]

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]

τ = 24 hours

NA = Not applicable

NC = Not calculable

(Table excerpted from Applicant's package)

Table 26 Toxicokinetics of Metabolite HM-136 during a 2-Year Oral Ospemifene Mouse Carcinogenicity Study

Occasion	Dose (mg/kg/day)	Sex	AUC _{0-τ} (ng.h/mL)	AUC _{0-τ} (norm)	C _{max} (ng/mL)	C _{max} (norm)	t _{max} (h)	t _{1/2} (h)	RA _{AUC}	RA _{Cmax}	MR _{AUC}	MR _{Cmax}
Day 1	100	Male	1100	11.0	134	1.34	2.00	NC	NA	NA	0.0292	0.0205
		Female	2280	22.8	310	3.10	4.00	NC	NA	NA	0.0538	0.0459
	400	Male	3230	8.06	309	0.773	6.00	NC	NA	NA	0.0335	0.0187
		Female	3710	9.26	582	1.45	6.00	3.32	NA	NA	0.0649	0.0841
	1500	Male	2750	1.83	374	0.250	4.00	3.43	NA	NA	0.0333	0.0375
		Female	7620	5.08	829	0.553	4.00	4.43	NA	NA	0.0617	0.0467
Week 13	100	Male	913	9.13	240	2.40	2.00	NC	0.832	1.79	0.0473	0.0464
		Female	1050	10.5	244	2.44	2.00	NC	0.459	0.788	0.0571	0.0566
	400	Male	1200	3.01	198	0.494	2.00	6.32	0.373	0.639	0.0375	0.0297
		Female	1280	3.21	127	0.319	8.00	NC	0.346	0.219	0.0370	0.0274
	1500	Male	1040	0.694	74.5	0.0497	2.00	NC	0.379	0.199	0.0344	0.0186
		Female	1560	1.04	103	0.0688	2.00	NC	0.205	0.125	0.0502	0.0287
Week 26	100	Female	517	5.17	99.0	0.990	2.00	NC	0.226	0.319	0.0317	0.0207
	400		734	1.84	95.3	0.238	6.00	NC	0.198	0.164	0.0381	0.0325
	1500		1180	0.784	121	0.0807	4.00	35.9	0.154	0.146	0.0406	0.0442
Week 39	100	Female	927	9.27	192	1.92	2.00	6.21	0.406	0.619	0.0464	0.0510
	400		1200	3.01	124	0.311	2.00	7.81	0.324	0.214	0.0397	0.0278
	1500		1290	0.860	107	0.0715	2.00	NC	0.169	0.129	0.0408	0.0379
Week 52	100	Female	444	4.44	92.6	0.926	2.00	4.66	0.195	0.299	0.0388	0.0290
	400		866	2.16	132	0.329	2.00	2.76	0.234	0.226	0.0399	0.0319
	1500		1090	0.728	68.6	0.0457	4.00	NC	0.143	0.0827	0.0429	0.0302

AUC (norm) = AUC [ng.h/mL] / dose [mg/kg/day]

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]

τ = 24 hours

NA = Not applicable

NC = Not calculable

(Table excerpted from Applicant's package)

Dosing Formulation Analysis

Homogeneity was assessed from top, middle and bottom of doses prepared on Days 1, 2, and 7 and was acceptable with all values within protocol defined range of 90 -110%. Test article concentrations were evaluated from middle of samples prepared in Weeks 1 and 4, Months 3, 6, 9, 12, 15, 18, 21, and 24 (co-incident with TK sampling) All sample results, except 3 out of 94, were within protocol defined range of 90 -110%.

**Study title: Ospemifene: 104 Week Oral (Gavage) Administration
Oncogenicity Study in the Rat**

Study no.: 15-44405
Study report location: EDR #0000, 4.2.3.4.1.1.15-44405
Conducting laboratory and location: (b) (4)
Date of study initiation: October 24, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Three lots of Ospemifene were used:
Lot 4-Batch #1052557, 99.6%
Lot 5-Batch #1052557, 99.6%
Lot 6-Batch #1195107, 100.2%
CAC concurrence: Yes

Key Study Findings**Adequacy of Carcinogenicity Study**

Doses based on MFD (saturation of exposure) were used with exCAC concurrence. There was sufficient survival to conduct a statistical evaluation. Neoplastic findings were consistent with known pharmacology in rats. The exposure based on AUC at termination did not achieve sufficient multiples of the clinical exposure at the proposed dose (30%, 60%, and 125%), but this was expected.

Appropriateness of Test Models

Species and strain were same as those used in repeat dose toxicity studies, and the oral route is the proposed clinical route. Both neoplastic and non-neoplastic treatment-related effects were related to pharmacology in estrogen target organs, including testes, ovary, uterus, mammary gland, bone, liver, adrenal, and pituitary.

Evaluation of Tumor Findings

Oral ospemifene was tumorigenic in male and female Han Wistar rats treated for two years. There were significant treatment-related increases in liver and thymus neoplasms in both sexes without a dose relationship. The incidences of liver and thymus neoplasms were above maximum historical control rates for untreated controls. The sponsor did not provide any historical data for the corn oil vehicle. Additionally, there were significant decreases in lymphocytic leukemia in males, mammary gland (females), pituitary (both sexes), skin (both sexes), thyroid (males), and testis uterus neoplasms. All findings, except reduced skin neoplasms, are consistent with the established pharmacology/toxicology of ospemifene, and the carcinogenicity of other mixed estrogen agonist/antagonists.

Methods

Doses: 0, 10, 50, 300, 0
 Frequency of dosing: daily
 Dose volume: 2 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: Corn oil
 Basis of dose selection: Saturation of absorption based on 13 week daily oral 50, 300, 2000 mg/kg DRF
 Species/Strain: Rat/CrlWI(Han)
 Number/Sex/Group: 50/sex/group
 Age: Approx. 6 weeks
 ♀: 118.2 - 199.5 g; ♂: 109.6 - 163.9 g
 Animal housing: single exclusive room, 5/cage
 Paradigm for dietary restriction: None, *ad libitum* to SQC Rat and Mouse Maintenance Diet No. 1, Expanded (b) (4)
 Dual control employed: Yes
 Interim sacrifice: No
 Satellite groups: Control 1: 5/sex, Treated Groups: 10/sex/group
 Deviation from study protocol: No major deviations reported

Mortality

There was a significant treatment-related effect on increased overall survival in both sexes at all doses. Slightly more treated and total males survived to termination than females. Survival in Week 104 at study termination was 68%, 74%, 96%, 86%, and 94% in Control 1, Control 2, LD, MD, and HD groups of males, respectively. Female survival was 72%, 58%, 92%, 82%, and 88% in Control 1, Control 2, LD, MD, and HD groups, respectively. The pattern of causes of death was consistent for rats of this strain and age.

Table 27 Statistical tests for Decreasing Drug Related Mortality during a 2-Year Oral Ospemifene Rat Carcinogenicity Study

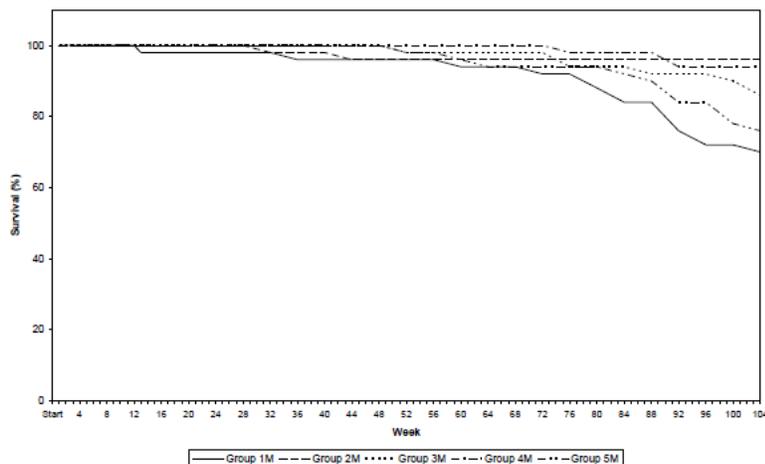
Sex	Type of death	Group					Results (P-values)			
		1 (C)	2 (L)	3 (I)	4 (H)	5 (C)	C _L I _L H	C _v L	C _v I	C _v H
M	Accident	0	0	0	0	0				
	Dead or Moribund	16	2	7	3	13	.004**	<.001***	.030*	<.001***
	Terminal Kill	34	48	43	47	37				
	Total	50	50	50	50	50				
F	Accident	0	0	1	1	0				
	Dead or Moribund	34	26	12	19	27	.007**	<.001***	.017*	.001**
	Terminal Kill	16	24	37	30	23				
	Total	50	50	50	50	50				

C=Control, L=Low dose, I=Intermediate dose, H=High dose

* P<0.05
 ** P<0.01
 *** P<0.001

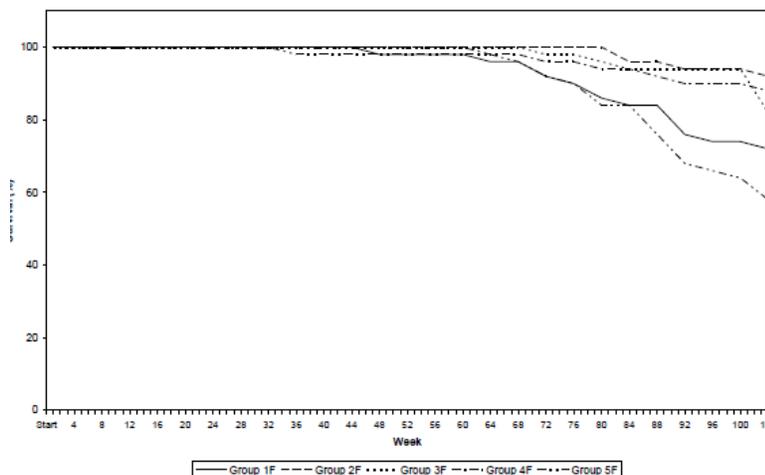
(Table excerpted from Applicant's package)

Figure 8 Group Mean Survival (%) of Male Rats during a 2-Year Oral Ospemifene Carcinogenicity Study



(Figure excerpted from Applicant's package)

Figure 9 Group Mean Survival (%) of Female Rats during a 2-Year Oral Ospemifene Carcinogenicity Study



(Figure excerpted from Applicant's package)

Clinical Signs- daily, weekly for tumor masses

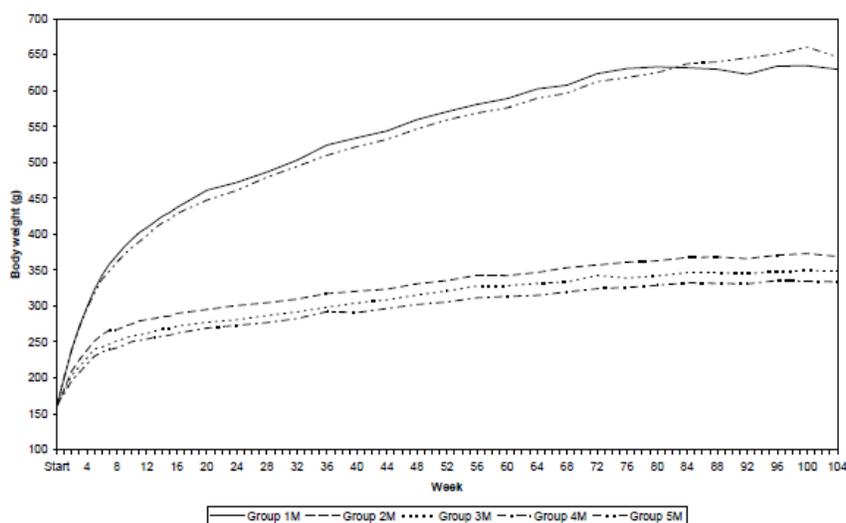
Small testes were observed in all treated groups of males beginning during Week 78 and continued through the end of the study with a duration-dependent increase in incidence. No control animals had this observation. In general, the incidence was lowest at LD (2-21%), highest at MD (17-69%), but not much higher than HD (12-49%). The highest incidence was observed in Week 104 for all treated groups. Thinning fur occurred at a greater incidence of roughly 50% in treated groups of both sexes compared to control groups. No other clinical signs showed a clear treatment-related effect.

There were decreased incidences of palpable masses in all treated groups of both sexes compared to the controls.

Body Weights- pre-dose, Day 1, weekly for 16 weeks, then monthly

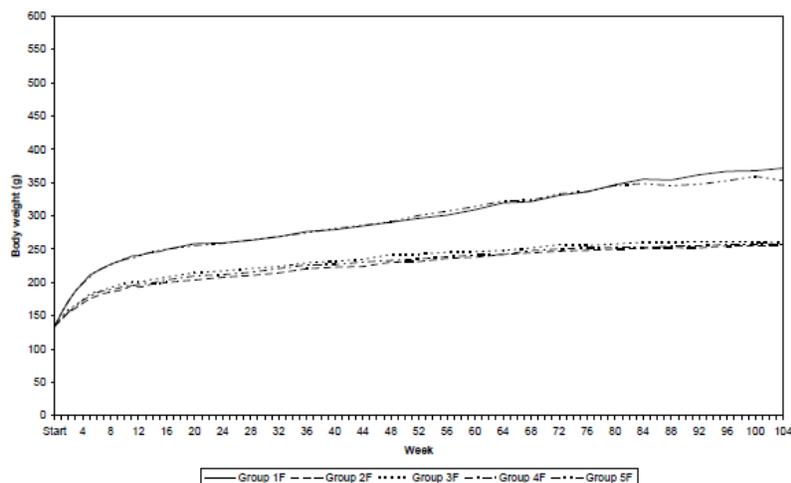
Body weight gain in the LD, MD, and HD groups of males was significantly and markedly reduced by 56%, 60%, and 64%, respectively. There was not a strong dose relationship and the pattern of weight gain was comparable for all treated groups. The reduced male body weight gain occurred starting in Week 1 and continued over the entire study period. The body weight gains were significantly reduced but slightly less marked in females with reductions of 49%, 47%, and 48% in the LD, MD, and HD groups, respectively. There was no dose relationship and the pattern of weight gain was comparable for all treated groups. This reduced weight gain may partly explain the greater survival in treated animals.

Figure 10 Group Mean Body Weights of Male Rats during a 2-Year Oral Ospemifene Carcinogenicity Study



(Figure excerpted from Applicant's package)

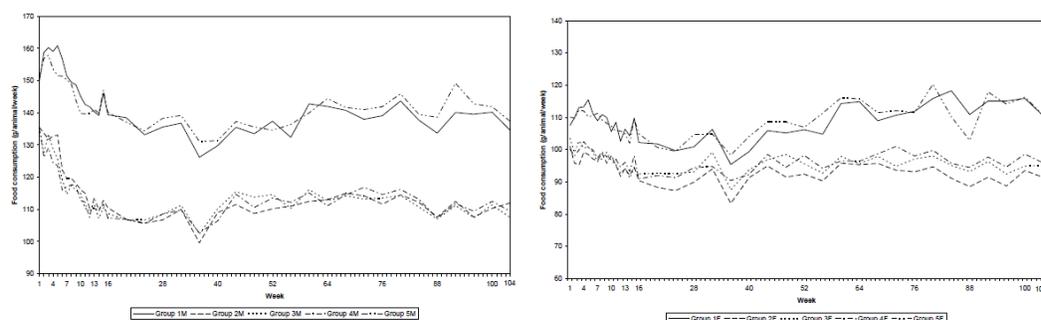
Figure 11 Group Mean Body Weights of Female Rats during a 2-Year Oral Ospemifene Carcinogenicity Study over Time



(Figure excerpted from Applicant's package)

Feed Consumption- weekly for 16 weeks, then monthly

Overall mean food consumption (g/animal/week) was significantly reduced in all treated groups. There was a reduction of 20% total food consumption in all treated male groups, while females consumed 13%, 11%, and 11% less, at LD, MD, and HD, respectively. The pattern of consumption was parallel to controls, but obviously reduced.



Ophthalmology- pre-dose, then Weeks 52 and 100 on the first ^t 20 control and HD animals.

Not remarkable.

Hematology- termination

There was a significant reduction in RBCs in males at all doses, whereas females increased- significantly at LD and MD. Neutrophils were reduced at all doses in both sexes, except LD females. However, Control 2 was increased compared to Control 1, suggesting high variability for this parameter.

Table 28 Selected Hematology Parameters at Termination of 2-Year Rat Oral Ospemifene Carcinogenicity Study

Parameter	10 mg/kg		50 mg/kg		300 mg/kg		0 mg/kg Control 2	
	M	F	M	F	M	F	M	F
RBCs ($10^{12}/L$)	-5***	+4**	-7***	+4**	-6***	+3	--	--
Neutrophils ($10^9/L$)	-36**		-45**	-29*	-45**	-29*	+45	+29

***, P<0.001; **, P<0.01; *, P<0.05

Gross Pathology

Differences in treated animals compared to controls were usually related to the pharmacology of the test article with little or no dose relationship at MD and HD. The gross pathological findings generally correlated with the reported microscopic and tumor findings. There was no notable difference in decedent or terminal kill findings. In general, females had greater increase or decrease in animals affected than males. In both males and females, there were increased incidences of fur loss on skin, dark adrenals, dark kidneys (except LD and MD males), pale area/foci on the lungs, small pituitary, firm, large and/or red thymus, mass on liver, and mass on thymus in all treated groups. There were decreased incidences of pale and/or mottled liver, large mandibular lymph node, large spleen, raised area on pituitary, and mass on pituitary.

Males had increased incidence of mottled kidney, large thyroid, abnormal, small, soft, and/or dark testes, small epididymides, seminal vesicles, preputial gland, and prostate; and decreased incidences of pale adrenal, dark mesenteric lymph node, large liver, and large thyroid. Females had increased incidences of thick mammary gland, cysts on ovary (except MD), small and/or pale ovary and thin uterus; and decreased firm uterus, large pituitary.

Table 29 Selected Macroscopic Findings of Note at Necropsy in 2-Year Rat Oral Ospemifene Carcinogenicity Study (n=50/group)

Tissue	Finding	mg/kg									
		Males					Females				
		0	10	50	300	0	0	10	50	300	0
Testes	Small	5	25	38	35	8					
	Dark	2	10	12	15	6					
	Soft	9	12	19	23	10					
	Abnormal	4	0	0	1	8					
Epididymides	Small	1	13	24	19	3					
Seminal vesicle	Small	8	46	49	47	3					
Preputial gland	Small	2	17	16	12	4					
Prostate	Small	4	24	26	35	1					
Ovary	Cyst						5	11	4	11	4
	Small						2	10	7	10	2
	Pale						1	4	5	6	3
Uterus	Thin						2	25	20	22	2
	Firm						5	2	1	1	6
Mammary Gland	Thick	0	1	0	0	0	3	1	0	0	13
Skin	Fur loss	5	5	19	16	2	7	16	29	25	5
Adrenal	Dark	2	11	8	8	2	3	7	6	5	1
	Pale	6	1	0	0	5	2	0	3	0	0
Kidney	Dark	1	1	1	4	0	4	14	10	13	1
	Mottled	0	2	2	4	0	2	0	2	0	3
Lung	Pale foci	9	15	16	21	11	23	16	12	15	12
	Pale area	18	33	32	28	23	19	35	36	38	27
Mandibular LN	Large	3	0	0	0	5	3	0	0	0	0
Mesenteric LN	Dark	7	1	1	4	8	1	0	3	1	4
Pituitary	Small	1	2	4	4	2	0	9	9	7	0
	Mass	5	0	0	1	5	13	0	0	0	15
	Large	1	0	0	0	0	6	0	0	0	6
	Raised area	2	1	0	0	1	5	0	0	0	1
Liver	Pale	23	12	5	11	30	14	6	3	6	6
	Mottled	37	12	11	9	40	23	8	3	3	21
	Large	6	0	1	1	7	1	0	0	0	0
	Mass	0	1	3	2	0	0	1	5	2	0
Spleen	Large	6	2	1	0	7	6	1	0	4	6
Thymus	Large	1	2	1	3	1	0	1	6	4	0
	Red	0	1	1	0	0	0	1	3	2	1
	Mass	0	1	6	2	0	2	7	13	6	2
	Firm	0	0	1	1	1	0	1	5	3	0
	Red focus	0	0	0	2	0	1	1	3	3	0
Thyroid	Large	8	0	1	2	3	1	0	3	1	3

Histopathology

Peer Review - Not stated

Neoplastic

There were treatment-related findings in the uterus, mammary gland, testes, liver, pituitary, lymphocytic leukemia, skin, and thyroid with increased neoplasms in liver and

thymus and decreased neoplasms in all other tissues. The thymus and liver findings will be discussed below in greatest detail, since they were significantly increased compared to control, are pharmacologically relevant, and were above Historical Control (HC) incidence rates. Except for skin, neoplastic findings are consistent with the known pharmacologic effects of a mixed estrogen agonist/antagonist on cell types that express the estrogen receptor. Other neoplastic findings in general were sporadic and consistent with the age, sex, and strain of rats.

- **Liver-** There was an increase in both benign and malignant hepatocellular tumors in the treated groups of both sexes. There were 2, 3, and 2 benign tumors in LD, MD, and HD males, respectively, and 1, 4, and 4 in LD, MD, and HD females, respectively, compared to none in any control group of either sex. The incidence rates exceeded or equaled the HC rate (1.7% for males, 2% for females) for all treated groups. There was 1 HD male and 2 MD females with malignant tumors compared to no concurrent or HC animals.
- **Thymus-** There was an increase in benign thymoma in both sexes and malignant thymoma in females. The incidence of benign thymoma was sex-dependent with twice as many treated female animals/group with 28% of treated females compared to 15% of treated males having this finding. There were 3 and 2 animals in the female control groups (2.5% incidence rate), with no control males with thymoma. Malignant thymoma was present in 2 MD females, but in no other group of either sex. HC data confirms this finding showing that females have about 2.5-fold background incidence compared to males.

The hemangioma in spleen occurred in 0, 6, and 2 of the LD, MD, and HD groups, respectively, compared to 1 each of the control in males. In females, there were 1, 2, and 3 tumors in the LD, MD, and HD groups, respectively, compared to none in the control groups. This finding is not recorded in the HC data, but was not significant in an appropriate merging and analysis for blood vessel tumors.

In the mammary gland of females, benign fibroadenomas were dramatically reduced from 27% of controls to 0% in treated animals (HC: 7.4%-35.6%). However, there was 1 LD and 1 HD in males, with none in concurrent and HC. There were 3 benign testis interstitial tumors in control with none present in treated groups. Uterine stromal tumors were reduced from 5 in controls to 1 in LD females. Pituitary adenoma/carcinomas decreased from 50% in female controls to 1 LD animals. In males, the incidence was 2%, 4%, and 4%, in LD, MD, and LD, respectively, compared to 23% for controls. Thyroid follicular cell tumors in males were reduced from 3 in each control group to 1 at LD.

Table 30 Selected Neoplastic Findings of Note* at Necropsy of the 2-Year Rat Oral Ospemifene Carcinogenicity Study

Tissue	Finding	mg/kg									
		Males					Females				
		0	10	50	300	0	0	10	50	300	0
Testes	B- Interstitial cell tumor	3	0	0	0	0					
Ovary	B- Cystadenoma						<i>0</i>	<i>0</i>	<i>1</i>	<i>1</i>	<i>0</i>
	B- Sex chord stromal tumor						<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>
	B- Granulosa cell tumor						<i>0</i>	<i>1</i>	<i>0</i>	<i>1</i>	<i>0</i>
Uterus	B- Stromal polyp						5	1	0	0	3
	M- Stromal sarcoma						1	0	0	0	1
	M- Adenocarcinoma						4	1	0	2	1
	M- Histocytic sarcoma						1	0	0	0	0
	M- Malignant schwannoma						<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>
	M- Squamous cell carcinoma						<i>0</i>	<i>0</i>	<i>1</i>	<i>1</i>	<i>0</i>
Mammary gland	B- Fibroadenoma	<i>0</i>	<i>1</i>	<i>0</i>	<i>1</i>	<i>0</i>	12	0	0	0	15
	B- Adenoma	0	0	0	0	0	1	0	0	0	1
Skin	B- Fibroma	1	0	0	0	6	0	0	0	0	3
	B- Dermal fibroma	0	0	0	0	2	0	0	0	0	0
	B- Benign hair follicle tumor	4	0	0	0	4	1	0	0	0	0
	M- Malignant basal cell tumor	2	0	0	0	0	0	0	0	0	0
	M- Fibrosarcoma	0	0	0	0	1	0	0	0	0	0
	M- Sarcoma NOS	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	0	0	0	0	0
Adrenal	B- Cortical adenoma	<i>0</i>	<i>1</i>	<i>1</i>	<i>1</i>	<i>0</i>	0	0	0	0	0
	M- Cortical carcinoma	1	0	0	0	0	0	0	0	0	0
Liver	B- Hepatocellular adenoma	<i>0</i>	<i>2</i>	<i>3</i>	<i>2</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>4</i>	<i>4</i>	<i>0</i>
	M- Hepatocellular carcinoma	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>2</i>	<i>0</i>	<i>0</i>
Pituitary	B- Adenoma	13	1	2	2	10	24	1	0	0	26
	M- Carcinoma	0	0	0	0	0	1	0	0	0	0
Thymus	B- Thymoma	<i>0</i>	<i>5</i>	<i>8</i>	<i>9</i>	<i>0</i>	<i>3</i>	<i>10</i>	<i>21</i>	<i>11</i>	<i>2</i>
	M- Malignant thymoma	<i>0</i>	<i>0</i>	<i>2</i>	<i>0</i>						
Kidney	B- Tubular cell adenoma	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	0	0	0	0	0
	M- Nephroblastoma	1	0	0	0	0	0	0	0	0	0
Spleen	B- Hemangioma	1	0	3	2	1	<i>0</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>0</i>
Thyroid	B- Follicular cell adenoma	3	1	0	0	3	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>
	M- Follicular cell carcinoma	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>	0	0	0	1	1
Blood	Lymphocytic leukemia	2	0	0	0	2	0	0	0	0	0

B, Benign; M, Malignant

*The values in grey represent where one sex had no tumors in any group, and the values in **bold** and *italics* represent where there was a numerical increase in treated groups compared to controls.

Table 31 Incidence Rates for Merged Tumors Observed Greater or Less Than Historical Control Rates

Merged Tumor Type	Maximal Observed Rate & Dose		Maximal Historical Control Rate Range	
	Males	Females	Males	Females
Greater than HC				
Thymus epithelial tumors	18% - HD	22% - MD	0 - 8.5%	3.4 - 12.5%
Hepatocellular adenoma/carcinomas	6% - MD	8% - MD&HD	0 - 1.7%	1.0 - 2.0%
Ovary adenoma/carcinomas	--	2% - MD&HD	--	0 - 1.6%
Ovary sex chord stromal; tumors	--	4% - LD	--	0 - 2.0%
Adrenal cortical tumors	2% - all	0%	0 - 1.7%	0 - 2.0%
Kidney tubular cell adenoma	2% - HD	0%	0%	0 - 1.0%
Less than HC				
Testis Interstitial tumors	0% - all	--	1.0 - 4.2%	--
Uterine stromal tumors	--	2% - LD	--	11.7 - 24.0%
Pituitary adenoma/carcinomas	4% - MD&HD	0%	14.0 - 34.3%	48.0 - 60.0%
Thyroid follicular cell tumors	2% - LD&MD	2% - MD	7.2 - 12.0%	8.1 - 22.0%
> in Males, < in Females				
Mammary gland epithelial tumors	2% - LD&HD	0%	0%	7.4 - 35.6%

The applicant's statistical analysis was conducted on combined neoplasms according to FDA Guidance and combined neoplasms were explicitly listed in the study report. All determinations of significance were made comparing to control. Two neoplasms had a significant increasing dose-response and 8 had a significant decreasing dose-response. The neoplasms were labeled (a)-(j), and whether they are considered common or rare, if increasing:

Increasing-

(a) Liver hepatocellular tumor - rare in males, common in females

For males, increases in liver hepatocellular tumor were found to be statistically significant for combined (fatal+nonfatal) tumors at MD and HD compared to controls. There was a significant increase in nonfatal hepatocellular tumors at all doses and nonfatal and combined tumors at MD and HD compared to controls in females.

(b) Thymus epithelial tumor - common in both sexes

For males, increases in thymus epithelial tumors were found to be statistically significant for nonfatal and combined (fatal+nonfatal) tumors at all doses and for overall dose-response compared to controls. Females had significant increases in nonfatal tumors at all doses and for overall dose-response compared to controls, and for combined tumors at all doses, but not for overall dose-response.

Table 32 Liver and Thymus Neoplastic Findings in Rats at Necropsy during the 2-Year Oral Ospemifene Carcinogenicity Study

		Incidence of liver tumours: liver									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Liver	No. examined:	50	50	50	50	50	50	50	50	50	50
hepatocellular adenoma	Finding present	0	2	3	2	0	0	1	4	4	0
hepatocellular carcinoma	Finding present	0	0	0	1	0	0	0	2	0	0
hepatocellular tumours combined		0	2	3*	3*	0	0	1	6**	4*	0

Key: Statistical analysis: * = P<0.05, ** = P<0.01

		Incidence of thymic tumours: thymus									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Thymus	No. examined:	50	50	48	49	48	49	49	49	49	50
benign thymoma	Finding present	0	5	8	9	0	3	10	21	11	2
malignant thymoma	Finding present	0	0	0	0	0	0	0	0	2	0
thymus epithelial tumours combined		0	5**	8***	9***	0	3	10*	21***	13**	2

Key: Statistical analysis: * = P<0.05, ** = P<0.01, *** = P<0.001

(Tables excerpted from Applicant's package)

Table 33 List of Statistically Significant Increasing Dose-Response Neoplasms and P-Values and % Incidence (Combined Fatal+Nonfatal) from a 2-Year Rat Oral Ospemifene Carcinogenicity Study

Tissue	Finding		Control vs. Dose Response			Control vs. LD			Control vs. MD			Control vs. HD		
			F	NF	All	F	NF	All	F	NF	All	F	NF	All
(a) Thymus	Epithelial tumor	♂		0.004	0.006		0.009	0.009		<0.001	<0.001		<0.001	<0.001
		♀		0.021			0.016	0.031		<0.001	<0.001		<0.001	<0.001
(b) Liver	Hepatocellular adenoma/carcinoma	♂								0.049			0.015	
		♀		0.048						0.007	0.003		0.024	0.024

♂, Male; ♀, Female

Decreasing-

- (c) Hemolymphoreticular lymphocytic leukemia in males
- (d) Testis interstitial cell adenoma in males
- (e) Pituitary adenoma/carcinoma in both sexes
- (f) Skin/appendage fibroblastic tumor in both sexes
- (g) Thyroid follicular cell tumor in males
- (h) Skin/appendage squamous cell tumor in males
- (i) Mammary gland epithelial tumor in females

(j) Uterus stromal tumor in females

Tumor types (d), (e), (g), (i), and (j) were found at rates below of HC rates, and there was no incidence of tumor types (c), (d) and (f, females).

Table 34 List of Statistically Significant Decreasing Dose-Response Neoplasms and P-Values and % Incidence (Combined Fatal and Nonfatal) from a 2-Year Rat Oral Ospemifene Carcinogenicity Study

Tissue	Finding		Control vs. Dose Response			Control vs. LD			Control vs. MD			Control vs. HD		
			F	NF	All	F	NF	All	F	NF	All	F	NF	All
(e) Pituitary	Adenoma/ carcinoma	♂		0.003	0.006		0.001	<0.001		<0.001	<0.001		<0.001	<0.001
		♀		<0.001			*	<0.001	<0.001	*	<0.001	<0.001	*	<0.001
(c) Hemolymphoreticular	Lymphocytic leukemia	♂			0.019									
		♀												
(d) Testis	Interstitial cell adenoma	♂			0.04									
(f) Skin	Fibroblastic tumor	♂		0.003			0.018			0.019				
		♀			0.042									
(g) Thyroid	Follicular cell tumor	♂		0.035										
		♀												
(h) Merged tissues (skin)	Squamous cell tumor	♂		<0.001	0.014			0.036		0.003	0.013		0.002	0.011
		♀												
(i) Mammary gland	Epithelial tumor	♂												
		♀		<0.001	<0.001		<0.001	<0.001		<0.001	<0.001		<0.001	<0.001
(j) Uterus	Stromal tumor	-												
		♀		<0.001	<0.001		0.021	0.03		0.029	0.008		0.023	0.006

♂, Male; ♀, Female; *, P=0.003

Non Neoplastic

Findings were generally low and sporadic, except in estrogen target organs such as testes, uterus, ovary, and vagina/clitoral gland. There was no NOEL set and the observed non-neoplastic findings were consistent with the observed neoplastic findings. In general, treatment-related effects showed little or no dose-dependency with the effects (or lack thereof) noted at all doses. This is likely due to saturation of exposure with only a 4- to 5-fold maximal increase in AUC from LD to MD and/or HD at 52 weeks.

Male Reproductive Organs- All male findings generally increased in overall severity from LD to MD, but MD severity was generally comparable to that at HD. There was a dose- and severity-dependent increase in minimal to severe germ cell depletion in the testes and oligospermia in the epididymides. Increased minimal to moderate cell debris was also noted in the epididymides. Minimal to severe atrophy was noted in the seminal vesicles of 84-94% of treated animals compared to 2% of controls. Minimal to severe atrophy was noted in the prostate of 84-96% of treated animals compared to 3% of controls. There was a slight increase in incidence and severity of inflammatory focus in treated groups compared to controls.

Mammary gland- There were two, sex-specific findings of note. In males only, there was an increased incidence of minimal to slight tubulo-alveolar differentiation of 48-56% in treated groups, but only 4% of controls. Severity, but not incidence was

dose-related. In females, cystic hyperplasia was not observed in any treated animals, but occurred in almost all examined controls.

Ovary- There were increased numbers of acyclic females in follicular phase and minimal to moderate sex chord stromal hyperplasia (SCSH) in treated groups compared to control. No incidence or severity dose-relationship was observed for these findings. The incidence of SCSH was slightly higher by 23-54% than controls, with 20, 17, and 16 in the LD, MD, and HD groups, respectively, and 13 in each control group.

Uterus- There was a dose-dependent increase in incidence and severity of minimal to moderately severe cervical squamous cell hyperplasia with 8, 20, and 34 in the LD, MD, and HD groups, respectively, compared to 4 and 1 in the control groups. There was increased minimal to moderately severe squamous cell metaplasia up to the MD compared to controls but the HD incidence was lower than MD; severity was not dose-related. Minimal to severe endometritis was observed at 4, 9, and 10 in the LD, MD, and HD groups, respectively, compared to 1 incidence of minimal findings each in the control groups. Severe glandular atrophy was present in essentially all treated animals and not observed in controls animals. Cystic glands and endometrial hyperplasia in treated groups were absent compared to controls.

Vagina/Clitoral gland- - There was an increase in minimal to slight inflammatory cell focus in treated groups with 6, 3, and 4 in the LD, MD, and HD groups, respectively compared to none in control. There was a slight decrease in duct ectasia noted in the clitoral gland. There were 8, 6, and 4 animals at LD, MD, and HD, respectively, with 7 and 16 in controls groups.

Bone- Minimal to moderate increased hematopoiesis of the femur and sternum of males only was slightly decreased compared to controls at all doses.

Adrenal- There was a slight increase in minimal to slight corticomedullary pigment with 84-94% of treated animals compared to 70-74% of controls. There were decreased incidences of eosinic, clear cell, and vacuolated foci in treated groups compared to controls. Vacuolated focus was fairly rare even in treated groups of both sexes. Clear cell focus decreased in all treated groups, but in males the decreases was more noted because more controls had the findings present, and there were fewer in the treated groups. The reverse occurred with eosinic focus reduced in all treated groups, but the difference in reduction was more pronounced in females.

Kidney- There was increased minimal to moderate pigment and decreased minimal to moderately severe chronic nephropathy observed in all treated groups. In males the findings of increased pigment were more dramatic than females with only 1 control male in each group compared to 7, 20, and 20 animals at LD, MD, and HD, respectively. In females there was a 2-3 fold increase with pigment, but about 25% of controls had this finding. In males, more control animals had findings of chronic nephropathy than females, but the reduction was less marked. The decreased incidence ranged from 1.7- to 2.5-fold in treated groups from 70% of controls for males, and ranged from 2.5- to 3.6-fold for females with control incidence at 25%. Minimal to slight corticomedullary mineralization showed a marked, male-specific increase in all treated groups with 28, 20, and 22 animals at LD, MD, and HD, respectively, compared to only 1 observed in controls. Females showed a greater background rate near 50%, but no treatment-related pattern. Females showed a dose-dependent decrease in incidences of minimal to moderate pelvic mineralization to 60-68% from 90% of

controls. Again, control rates were sex-dependent and only 60% of control males had the finding.

Heart- Minimal to slight cardiomyopathy was reduced in male rats only with 15, 12, and 9 animals at LD, MD, and HD, respectively compared to 28 and 20 in control groups. Females had reduced incidences ranging from 5 to 10, regardless of group.

Liver- Overall, the incidence of notable findings was reduced in treated groups of both sexes with no dose-related effect on incidence or severity. There was a decrease in minimal to moderate hepatocyte vacuolation, with males having greater rate of control animals with a finding compared to females (75% vs. 25%, respectively). Minimal to moderately severe basophilic focus was reduced, with the treatment-related effects marked in females compared to males. There were only 3 LD females compared to 81 control females with basophilic focus. In males, there were 2, 0, and 2 in LD, MD, and HD animals, respectively, compared to 9 and 12 animals in controls groups. Minimal to moderate clear cell/eosinophilic focus was reduced to 10, 15, and 15 in LD, MD, and HD animals, respectively, compared to 25 and 28 control animals. The background incidence was reduced to 6 and 8 in female controls, with 6, 1, and 0 in LD, MD, and HD animals, respectively. Minimal bile duct hyperplasia was reduced from 15% of control animals to 3% and 2% of males and females, respectively. Minimal to slight vacuolated focus occurred only in a single LD male compared to 6 male and 5 female control animals.

Lung- There was an increase in minimal to severe foamy macrophages present with a slight dose-related increase in severity. Males increased from 80% incidence rate in controls to 96-95% of treated animals, and females increased from 85% to 94-98%, respectively.

Thymus- There was a decrease in minimal to severe thymic atrophy in all treated males and MD and HD females compared to controls. The reduction was greater in males compared to females, mainly based on the higher rate in controls with 38% of males and 22% of females with atrophy noted. In males only, there was an increase of cysts present with 24, 12, and 17 in LD, MD, and HD animals, respectively, compared to 7 animals in each control group. Females showed no treatment-related effect. Incidence of epithelial hyperplasia had opposite findings in males and females, with an increase of 4.1-fold in males and a decrease of 2.3-fold in females.

Pituitary- Focal hyperplasia was reduced in all treated groups compared to controls. Although the reduction was consistent, there was large difference in the incidence in controls of both sexes.

Skin- There was an increase in minimal to moderate acanthosis/adnexal atrophy in males at MD and HD and females at all doses. Although the control rates were identical (5%), there was increase of up to 7-fold for females compared to 2-fold for males.

Spleen- Minimal to severe hematopoiesis incidence was reduced in treated groups, albeit slightly in LD and HD males and LD females compared to control groups. The control incidence rates were around 80%, ranging from 74-88%, and incidences in treated groups ranged from 48-74%.

Stomach- Minimal to slight cystic glands incidence was decreased in MD and HD males and all treated groups of females. The effect was slightly greater in females, and appeared to be dose-related in females, but not males

Thyroid- There was a decrease in minimal to moderate C-cell hyperplasia in all treated male groups and LD and HD female groups (compared to overall control rate, the MD was lower as well). Follicular cell hypertrophy was present in 1, 2, and 2 of LD, MD, and HD males and 1 each of all treated female groups compared to none in any control group.

Table 35 Selected Non-neoplastic Microscopic Findings of Note at Necropsy in the 2-Year Rat Oral Ospemifene Carcinogenicity Study (n=50/group)

Tissue	Finding	mg/kg									
		Males					Females				
		0	10	50	300	0	0	10	50	300	0
Testes	Germ cell depletion	0	11	21	25	0					
Epididymides	Oligospermia	4	9	20	13	10					
	Cellular debris	2	9	8	16	0					
Seminal vesicle	Atrophy Min-sev	2	42	46	43	0					
Preputial gland	Duct ectasia	17	8	3	0	9					
Prostate	IC focus	4	11	13	9	5					
	Atrophy	3	48	47	42	0					
Ovary	Acyclic -follicular phase						8	44	45	47	9
	Sex chord stromal hyperplasia						13	20	17	16	13
Uterus	Cystic glands						10	0	0	0	6
	Endometritis						1	4	9	10	1
	Squamous cell metaplasia						0	3	12	9	1
	Glandular atrophy						0	50	50	49	0
	Squamous cell hyperplasia - cervix						4	8	20	34	1
	Endometrial hyperplasia						3	0	0	0	1
Vagina	IC infiltration						0	6	3	4	0
Mammary gland	Tubulo-alveolar differentiation	4	28	24	25	4	0	0	0	0	0
	Cystic hyperplasia	2	1	0	1	2	26	0	0	0	33
Skin	Acanthosis/ adnexal atrophy	3	1	7	7	2	3	13	21	21	2
Femur + marrow	↑ hematopoiesis	5	0	2	2	4	8	3	1	5	4
Sternum + marrow	↑ hematopoiesis	4	0	1	2	4	4	3	0	5	4
Adrenal	Corticomedullary pigment	37	42	43	47	37	34	44	44	42	35
	Clear cell focus	18	0	7	4	20	10	9	4	2	11
	Eosinic focus	4	5	2	3	2	13	7	6	3	11
	Vacuolated focus	8	2	1	0	6	3	1	1	0	3
Kidney	Pigment	1	7	20	20	1	14	28	37	30	10
	Corticomedullary mineralization	1	28	20	22	0	26	34	27	17	23
	Pelvic mineralization	30	31	35	36	29	44	38	33	30	46
	Chronic nephropathy	36	14	18	21	36	25	7	10	8	25
Heart	Cardiomyopathy	28	15	12	9	20	7	8	5	7	10
Liver	Hepatocyte vacuolation	36	19	10	13	38	13	3	0	2	12
	Pigmented macrophages	0	0	1	2	1	6	1	2	1	7
	Bile duct hyperplasia	9	3	0	1	6	9	0	3	0	6
	Vacuolated focus	1	1	0	0	5	2	0	0	0	3
	Basophilic focus	9	2	0	2	12	39	3	0	0	42
	Eosinic focus	25	10	15	15	28	6	6	1	0	8
Lung	Foamy macrophages	39	48	49	48	41	42	47	48	49	43

Pituitary	Focal hyperplasia	6	3	0	1	14	4	2	2	2	10
Spleen	Hematopoiesis	38	35	30	37	42	44	35	24	30	37
Stomach	Cystic glands	27	23	16	17	24	23	20	16	12	25
Thymus	Cyst	7	24	12	17	7	19	22	9	11	14
	Atrophy	18	5	1	3	20	12	10	4	4	11
	Epithelial hyperplasia	3	12	12	19	4	12	5	8	8	13
Thyroid	C-cell hyperplasia	10	4	4	4	16	7	4	7	4	12
	Follicular cell hypertrophy	0	1	2	2	0	0	1	1	1	0

IC, Inflammatory Cell

Toxicokinetics- Day 1, Months 3, 6, 9, and 12 at 2, 4, 6, 8, 12 and 24 hrs post-dose. Three animals/group were used, with no animal bled twice during any given TK period- usually every other time.

Ospemifene was rapidly absorbed at all doses with a t_{max} of 2 - 4 hr. Excluding Day 1 values (most were not calculable), ospemifene was eliminated fairly rapidly with $t_{1/2}$ range of 3.0 - 13.8 hr, with no obvious sex-, dose- or time-related pattern. AUC exposure from 10 mg/kg to 50 mg/kg increased at less than a dose-proportional manner (~4-fold at Week 52), except for Day 1 where it was dose-proportional; exposure based on C_{max} always increased less than dose-proportional. There was a less than dose-proportional increase in both AUC and C_{max} exposure from 50 mg/kg to 300 mg/kg (~1.2-fold at Week 52), which decreased in a duration-dependent manner until AUC exposure plateaued from Weeks 39 to 52 and C_{max} exposure plateaued at Week 13, onward. Ospemifene showed no obvious pattern of accumulation or of a sex-dependent relationship.

Metabolite HM-187 showed some delay in t_{max} compared to parent with values ranging from 2 - 8 hr. Similar to parent, the $t_{1/2}$ ranged from 2.81 - 11.6 hr, with an occasional value not calculable. The increase in exposure was initially comparable to dose-proportional, but was less than dose-proportional over time. The increase in exposure was greater going from 10 mg/kg to 50 mg/kg than from 50-mg/kg to 300 mg/kg. In general, exposure increased in a duration-dependent manner and was higher in females than males at most doses and times. Males had less HM-187 than parent with AUC ratios ranging from 0.24 to 0.95, whereas females had levels of HM-187 ranging from 0.8 to 2.0 of the parent. Overall, there was evidence of accumulation of HM-187.

Metabolite HM-136 showed a delay in t_{max} compared to parent with values ranging from 4 - 8 hr and $t_{1/2}$ was not calculable, likely due to the generally low levels measured. The levels of HM-136 exposure were low compared to parent and HM-187, and AUC were not calculable at 10 mg/kg, except during Week 52. Exposure from Week 13 onward increased in a less than dose-proportional manner from 50 mg/kg to 300 mg/kg, when it could be calculated. The ratios of HM-136 to parent ranged from 0.016 to 0.071. There were no duration- or sex-dependent differences observed.

Table 36 Toxicokinetics of Ospemifene during the 2-Year Oral Ospemifene Rat Carcinogenicity Study

Occasion	Dose (mg/kg/day)	Sex	AUC _{0-τ} (ng.h/mL)	AUC _{0-τ} (norm)	C _{max} (ng/mL)	C _{max} (norm)	t _{max} (h)	t _{1/2} (h)
Day 1	10	Male	838	83.8	176	17.6	2.00	NC
		Female	754	75.4	132	13.2	2.00	NC
	50	Male	5130	103	535	10.7	2.00	2.98
		Female	3550	71.0	555	11.1	2.00	NC
	300	Male	15200	50.6	1610	5.36	2.00	7.44
		Female	13500	44.9	1010	3.37	2.00	NC
Week 13	10	Male	1470	147	306	30.6	2.00	6.91
		Female	1000	100	188	18.8	2.00	5.70
	50	Male	4920	98.4	706	14.1	2.00	7.44
		Female	4360	87.2	590	11.8	2.00	6.48
	300	Male	7760	25.9	1170	3.89	2.00	6.22
		Female	6920	23.1	690	2.30	2.00	13.8
Week 26	10	Male	1590	159	350	35.0	2.00	9.85
		Female	1010	101	215	21.5	2.00	13.5
	50	Male	4040	80.9	566	11.3	4.00	6.17
		Female	3590	71.8	454	9.09	4.00	7.96
	300	Male	6280	20.9	545	1.82	2.00	8.54
		Female	6310	21.0	486	1.62	4.00	13.4
Week 39	10	Male	1620	162	330	33.0	2.00	9.59
		Female	1440	144	293	29.3	2.00	7.21
	50	Male	4100	82.0	469	9.39	4.00	7.20
		Female	4490	89.8	561	11.2	2.00	6.21
	300	Male	6510	21.7	576	1.92	4.00	6.29
		Female	7110	23.7	634	2.11	2.00	8.94
Week 52	10	Male	1500	150	215	21.5	2.00	NC
		Female	1470	147	242	24.2	2.00	9.34
	50	Male	5440	109	729	14.6	2.00	7.58
		Female	5400	108	746	14.9	2.00	7.50
	300	Male	7170	23.9	771	2.57	2.00	6.35
		Female	6470	21.6	492	1.64	2.00	8.85

AUC (norm) = AUC [ng.h/mL] / dose [mg/kg/day]

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]

τ = 24 hours

NA = Not applicable

NC = Not calculable

(Table excerpted from Applicant's package)

Table 37 Toxicokinetics of Metabolite HM-187 during the 2-Year Oral Ospemifene Rat Carcinogenicity Study

Occasion	Dose (mg/kg/day)	Sex	AUC _{0-τ} (ng.h/mL)	AUC _{0-τ} (norm)	C _{max} (ng/mL)	C _{max} (norm)	t _{max} (h)	t _{1/2} (h)	RA _{AUC}	RA _{C_{max}}	MR _{AUC}	MR _{C_{max}}
Day 1	10	Male	259	25.9	42.1	4.21	2.00	6.07	NA	NA	0.309	0.240
		Female	1530	153	137	13.7	2.00	NC	NA	NA	2.03	1.04
	50	Male	2380	47.7	255	5.10	4.00	3.84	NA	NA	0.465	0.476
		Female	6270	125	629	12.6	4.00	4.06	NA	NA	1.77	1.13
	300	Male	5650	18.8	559	1.86	6.00	11.6	NA	NA	0.372	0.348
		Female	20400	68.1	2010	6.69	4.00	NC	NA	NA	1.51	1.99
Week 13	10	Male	829	82.9	116	11.6	4.00	2.81	3.20	2.76	0.566	0.380
		Female	1590	159	157	15.7	2.00	5.00	1.04	1.15	1.59	0.835
	50	Male	4650	93.1	523	10.5	6.00	2.92	1.95	2.05	0.946	0.741
		Female	6760	135	982	19.6	6.00	4.01	1.08	1.56	1.55	1.67
	300	Male	5520	18.4	654	2.18	6.00	NC	0.977	1.17	0.711	0.561
		Female	10600	35.4	1130	3.78	6.00	9.54	0.520	0.565	1.54	1.64
Week 26	10	Male	971	97.1	160	16.0	4.00	6.48	3.75	3.80	0.611	0.458
		Female	2070	207	253	25.3	4.00	7.15	1.35	1.85	2.04	1.18
	50	Male	5510	110	598	12.0	8.00	NC	2.31	2.35	1.36	1.06
		Female	5230	105	605	12.1	4.00	4.23	0.834	0.961	1.46	1.33
	300	Male	10400	34.8	1060	3.54	6.00	8.22	1.85	1.90	1.66	1.95
		Female	13600	45.2	1340	4.47	6.00	7.14	0.664	0.668	2.15	2.76
Week 39	10	Male	1200	120	147	14.7	6.00	NC	4.63	3.50	0.740	0.447
		Female	1830	183	270	27.0	4.00	3.89	1.19	1.98	1.27	0.923
	50	Male	4280	85.5	471	9.42	4.00	3.75	1.79	1.85	1.04	1.00
		Female	5290	106	636	12.7	4.00	3.45	0.844	1.01	1.18	1.13
	300	Male	9780	32.6	1380	4.60	6.00	3.27	1.73	2.47	1.50	2.39
		Female	14200	47.4	1240	4.14	6.00	3.63	0.697	0.620	2.00	1.96
Week 52	10	Male	3550	355	313	31.3	6.00	4.66	13.7	7.43	2.36	1.46
		Female	5320	532	514	51.4	4.00	5.83	3.47	3.76	3.61	2.12
	50	Male	11300	226	1050	21.1	8.00	NC	4.74	4.13	2.08	1.44
		Female	11100	222	814	16.3	4.00	7.79	1.77	1.29	2.06	1.09
	300	Male	13200	43.9	1250	4.17	8.00	NC	2.33	2.24	1.84	1.62
		Female	13900	46.4	1290	4.31	6.00	5.47	0.681	0.645	2.15	2.63

AUC (norm) = AUC [ng.h/mL] / dose [mg/kg/day]

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]

τ = 24 hours

NA = Not applicable

NC = Not calculable

(Table excerpted from Applicant's package)

Table 38 Toxicokinetics of Metabolite HM-136 during the 2-Year Oral Ospemifene Rat Carcinogenicity Study

Occasion	Dose (mg/kg/day)	Sex	AUC _{0-τ} (ng.h/mL)	AUC _{0-τ} (norm)	C _{max} (ng/mL)	C _{max} (norm)	t _{max} (h)	t _{1/2} (h)	RA _{AUC}	RA _{Cmax}	MR _{AUC}	MR _{Cmax}
Day 1	10	Male	NC	NC	<5.00	NC	NC	NC	NA	NA	NC	NC
		Female	NC	NC	<5.00	NC	NC	NC	NA	NA	NC	NC
	50	Male	122	2.43	21.3	0.425	4.00	NC	NA	NA	0.0237	0.0397
		Female	80.8	1.62	17.2	0.344	4.00	NC	NA	NA	0.0227	0.0310
	300	Male	687	2.29	71.8	0.239	6.00	10.6	NA	NA	0.0452	0.0447
		Female	673	2.24	71.8	0.239	4.00	NC	NA	NA	0.0499	0.0711
Week 13	10	Male	NC	NC	<5.00	NC	NC	NC	NC	NC	NC	NC
		Female	NC	NC	<5.00	NC	NC	NC	NC	NC	NC	NC
	50	Male	126	2.52	34.4	0.688	6.00	NC	1.04	1.62	0.0256	0.0487
		Female	NC	NC	26.3	0.527	6.00	NC	NC	1.53	NC	0.0447
	300	Male	160	0.534	24.5	0.0816	8.00	NC	0.233	0.341	0.0206	0.0210
		Female	241	0.802	30.8	0.103	6.00	NC	0.358	0.429	0.0348	0.0446
Week 26	10	Male	NC	NC	<5.00	NC	NC	NC	NC	NC	NC	NC
		Female	NC	NC	<5.00	NC	NC	NC	NC	NC	NC	NC
	50	Male	125	2.5	17.7	0.354	6.00	NC	1.03	0.832	0.0309	0.0313
		Female	91.1	1.82	15.6	0.313	4.00	NC	1.13	0.909	0.0254	0.0344
	300	Male	354	1.18	34.5	0.115	8.00	NC	0.515	0.481	0.0564	0.0633
		Female	401	1.34	33.8	0.113	6.00	NC	0.596	0.470	0.0635	0.0694
Week 39	10	Male	NC	NC	<5.00	NC	NC	NC	NC	NC	NC	NC
		Female	NC	NC	6.33	0.633	4.00	NC	NC	NC	NC	0.0216
	50	Male	64.5	1.29	14.5	0.289	4.00	NC	0.530	0.680	0.0157	0.0308
		Female	124	2.47	18.5	0.369	4.00	NC	1.53	1.07	0.0275	0.0329
	300	Male	245	0.818	34.0	0.113	4.00	NC	0.357	0.474	0.0377	0.0590
		Female	353	1.18	32.5	0.108	6.00	NC	0.525	0.452	0.0497	0.0512
Week 52	10	Male	NC	NC	11.0	1.10	4.00	NC	NC	NC	NC	0.0511
		Female	NC	NC	12.6	1.26	4.00	NC	NC	NC	NC	0.0521
	50	Male	279	5.57	38.4	0.769	4.00	NC	2.29	1.81	0.0512	0.0527
		Female	268	5.36	28.3	0.566	2.00	6.07	3.31	1.65	0.0496	0.0379
	300	Male	342	1.14	29.2	0.0973	8.00	NC	0.497	0.407	0.0477	0.0379
		Female	379	1.26	30.6	0.102	8.00	NC	0.564	0.426	0.0587	0.0623

AUC (norm) = AUC [ng.h/mL] / dose [mg/kg/day]

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]

τ = 24 hours

NA = Not applicable

NC = Not calculable

(Table excerpted from Applicant's package)

Dosing Formulation Analysis

Homogeneity was assessed from top, middle and bottom of doses prepared on Days 1, 2, and 7 and was acceptable with all values within protocol defined range of 90 -110%. Test article concentrations were evaluated from middle of samples prepared in Weeks 1 and 4, Months 3, 6, 9, 12, 15, 18, 21, and 24 (co-incident with TK sampling). All sample results, except 1 out of 95, were within the protocol defined range of 90 -110%.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not conducted.

9.2 Embryonic Fetal Development

Study Title: "FC-1271a: Embryo-fetal development study of Fc-1271a in rats by oral (gavage) administration"

Key study findings:

- An increase in placental weight at ≥ 1 mg/kg/day. Maternal NOAEL=0.1 mg/kg/day
- An increase in testes displacement was seen in all treated groups. Male fetal NOAEL <0.1 mg/kg/day; female fetal NAOEL >4 mg/kg/day.

Study no: 1504011 (15-IIC-001); (b) (4) # TOX-95018

Volume # and page #: Vol. 12, section 21 (page # 007338)

Conducting laboratory and location: (b) (4)

Date of study initiation: February 02, 1995 (Start of dosing)

GLP compliance: Yes

QA Report: Yes

Drug, lot # and % purity: Fc-1271a, batch #10735V, purity not provided

Methods

Doses: 0 (vehicle alone), 0.1, 1 or 4 mg/kg/day dissolved in 0.5% carboxymethylcellulose (CMC).

Species/Strain: Female Sprague Dawley rats were 10-12 weeks old and weighed 170-230 g (females) at the time of mating. Untreated male rats (same strain & source) used for mating were 10-12 weeks old and weighed 300-350 g.

Number/sex/group: 19/group. In addition, 2 sets of satellite dams comprising 3 dams/treatment group were used for TK on GD6 and GD16.

Route, formulation, volume and infusion rate: Oral gavage, aqueous solution in 0.5% methylcellulose (w/v), 10mL/kg.

Study Design: Daily dosing from GD 6-16. Animals were killed on GD20 for C-section. Parameters and endpoints evaluated: Clinical observations, morbidity and mortality were conducted twice daily. Body weight was done on most days starting prior to mating until On GD 20, cesarean section was performed and data for each litter was collected: gravid uterine weight, number of corpora lutea, number of live/dead fetuses, and number of implantation sites were recorded (early/late resorption); sex & body weight of each live fetus. External and palatine abnormalities of all term fetuses were recorded. Maternal toxicity was assessed by clinical appearance, body weight and food consumption changes, gross

necropsy and reproductive outcome. Developmental toxicity was assessed by effects on embryo/fetal survival (pre & postimplantation loss), fetal body weight, sex distribution and external findings.

RESULTS

Mortality (dams): All dams survived to terminal kill.

Clinical signs (dams): No treatment-related signs were noted. Labored breathing was noted in 1 rat each of LD and HD groups on single occasions after dosing.

Body weight (dams): HD-treated dams showed a significant decrease between GD10-16 and on GD20 but the overall weight gain was not significantly reduced (change did not exceed 5% difference from control dams). A dose-related, non-significant decrease in body weight was also noted for the LD and MD groups.

Food/water consumption (dams): No treatment-related food or water consumption effect was noted among treated dams.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): There were no treatment-related maternal gross pathology findings, effects on the corpora lutea, difference in the number of implantations or live/dead fetuses. An increase in placental weight was found in MD (13.5%) and HD (21%)-treated dams compared to controls.

Offspring (malformations, variations, etc.): No change in mean fetal body weight was seen among treated groups. There were no fetal external or palatine findings, difference in sex ratio, or skeletal malformations. Visceral examination showed a non-statistically significant increase in testes displacement in all treated groups (Control: 11, LD: 20, MD: 24 & HD: 22) compared to controls. Plasma levels increased with dose and no difference was noted between GD6 and 16. PK data were not calculated due to the small sample number. Maternal NOAEL=0.1 mg/kg/day (placental finding); male fetal NOAEL <0.1 mg/kg/day; female fetal NAOEL >4 mg/kg/day.

Study title: Dose Range-Finding Study for Effects on Embryo-fetal Development after Oral Administration (Gavage) in the Himalayan Rabbit

Study no.: 15-44502
 Study report location: EDR #0000 4.2.3.5.2.1.15-44502
 Conducting laboratory and location: (b) (4)
 Date of study initiation: February 7, 2001
 GLP compliance: Yes
 QA statement: Yes
 Drug, batch #, and % purity: Ospemifene, 00E28, 99.9%

Key Study Findings

- There was reduced food consumption and body weight gains at ≥ 10 mg/kg compared to control.
- No clear test article-related effects were noted on C-section endpoints and on the offspring.

- Maternal NOAEL was set at 3 mg/kg and fetal NOAEL was set at 30 mg/kg.

Methods

Doses: 0, 1, 3, 10, and 30 mg/kg
Frequency of dosing: Once daily
Dose volume: 4 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: 5% CMC in distilled water
Species/Strain: Himalayan rabbits/ (b) (4) (b) (4)
Number/Sex/Group: 5 ♀/group
Weight: 2.2 - 3.1 kg
Age: ≥16 weeks
Satellite groups: None

Study design: F₀ Dams treated from Gestation Day (GD) 6 to GD20 inclusive; F₁ animals were never directly treated at any point, but exposure potentially may have occurred *in utero* and through nursing (test article was confirmed present in milk). Necropsy occurred on GD28 for F₀ ♀ dams and F₁ pups.

Observations and Results

Mortality: None

Clinical signs: Twice daily
Not remarkable.

Body weight: Daily

Body weight losses were observed from GD6-11 at ≥10 mg/kg (-32g and -47g at 10 and 30 mg/kg, respectively). This resulted in overall reductions in body weight gains of -0.7% and 1.9% compared to 3.7% increase for controls.

Feed Consumption: Daily

There was a marked reduction in food consumption by ~35% at ≥10 mg/kg over the treatment period (-34.8% and -35.7% at 10 and 30 mg/kg, respectively). Consumption recovered partially during GDs 21-29.

Toxicokinetics: TK samples collected from 3 dams/group at pre-dose, and at 4, 8, and 24 hrs on GDs 6 and 18; the results were reported separately.

Dosing Formulation Analysis: Test-article formulations were stable and homogenous and within ±10% of target formulation.

Necropsy: No test article-related findings were noted.

Cesarean Section Data: One vehicle control dam had total implantation loss (likely an early post-implantation loss rather than abortion). Post-implantation loss in dams with live fetuses was increased at ≥ 3 mg/kg with 0%, 10.0%, 20.7%, and 18.9% compared to control value of 3.8%. If total loss female is included, the value for control increases to 13.8%. There were significantly increased implantation sites and decreased pre-implantation and fetal weight (due to males) noted at 10 mg/kg, but not at 3 or 30 mg/kg.

Offspring: No test article-related findings were noted.

Study Title: "Study for the effects on embryo-fetal development in the Himalayan rabbit"

Key study findings:

- A reversible, non-significant decrease in food consumption and mean body weight gain was noted in treated dams from GD6-18 in all treatment groups. Total resorption was seen in 3 HD treated dams. Maternal NOAEL = 10 mg/kg/day
- A decrease (non-significant) in fetal weight was seen in HD-treated groups. Fetal NOAEL=30 mg/kg/day.

Study no: 15-44503 (former #1504010 & 15-IIC-003); (b) (4) # 796500

Volume # and page #: Vol. 12/section 22 (page 007489)

Conducting laboratory and location: (b) (4)

Date of study initiation: June 12, 2001 (in life phase)

GLP compliance: Yes

QA Report: Yes

Drug, lot # and % purity: FC-1271a, lot # 00E28, 99.9%

Methods

Doses: 0 (vehicle alone), 3, 10 & 30 mg/kg/day dissolved in 0.5% carboxymethylcellulose (CMC).

Species/Strain: Female Himalayan rabbits (b) (4) were 22 weeks old and weighed 1907-3495 g at the time of arrival. Fertile male rabbits kept in house were used for mating.

Number/sex/group: 5/group. In addition,

Route, formulation, volume and infusion rate: Oral gavage in aqueous solution of 0.5% methylcellulose (w/v), 4 mL/kg

Satellite groups used for toxicokinetics: 3 additional animals/group were bled on GD6 and 18 for TK analysis.

Study Design: Pregnant dams were administered daily dosing from GD 6-18. Animals were killed and a c-section was performed on GD28.

Parameters and endpoints evaluated: Clinical observations, mortality and morbidity were observed twice daily on dosing days and once daily on nondosing days. Body weights were recorded daily from GD0-28. Individual food consumption was recorded on GD 0-6, 6-11, 11-15, 15-19, 19-24 and 24-28. On

GD 28, gross pathology with emphasis on uterus and ovaries, uterine contents, position of fetuses in uterus, number of corpora lutea, gravid uterine weight, number of live/dead fetuses, and number of implantation sites were recorded (early/late resorption); sex & body weight of each live fetus. External and palatine abnormalities of all term fetuses were recorded. Maternal toxicity was assessed by clinical appearance, body weight and food consumption changes, gross necropsy and reproductive outcome. Developmental toxicity was assessed by effects on embryo/fetal survival (pre & postimplantation loss), fetal body weight, sex distribution and external findings.

RESULTS

Mortality (dams): One rabbit treated with 3 mg/kg/day showed signs of apathy and salivation on GD18 and was found dead in the morning of GD19.

Clinical signs (dams): None.

Body weight (dams): A decrease in mean body weight gain was seen for dams treated with \geq MD compared to vehicle controls between GD6-18. BW increased between GD19-30 among treated groups and was comparable to vehicle control group.

Food consumption (dams): A decrease in mean food consumption was noted during the dosing period for all treated dams (LD: -18%, MD: -24% and HD -33% compared to vehicle control) that was significant between GD6-11 for all treatment groups. Food consumption recovered after the end of the dosing period compared to vehicle control dams. BW changes correlated with food consumption patterns.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Total litter resorption was seen in dams treated with 3 (n=1), 10 (n=2) & 30 (n=3) mg/kg/day. Consequently, the mean number of fetuses/dam was slightly decreased in HD-treated dams. No other changes were noted.

Offspring (malformations, variations, etc.): Mean fetal body weight was decreased at >100 mg/kg/day. There were no substantial fetal external, visceral or skeletal findings. (NOAEL=4 mg/kg/day).

TK: Plasma ospemifene levels increased with dose but with large variability among dams. Levels increased over time as GD18 levels increased compared to those measured after a single dose exposure.

GD 6	3	10	30
AUC	21 \pm 19	130 \pm 53	214*
C _{max}	4.8 \pm 5.4	15.9 \pm 2.7	24.7 \pm 12
T _{max}	1.7 \pm 0.6	2.7 \pm 1.2	3.3 \pm 1.2

*, SD was not calculated

GD 18	3	10	30
AUC	60 \pm 34	327 \pm 211	731 \pm 506
C _{max}	8 \pm 3.2	55.4 \pm 25	71 \pm 26
T _{max}	3.7 \pm 3.8	1.7 \pm 0.6	2.7 \pm 1.2

9.3 Prenatal and Postnatal Development

Study title: Dose Range-Finding Study for Effects on Pre- and Postnatal Development in the Han Wistar Rat

Study no.: 15-44506
 Study report location: EDR #0000 4.2.3.5.3.1.15-44506
 Conducting laboratory and location: (b) (4)
 Date of study initiation: June 5, 2006
 GLP compliance: Yes
 QA statement: Yes
 Drug, batch #, and % purity: Ospemifene, 1041768, 100.7%

Key Study Findings

- There was dose-related decrease of body weight gain and feed consumption at ≥ 0.1 mg/kg that was significant at 1 mg/kg.
- There was a dose-related significant increase in post-implantation loss at ≥ 0.1 mg/kg, with increases in dead pups and pups that died before PND 21.
- Test article was detectable in milk at ≥ 0.1 mg/kg; test article was below Lower Limit of Quantification in serum of nursing pups.

Methods

Doses: 0, 0.01, 0.1, and 1 mg/kg
 Frequency of dosing: Once daily
 Dose volume: 5 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: Corn oil
 Species/Strain: Han Wistar Rat - Han (b) (4) WIST(SPF)
 Number/Sex/Group: 5♀/group
 Weight: 183 g to 215 g
 Age: ~11 weeks
 Satellite groups: TK - 0, 0.1, and 1 mg/kg, 4♀ vehicle, 9♀/ test article group, performed on Postnatal Day (PND) 7
 Study design: F₀ Dams treated from Gestation Day (GD) 6 to Lactation (LD) Day 20) inclusive; F₁ animals were never directly treated at any point, but exposure potentially may have occurred *in utero* and through nursing (test article was confirmed present in milk). On PND 7, satellite groups serum and milk were sampled for PK, then animals were sacrificed.
 -Necropsy occurred on PND 21 for F₀ ♀ dams and F₁ pups.

Observations and Results

F₀ in-life:

Mortality: None

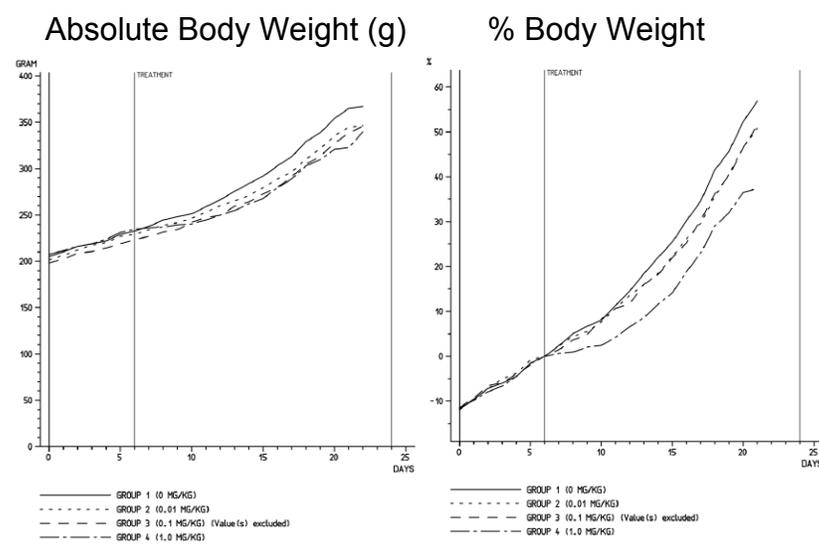
Clinical signs: Twice daily

Not remarkable.

Body weight: Daily

One animal at 0.1 for all BWts measurements (#11) and one animal at 1 mg/kg for lactation period (#17) were excluded (see below in Mating/Fertility). At ≥ 0.1 mg/kg, there was a dose-dependent decrease in BWt gain. BWt gain at 1 mg/kg was significantly decreased by -12% compared to control on GD 21. Significant effects on BWt gain occurred on GDs 12-21 compared to control, but reduced BWt gain was noted on all dosing days. The BWt gain decrease at 0.1 mg/kg was -7%

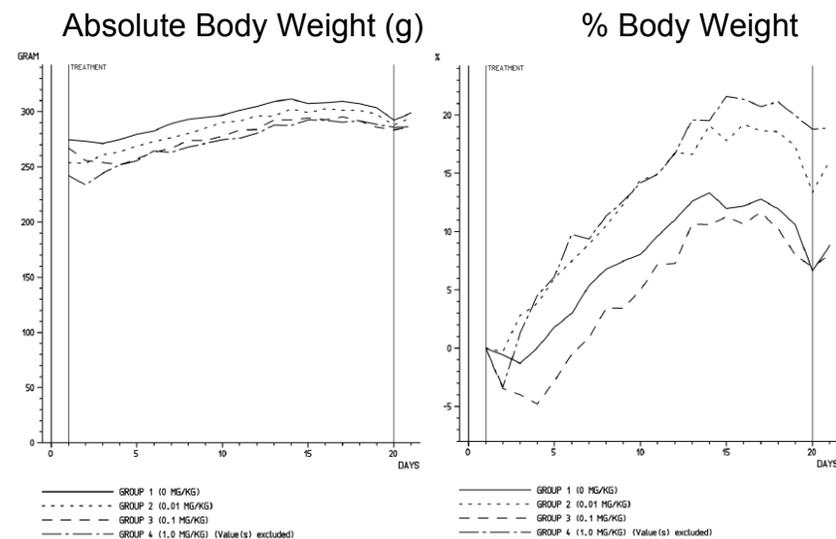
Figure 12 Mean Absolute and % Body Weight (BWt) Gains in Pregnant Rats Given Oral Ospemifene



(Figures excerpted from Applicant's package)

BWts at all doses remained reduced compared to control during the lactation phase. In general there was recovery at 0.01 and 1 mg/kg but not for the 0.1 mg/kg group. The % change for 0.1 mg/kg was comparable to the control group (7% vs. 9%), but the lack of a greater recovery was driven by a single dam (#13) that failed to consistently gain weight. All groups had BWt gain reductions noted during LDs 14-21 with most of reduction occurring on LDs 17-20.

Figure 13 Mean Absolute and % Body Weight (BWT) Gains in Rats Given Oral Ospemifene during Lactation Period



(Figures excerpted from Applicant's package)

Feed consumption: There was a dose-related decrease in feed consumption by dams during pregnancy, reaching significance at 1 mg/kg. However, the % reduction in consumption was greater than the observed BWt reductions by ~2-fold greater (-12% and -24% at 0.1 and 1 mg/kg, respectively). The amount of feed consumed by pups from PND14-21 was reduced by -22% and 15% at 0.1 and 1 mg/kg, respectively.

Mating/Fertility: One dam at 0.1 mg/kg was not pregnant and one dam at 1.0 mg had total litter loss (TLL). The dam at 1.0 mg/kg with TLL delivered one viable pup, which died before PND4. There was a significant increase in post-implantation loss at ≥ 0.1 mg/kg with increases in dead pups and pups that died before PND21.

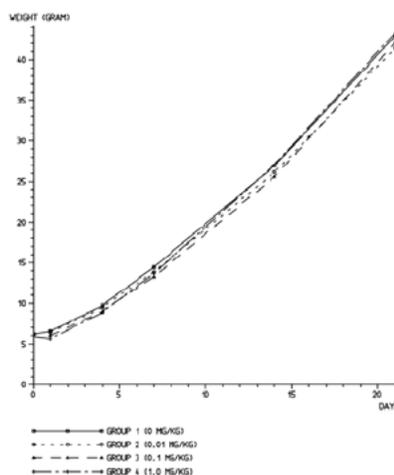
Table 39 Selected Mating and Fertility Parameters of Pregnant Rats Administered Oral Ospemifene during the Peri-Postnatal Development DRF Study

Parameter	Dose (mg/kg)			
	0	0.01	0.1	1
# Mated	5	5	5	5
# Pregnant	5	5	4	5
# with Total Litter Loss	0	0	0	1
# Litters with Liveborn	5	5	4	4
Gestation (Days)	21.6	21.4	21.8	21.4
Mean Implantations	14.0	12.6	12.3	13.0
Mean Viable Pups - 1 st Evaluation	12.4	12.2	8.3	7.6
Post-implantation Loss (%)	11.4	3.2	32.7**	41.5**
# Litters w/ Dead Pups- 1 st Evaluation	0	0	1	3
Mean Dead Pups- 1 st Evaluation	0	0	0.8	2.6
PND1-4 Losses/ #Litters	0/0	0/0	1/1	2/2
Mean Living Pups PND21	12.4	12.2	8.0	7.0

** , P<0.01 v. control

F₀ and F₁ Necropsy: Not remarkable

F₁ physical development: Mean pup weight at 0.1 and 1 mg/kg was decreased on PND 1 by -10% and -13%, respectively. However, these groups gained weight from PND 1 through PND 21 and were comparable to control by PND21. No other findings were considered remarkable.

Figure 14 Mean Bwt of F1 Pups from Postnatal Day 1 to 21

(Figure excerpted from Applicant's package)

Toxicokinetics - On PND 7, serum and milk samples from F₀ females or serum from pups were obtained 4 hr post-dose.

No test article was measurable the serum of F₀ females or pups at 0.01 mg/kg and 0.1 mg/kg. At 1 mg/kg only a single pup had detectable levels in serum (0.3 ng/mL).

Animal	Analyte	Dose (mg/kg)	Concentration (ng/mL)
Dams	Serum	0.01	<LLQ
		0.1	<LLQ
		1.0	1.97
	Milk	0.01	<LLQ
		0.1	32.0
		1.0	180.6
Pups	Serum	0.01	<LLQ
		0.1	<LLQ
		1.0	<LLQ*

only 1/6 had quantifiable amounts
<LLQ, below Lower Limit of Quantification

Study title: Study for Effects on Pre- and Postnatal Development including Maternal Function in the Han Wistar Rat

Study no.: 15-44505
 Study report location: EDR #0000 4.2.3.5.3.1.15-44505
 Conducting laboratory and location: (b) (4)
 Date of study initiation: September 8, 2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, batch #, and % purity: Ospemifene, 1254369, 100.7%

Key Study Findings

- There was an increase in F₀ female mortality at 0.25 mg/kg preceded by clinical signs of difficult parturition such as dystocia, vaginal bleeding, ruffled fur, lethargy, hypothermia, and/or uterine prolapse. Three dams were found dead pre-partum on Gestational Days 24-25, one was found dead on Lactation Day (LD) 3, and one was euthanized on LD 3.
- At ≥0.05 mg/kg, there was a significant decrease mean viable pups born and increased post-implantation loss (total and %). There was also a significantly increased length of gestation at ≥0.05 mg/kg consistent with mortality and dystocia.
- At 0.25 mg/kg, there were 3 dams with total litter loss compared to none in all other groups.
- There was significantly early incisor eruption and opening of eyes at ≥0.05 mg/kg, and pinna unfolding and coat development onset at 0.05 mg/kg only.
- Maternal NOAEL was set at 0.01 mg/kg, and F₁ NOAEL was set at 0.01 mg/kg.

Methods

Doses: 0, 0.01, 0.05, and 0.25 mg/kg
 Frequency of dosing: Once daily
 Dose volume: 5 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: Corn oil
 Species/Strain: Han Wistar Rat - Han^{(b) (4)}:WIST(SPF)
 Number/Sex/Group: 22♀/group
 Weight: 181 g to 224 g
 Age: ~11 weeks
 Satellite groups: TK (serum and milk)- 4♀/group
 Study design: F₀ Dams treated from Gestation Day (GD) 6 to Lactation (LD) Day 21) inclusive; F₁ animals were never directly treated at any point, but exposure may have occurred *in utero* and through nursing (test article was confirmed present in milk). On Postnatal Day (PND) 4, litter size reduction/ standardization occurred with gross necropsy on any dead or euthanized pups (4/sex/litter randomly selected). F₁ were mated 1:1 at 10 weeks.
 -Necropsy occurred on following schedule: F₀ ♀ after weaning of F₁ pups on PND 21; F₀ ♀ that failed to produce a viable litter by Day 26 *post-coitus*; unselected F₁ pups on PND 4 or after weaning on PND 21; All F₁ pups not mated after behavioral evaluations. Mated F₁ ♂, after necropsy of the F₁ ♀ on Day 14 *post coitum*.
 -Live evaluations: tonic neck reflex (PND 14), palmar grasp (PND 16), photophobotaxis, cliff avoidance, exploratory locomotor pattern, papillary reflex, acoustic startle, negative geotaxis (PND 22). water maze tests- learning and memory (PND 35-42).

Observations and Results

F₀ in-life:

Mortality: In the main study at 0.25 mg/kg, there were 3 dams found dead near term (GDs 24, 25, and 25) with delayed delivery compared to other dams. During the lactation phase, 1 female euthanized *in extremis* (LD 3) and 1 was found dead on LD3. The deaths were test article-related.

The clinical signs preceding death in these dams included ruffled fur, lethargy, hypothermia, and/or vaginal bleeding (see Clinical Signs below for details). A single dam each also exhibited dystocia or prolapsed uterus.

Table 40 **Unscheduled Deaths at 0.25 mg/kg in a Rat PPND Toxicity Study of Daily Oral Ospemifene Administration**

Dam #	Disposition	Day of Death	# Pups Delivered	# Retained Fetuses	Precedent Clinical Signs
68	Found Dead	GD 24	n/a	n/a	ruffled fur, lethargy, red bedding
71	Found Dead	GD 25	n/a	n/a	ruffled fur, lethargy, red bedding
74	Found Dead	GD 25	n/a	n/a	dystocia, vaginal bleeding, hypothermia
67	Found Dead	LD 3	2	9	vaginal bleeding
75	Euthanized	LD 3	3	3	vaginal bleeding, uterine prolapse

GD, Gestation Day; LD, Lactation Day

Clinical signs: Once daily

As stated above, almost all clinical signs occurred in dams treated at 0.25 mg/kg and preceded unscheduled deaths; there were no relevant clinical observations at ≤ 0.1 mg/kg. All occurrences were within 2 days of delivery or death.

Table 41 **Notable Clinical Signs Observed from Gestation Day 6 to Lactation Day 20 in a Rat PPND Toxicity Study of 0.25 mg/kg Daily Oral Ospemifene Administration (N=15-22/group)**

Sign	# Observations	# Animals
Hypoactivity	2	2
Hypothermia	2	2
Ruffled Fur	6	4
Vaginal Bleeding	9	9
Dystocia	1	1
Uterine Prolapse	3	1

Body weight: Daily

Overall, there was not a pronounced effect on body weight or body weight gain. There was a significant decrease in % BWt gain at 0.25 mg/kg compared to control occurring from GDs 8-17, but this did not result in a significant BWt reduction compared to control. There was a slight increase in BWt gain during the lactation period, but this was not significantly greater than control.

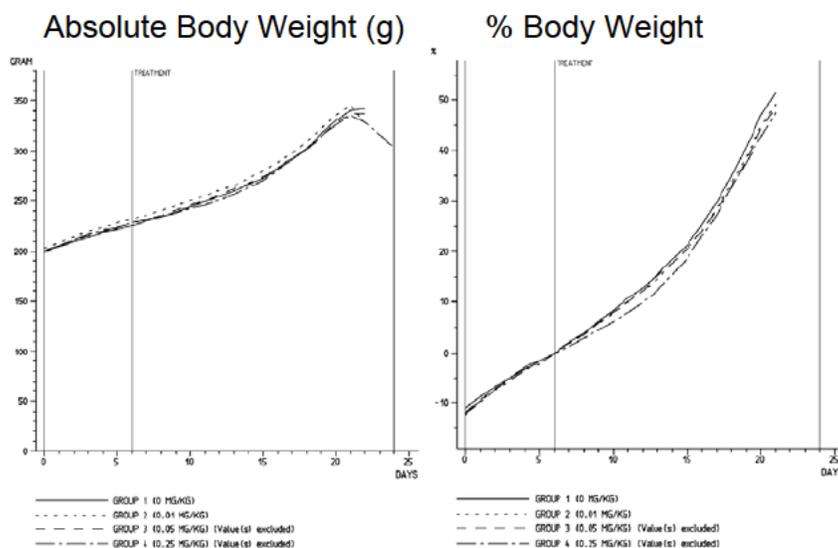
Table 42 Body Weight (BWt) Changes (% Gain) and Differences from Control (%Δ) in Female Rats Administered Oral Ospemifene from GD6 through LD21 N=15-22/sex)

Dose (mg/kg)	Gestation				Lactation			
	GD 6 BWt (g)	GD 21 BWt(g)	% Gain	% Δ	LD 1 BWt (g)	LD 21 BWt (g)	% Gain	% Δ
0	225	341	52%	--	253	286	13%	--
0.01	232	346	49%	1%	258	293	14%	2%
0.05	228	337	48%	-1%	254	288	13%	1%
0.25	228	336	47%	-1%	248	285	15%	--

Table 43 Body Weight Gain in Rats Administered Oral Ospemifene from GD6 through LD21 (N=15-22/sex)

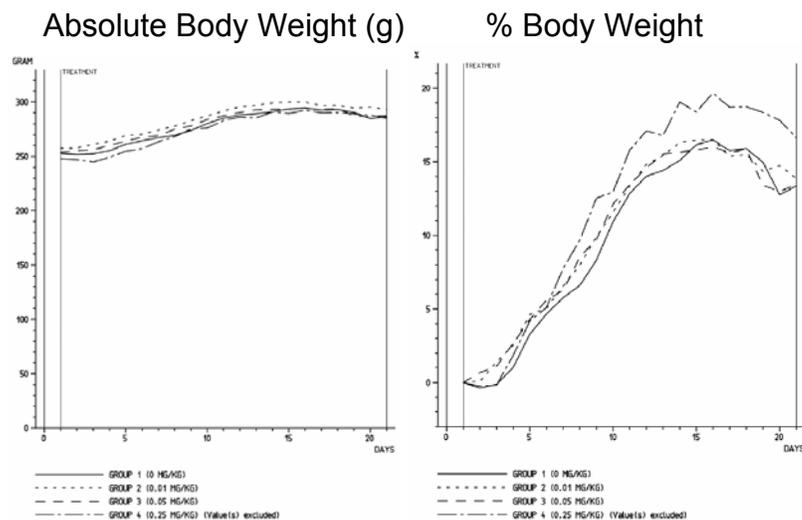
Dose (mg/kg)	BWt Gain (g) GD 6 to GD 21	% Δ	BWt Gain (g) LD 1 to LD 21	% Δ
0	116	--	33	--
0.01	114	-2%	35	6%
0.05	109	-6%	34	3%
0.25	108	-7%	37	12%

Figure 15 Mean Absolute and % Body Weight (BWt) Gains in Pregnant Rats Given Oral Ospemifene



(Figures excerpted from Applicant's package)

Figure 16 Mean Absolute and % Body Weight (BWT) Gains in Rats Given Oral Ospemifene during Lactation



(Figures excerpted from Applicant's package)

Feed consumption: Consumption during the gestation period (GDs 6-21) was slightly decreased by 4% (non-significant) at 0.25 mg/kg compared to control, consistent with the observed BWt gain effects. Consumption during lactation was consistent with observed BWt gains.

F₀ necropsy: Other than the noted clinical signs of vaginal bleeding and uterine prolapse, no F₀ animal had notable findings.

F₀ Mating/Fertility: At ≥ 0.05 mg/kg, there was a significant decrease in Birth Index compared to control, consistent with findings of significantly decreased mean viable pups born and increased post-implantation loss (total and %). There was also a significantly increased length of gestation at ≥ 0.05 mg/kg that was consistent with the observed clinical findings at 0.25 mg/kg (but *not* at 0.05 mg/kg) that preceded mortality in 3 dams, including vaginal bleeding, dystocia, and uterine prolapse. At 0.25 mg/kg, there were 3 dams with total litter loss compared to all other groups, which had none noted. Two of these females died (see Mortality), and the third only had a single dead pup (#84). No effects were noted at 0.01 mg/kg on any parameter. Significant changes were generally outside the reported historical control ranges for the conducting laboratory and consistent with the known effects of this compound class.

Table 44 Selected Mating and Fertility Parameters of Pregnant Rats Administered Oral Ospemifene during the Peri-Postnatal Development Final Study

Parameter	Dose (mg/kg)			
	0	0.01	0.05	0.25
Birth Index (%)	92.9	94.3	84.4**	81.5**
# Mated	22	22	22	22
# Pregnant	22	22	20	21
# Dams Died Pre-partum	0	0	0	3
# Dams with Total Litter Loss	0	0	0	3
# Dams that Reared Pups to LD 21	22	22	20	15
Gestation (Days)	21.3	21.4	21.8*	21.9*
Mean Implantations	13.4	12.7	12.2	13.2
Total Post-implantation Loss	21	16	38**	44**
# Litters Affected	12	10	16	14*
Post-implantation Loss (%)	7.1	5.7	15.6	20.9
Mean Viable Pups - 1 st Evaluation	12.4	12.0	10.3*	9.6*

*, P<0.05 v. control; **, P<0.01

Birth Index = (# pups born alive / # implantations) * 100

F₁ survival and clinical signs: There was a significant decrease in mean number of pups delivered and mean live litter size and at ≥0.05 mg/kg compared to the control group. Consistent with these findings, there were increases in mean dead pups and number of litters with dead pups at ≥0.05 mg/kg. There were 4 pups partially cannibalized by the dam with dystocia (#74). There were no significant clinical signs, BWt changes or pup losses after PND 1 through weaning and maturation in any group.

Table 45 Selected F₁ Litter Parameters Following Administered Oral Ospemifene during the Peri-Postnatal Development Final Study

Parameter	Dose (mg/kg)			
	0	0.01	0.05	0.25
# Litters	22	22	20	18
# Dams Died after Delivery	0	0	0	3
Mean Viable Pups - 1 st Evaluation	12.4	12.0	10.3*	9.6*
# Litters w/ Dead Pups- 1 st Evaluation	1	0	3	5
Mean Dead Pups- 1 st Evaluation	0.1	0.0	0.3	0.8
PND1-4 Losses/ #Litters	3/2	5/3	7/3	6/3
Viability Index (%)	98.9	98.1	96.6	96.5
Mean Living Pups PND21	7.9	7.6	7.5	6.5
# Dams that Reared Pups to LD 21	22	22	20	15
Weaning Index (%)	100	99.4	100	100
Mean BWt at PND 1 (g)	6.1	6.3	6.4	6.3
Mean BWt at PND 21 (g)	48.6	49.4	48.9	50.1

*, P<0.05; **, P<0.01

Viability Index = (# of alive pups on day 4 p.p. / # pups born alive) * 100

Weaning Index = (# alive pups on day 21 p.p. / # alive pups on day 4 p.p.) * 100

F₁ physical development: There was significantly early incisor eruption and opening of eyes at ≥0.05 mg/kg, and pinna unfolding and coat development onset at 0.05 mg/kg only (although these values 0.25 mg/kg were earlier than control, they were not

significant). The sponsor states the effect is due to delayed parturition in these groups making these parameters appear to be more advanced than control. Later occurring and sex-hormone controlled milestones such as balano-preputial separation, testicular descent, and vaginal opening showed no significant difference from control. BWt gain and food consumption was not remarkable up to F₁ mating.

Parameter (days)	Dose (mg/kg)			
	0	0.01	0.05	0.25
Pinna unfolding	3.0	3.0	2.5*	2.6
Incisor eruption	7.7	7.3	6.7*	7.1*
Coat development onset	9.2	8.8	8.7*	8.9
Opening of eyes	15.2	15.1	14.7*	14.6*
Testicular descent	22.2	22.4	22.4	22.5
Balano-preputial separation	27.6	27.6	27.4	27.1
Vaginal opening	35.1	35.5	35.9	35.7

*, P<0.05

F₁ behavioral evaluation: Not remarkable.

F₁ reproduction: No remarkable effects were noted. There was a significantly higher post-implantation rate of 11.1% and 8.5% for 0.01 mg/kg and 0.25 mg/kg, respectively, compared to control (4.5%), but within the historical control range of 3.2% to 14.8%.

F₁ necropsy: Not remarkable.

F₂ findings: Gross evaluation of fetuses was not remarkable.

Toxicokinetics: Breast milk and serum from F₀ females was sampled 4 hr post-dose on PND 21 from 3-4/group for serum and 3/group for milk.

No test article was measurable the serum of F₀ females at 0.01 mg/kg and 0.05 mg/kg, except a single dam (0.56 ng/mL). Milk was quantifiable at ≥0.05 mg/kg, except in one animal at 0.05 mg/kg. Exposure in milk was approximately dose-proportional from 0.05 to 0.25 mg/kg.

Analyte	Dose (mg/kg)	Concentration (ng/mL)
Serum	0.01	<LLQ
	0.5	<LLQ*
	0.25	0.51
Milk	0.01	<LLQ
	0.5	4.6**
	0.25	24.0

*only 1/3 had quantifiable amounts

**1/3 was not quantifiable

<LLQ, below Lower Limit of Quantification

Dosing Formulation Analysis

There was an adequate concentration and homogeneity analysis. The test article was homogenous and stable up to 7 days at RT and 30 days at 4°C.

10 Special Toxicology Studies

Study title: Ospemifene: 8 Week Oral (Gavage) Administration Age Comparison Toxicity Study in the Mouse

Study no.: 15-44407
 Study report location: eCTD 4.2.3.7.7
 Conducting laboratory and location: (b) (4)
 Date of study initiation: March 12, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Ospemifene, #1052557, 99.6%

Key Study Findings

- Treated mice in all groups had urogenital swelling and epididymal fat pads compared to none observed in controls.
- Mice aged 35-37 were most sensitive to the effects of ospemifene-caused urogenital swelling compared to older animals and control with greater incidence and severity of urogenital swelling and epididymal fat pads resulting in morbidity and early sacrifice of 6 animals.

Methods

Doses: 1500 mg/kg
 Frequency of dosing: daily
 Route of administration: Oral gavage
 Dose volume: 6 mL/kg
 Formulation/Vehicle: Corn oil
 Species/Strain: Mice/ Crl:CD1(ICR)
 Number/Sex/Group: 15♂/group
 Age: See below
 Weight: Age-dependent
 Satellite groups: none
 Unique study design: The sponsor used different aged males to determine if age was a factor in unanticipated morbidity

Deviation from study protocol: None reported

Group Number	Description	Cohort	Age at start of dosing	Dose level (mg/kg/day)	Number of animals (males only)
1	Control	Cohort 1	35-37 days	0	15
2	Test	Cohort 1	35-37 days	1500	15
3	Test	Cohort 2	56-58 days	1500	15
4	Test	Cohort 3	70-72 days	1500	15

Observations and Results

Mortality

Six unscheduled deaths by humane sacrifice occurred in the youngest aged group. All deaths were test article-related with severe urogenital swelling, as previously observed in the mouse carcinogenicity study.

Table 46 Unscheduled Deaths in Male Mice Aged 35-37 Days at Study Start

Animal number	Day of death	Clinical signs prior to death	Reason for sacrifice	Macroscopic findings	Microscopic findings
23	18	Urogenital area (right side) swollen, from Day 15	Severity of urogenital swelling	Caecum, ileum and colon – protruding, moderate, into scrotal sac.	Caecum, ileum and colon – unremarkable.
29	31	Urogenital area (left side) swollen, from Day 15	Severity of urogenital swelling	Caecum – protruding, moderately severe, into scrotal sac. Connective tissue – soft and pale epididymal fat pad	Caecum – unremarkable. Epididymal fat pad – present.
19	34	Urogenital area (left side) swollen, from Day 15	Severity of urogenital swelling	Caecum – protruding, moderately severe, into scrotal sac.	Caecum – unremarkable.
16	37	Urogenital area swollen, from Day 18	Severity of urogenital swelling	Caecum – protruding, moderately severe, into scrotal sac. Connective tissue – soft and pale, moderately severe, epididymal fat pad	Caecum – unremarkable. Epididymal fat pad – present.
22	37	Urogenital area swollen, from Day 19; sores/lesions on neck	Severity of urogenital swelling	Caecum – protruding, moderately severe, into scrotal sac. Ileum and colon – protruding, moderate, into scrotal sac. Skin and subcutis – sore, moderate, on neck.	Caecum, ileum and colon – unremarkable. Skin and subcutis – dermatitis, slight.
25	37	Urogenital area (left side) swollen, from Day 15	Severity of urogenital swelling	Caecum – protruding, moderately severe, into scrotal sac. Urinary bladder – distension, moderate.	Caecum – unremarkable. Urinary bladder – missing.

(Table excerpted from Applicant's package)

Clinical Signs

Urogenital swelling occurred in all treated groups compared to none observed in control males. Onset was most rapid in the youngest males.

Table 47 Urogenital Swelling Incidence and Onset in Male Mice Treated with Oral Ospemifene for 8 Weeks

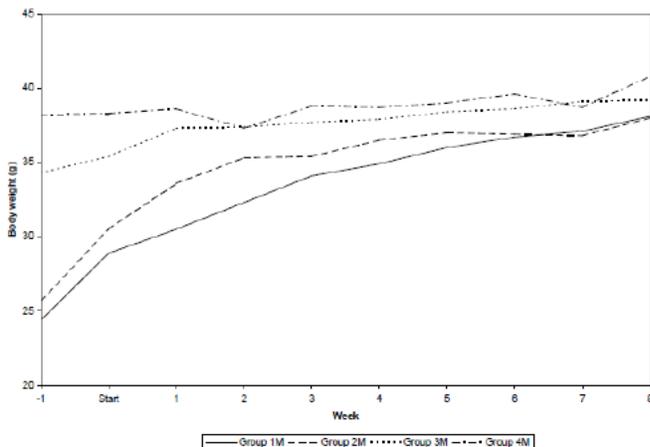
Group	Study Start Age (Days)	# Affected	% Affected	Onset (# / Week)	Severity
1	35-37	0	0	--	
2	35-37	12	80	6 / Wk 3 5 / Wk 4 1 / Wk 5	Slight-Severe

3	56-58	10	67	3 / Wk 4 2 / Wk 5 3 / Wk 7 2 / Wk 8	Minimal-moderate
4	70-72	10	67	3 / Wk 4 6 / Wk 6 1 / Wk 8	Minimal-moderate

Body Weights

All groups gained body weight during treatment, showing that ospemifene did not adversely affect weight gain. Since Groups 3 and 4 were now age-matched, direct comparison is not useful between the controls. The treated animals aged 35-37 days gained 16% less weight than the untreated age-matched controls.

Table 48 Group Mean Body Weights of Male Mice Treated with Oral Ospemifene for 8 Weeks



(Figure excerpted from Applicant's package)

Feed Consumption

Food consumption was higher in treated males aged 35-37 days than untreated controls.

Gross Pathology

Group (Study Start Age)	Finding	# Affected/Total
2 (35-37 Days)	Thick Connective Tissue	6/9
2 (35-37 Days) - decedents	Protruding Caecum	6/6
	Protruding Ileum	3/6
	Protruding Colon	3/6
	Distended Urinary Bladder	1/6
	Thick Connective Tissue	1/6
4 (70-72 Days)	Thick Connective Tissue	7/15
	Large Connective Tissue	3/15

Histopathology

Adequate Battery - No, but adequate for purpose of examining urogenital organs.

Tissue list

animal identification

gross lesions (§)

kidney

prostate

seminal vesicles

testes + epididymides

ureters

urinary bladder

fixative = 10% neutral buffered formalin

Peer Review - No

Histological Findings

Epididymal fat pad was present in 8, 12, and 9 males of each treated group, compared to none observed in the control group.

11 Integrated Summary and Safety Evaluation

Ospemifene was developed to treat Vulvar and Vaginal Atrophy in postmenopausal women. This SERM is the first that has an agonist (estrogenic) effect on the human vagina, thus showing slightly more estrogen agonism than raloxifene, but less than tamoxifen (obvious endometrial agonism). The sponsor conducted a full nonclinical program including an extensive number of pharmacology, pharmacokinetic, and toxicology studies. The toxicology program used rats and cynomolgus monkeys for the chronic toxicology studies.

Overall Nonclinical Finding Summary: Ospemifene demonstrated expected pharmacology of a mixed estrogen agonist/antagonist with no unexpected safety signals. When comparable, the findings from nonclinical studies were consistent with the findings from clinical studies with only minor differences observed. The greatest clear risk of ospemifene is if pregnant women or women of child bearing potential take this drug. Embryofetal lethality was observed in rats and rabbits, and testicular displacement was observed in male offspring of treated pregnant rats. Additional detrimental effects were noted in parturition of rats. These effects occurred at doses that are well below human exposure. This should not be a concern if used as indicated, since this drug is to treat postmenopausal women only. Ospemifene is tumorigenic in rodents like all other approved estrogens, anti-estrogens or SERMs. The tumors were noted at doses at human exposure, and no dose was observed free of treatment-related tumors. Based on other approved drugs that are estrogen receptor ligands and especially SERMs, there appears to be a low risk for human carcinogenicity. Since rodents were treated for a lifetime, the studies likely exaggerate the risk to postmenopausal women. In humans, the most common estrogen-dependent tumors originate in the uterus and breast, whereas these were reduced in rodents, consistent

with the established pharmacology of ospemifene. Overall, animals treated with ospemifene appear to have more tumors types compared to other SERMs but did not have an adverse impact on survival, since ospemifene appeared to have prevented many of the tumors that normally do kill rodents, e.g., mammary tumors.

Pharmacology: Ospemifene is a mixed estrogen receptor agonist/antagonist that competitively and reversibly binds to both estrogen receptors. Ospemifene has predominantly estrogen agonist activities on the vagina and bone, mixed activities on the uterus, and antagonistic activities on the mammary gland.

Safety Pharmacology: No significant findings were reported from safety pharmacology studies. The in vitro hERG assay suggested that ospemifene and the predominant metabolite 4-hydroxy ospemifene (designated M1) had slight potential for QT prolongation based on hERG-1 tail current inhibition at ≥ 1 μM with maximal inhibition of 35% and 86%, respectively. In dog and monkey nonclinical studies, no effect on QT was observed in vivo. A dedicated clinical study showed no relationship between treatment between placebo-corrected QTcI change from the baseline and time-matched concentrations.

PK/ADME: Ospemifene is highly protein bound in all species, ranging from 83 - 95% ex vivo, depending on species, timing, and assay, but $>97\%$ bound in vivo. Absorption in humans and monkeys was more rapid than rodents with T_{max} values of ~ 2 hrs and ~ 8 hrs, respectively. However, bioavailability (F) was only 2.6% and 2.7% for rats and monkeys; human F was not calculated. The poor absorption/F of ospemifene was not explained by in vitro drug transporter assays. Ospemifene was highly permeable across CaCo-2 and MDR-MDCK cell monolayers with no measurable efflux and was not a substrate of the P-glycoprotein transporter. One potential source for the apparent low F is extensive first-pass metabolism in the intestine and/or liver and conversion to the M1 metabolite (see below), since the radioactivity F is 49% in rats and 11% in monkeys. Decreasing particle size had only a modest effect on increasing exposure, and data from different vehicles provided no clear explanation.

^3H -Ospemifene was widely distributed to most tissues but at low levels, except for gastrointestinal tissues, liver, and bile duct. Radioactivity in most tissues declined, but was still detectable at 72 hrs suggesting long-lived metabolites. These findings were consistent with elimination of ospemifene in rat and monkey predominantly through the liver with hepatobiliary recirculation observed. Excretion is predominantly through the feces in rats, monkeys, and humans (70-80%), with very little excreted in urine ($\leq 7\%$).

Accumulation was not observed in rats, mice or monkeys, but there was a ~ 2 -fold increase in AUC for humans. This is likely due to the longer half-life observed in humans (25 hrs) compared to rodents (~ 2 hrs) and monkeys (~ 8 hrs).

Ospemifene is extensively metabolized in the liver in all species by CYP enzymes and most metabolites are hydroxylated and glucuronidated products. Based on in vitro studies using liver microsomes and isolated hepatocytes, the predominant CYPs

involved in the metabolism are CYP3A4, CYP2C9, CYP2C19 and CYP2B6. In humans, ospemifene is primarily metabolized by CYP3A4, CYP2C9 and CYP2C19, with CYP3A4 likely the most prominent in activity.

The metabolites 4-hydroxy ospemifene (M1) and 4'-hydroxy ospemifene (designated M2) are the predominant metabolites by exposure in humans, mice, rats, and monkeys. M1 and M2 have similar pharmacological profiles as ospemifene. Exposure of M1 exceeds that of the parent in rodents, dogs and monkeys, but is only 25% of parent exposure in humans. The ratios of nonclinical exposures to human exposures range from 10.2 in rats to 21.8 in monkeys. M1 exposure profile and T_{max} is comparable to ospemifene, suggesting rapid first-pass metabolism in the liver. M2 exposure is comparable across these species, ranging from 4-8%; the ratios are 1 for rats and 6 for monkeys.

In clinical studies that investigated co-administration of known CYP inhibitors, the CYP3A4 inhibitor ketoconazole, the CYP2C19 inhibitor omeprazole, and the CYP3A/CYP2C9/CYP2C19 inhibitor fluconazole increased AUC exposure of ospemifene by 42%, 17%, and 174%, respectively. Co-administration of drugs that inhibit both CYP3A4 and CYP2C19 are likely to increase the exposure of ospemifene. Ospemifene does not apparently inhibit CYP activities. In vitro experiments showed potential inhibition of CYP2B6, CYP2C9, CYP2C19, CYP2C8 and CYP2D6 activities by ospemifene with IC_{50} values $\geq 8 \mu M$. However, the mean C_{max} of ospemifene in women after taking the proposed 60 mg dose of ospemifene is $\sim 3 \mu M$.

Ospemifene did not greatly induce CYP activities in isolated human hepatocytes. Ospemifene at 20 μM induced CYP1A2, CYP2B6 and CYP3A4 activities in hepatocytes from only 1/4, 2/4, and 1/3 responsive donor livers. A lack of induction was confirmed in clinical trials using probes for CYP2C9 (S-warfarin), CYP2C19 and CYP3A4 (omeprazole) and a CYP2B6 (bupropion). Conversely, the inducer of CYP3A4 and CYP29C activity, rifampin, decreased the AUC exposure by 58% in humans.

General toxicology: In rats, mice, female dogs, and female monkeys, there were no unexpected toxicities in repeat dose studies up to 26, 13, 13, and 39 weeks, respectively. The main effects noted were mild and related to the exaggerated pharmacological effect of ospemifene on reproductive organs. Organ weight changes, gross pathological and histopathological effects were noted on the ovary, uterus, vagina, mammary gland, liver (see **Liver** below), prostate, testis, and epididymis in most species and studies. Sporadic findings were reported in the adrenal and pituitary and on hematopoiesis in rodents, consistent with known estrogenic and SERM profiles (these sporadic findings were more pronounced in the carcinogenicity studies). The ovary, uterus, mammary gland, and male reproductive organs showed a predominantly antagonistic profile, whereas the vagina showed agonism. However, some studies showed agonism, demonstrating the potential cell- and tissue-selective effects of ospemifene. Except in mice, there was little of note in bone in the toxicology studies. All findings noted were at exposures comparable to human exposure at the proposed dose, but other than vaginal effects, no similar clear signals were noted in clinical trials.

Tissues:

Ovary- There were decreased ovarian weights with ovarian cysts, with disruption of reproductive cycling secondary to the ovarian effects in mice, rats, dogs, and monkeys. Mice and dogs had absent antral follicles, and rats and monkeys had decreased corpora lutea. In rats at 26 weeks, some HD females had cystopapillary hyperplasia of the granulosa cells within cystic follicles, which appears to be a neoplastic precursor to the ovarian adenomas observed. Rats and monkeys had marked increases in estradiol and modest increases in FSH in the chronic studies compared to control; rats had small increases in LH.

Vagina- Mucification was noted in rats and monkeys.

Uterus- There was decreased uterine weight with endometrial atrophy, cystic dilatation of glands in mice and rats. Additionally noted in rats were squamous cell metaplasia, minimal endometrial hyperplasia, luminal epithelium hypertrophy, and myometrial atrophy. In rats at 26 weeks, there was atrophy of endometrium stroma, but also hyperplasia/hypertrophy of endometrial epithelium. In the monkey at 39 weeks, there was a spectrum of activities based on dose. Cystic glandular changes and endometrial hyperplasia were observed in most animals in the mid or high dose. Uterine decidual reaction was observed in half of the low dose animals, and cervical squamous metaplasia in most animals at the mid dose level.

Liver- Liver weight increases correlating with centrilobular hepatocyte hypertrophy were noted in mice, rats, dogs, and monkeys. The pattern of liver enzymes showed a species-specific pattern. Rats had slight increases in ALT, ALP, and decreased serum cholesterol. Dogs had increased ALP and TGs. Monkeys had ALT increases. These findings are consistent with metabolic adaptation and induction of the CYP enzymes that metabolize ospemifene and M1. The liver is a potential estrogen target organ both through pharmacology and metabolism. It is unknown if the increase in liver neoplasms observed for ospemifene is rodent-specific.

Mammary Gland- The mammary gland showed sex-, dose- and species- specific effects, considered to be pharmacological and antagonistic in female rats and monkeys. In male rats, there was tubulo-alveolar differentiation; this is normally only observed in female rats as a function of estrogenic actions in concert with progesterone. Ductal hypertrophy was observed in female rats at 26 weeks, but most animals had atrophied mammary glands. In female monkeys after 39 weeks, some animals at LD and HD were noted with mammary glandular atrophy; males were not evaluated. Similar to the uterus the predominant activity was antagonism, but not completely so.

Male Reproductive Organs- Decreased organ weights with atrophy of the prostate, testis, and epididymis were noted in rodents and dogs; male monkeys were not evaluated.

Other Notable Species Effects:

Rats- Decreases in body weight and food consumption were noted in rats at doses from 1 to 3 times human exposure.

Mice- In general, findings were mild in mice treated with oral ospemifene at doses from 1 to 5 times human exposure. No unique finding was reported.

Monkeys- In general, findings were mild in female monkeys treated for up to 39 weeks with oral ospemifene at doses less than human exposure. No unique finding was reported.

Dogs- The dog was not selected as the second species due to the unique endometrial toxicity associated with estrogenic compounds- endometrial infections called pyometras. Consistent with this, there were increases in uterine weight with cystic uterine endometrial hyperplasia noted in all treated animals; and with cervix and/or uterus squamous metaplasia and endometrial inflammation in most of the animals.

By Dr. Wafa Harrouk (DARRTS, 9/28/2005):

In the rat, decreases in body weight and food consumption were noted. Other findings were limited to the exaggerated pharmacological effect of ospemifene on reproductive organs (ovarian cysts, thin uterus with endometrial stromal atrophy and epithelial hypertrophy/hyperplasia, vaginal mucification, mammary ductal hypertrophy) and pituitary vacuolation and were in line with known SERM effects. NOEL for the rat was set at 300 mg/kg/day.

In the dog, there was no effect on BW, food consumption, ECG, or blood pressure. Findings were limited to exaggerated pharmacological effects of ospemifene on reproductive organs (testis, epididymis & prostate atrophy, dilated endometrial glands). NOEL was set at 8 mg/kg/day. No target organs were identified in the dog. However, due to the potential effect of SERMs on the dog model (e.g., uterine abscesses), sponsor has replaced the dog model with the monkey.

In the monkey, an increase in liver and ovarian weight and presence of ovarian cysts were noted. Histopathological changes in the ovaries, uterus, vagina and mammary glands were also noted in the monkey, all of which are considered exaggerated pharmacological effects. The NOEL was set at 150 mg/kg/day.

Table 49 Exposure Multiples Based on AUC and Body Surface Area (BSA) for Ospemifene Following Repeat Dose Oral Administration to Rats, Mice, Rabbits and Monkeys in the Pivotal Toxicology Studies Compared to Humans

Species	Sex	Study	Dose (mg/kg)	NOEL, LOAEL, or NOAEL	AUC _{0-inf} (ng*hr/mL)	Exposure Multiple	
						AUC	BSA
Rat	F	6 month	3	NOEL*	ND	ND	0.5x
	F	6 month	30	--	ND	ND	5x
	F	6 month	300	NOAEL#	ND	ND	49x
	M/F	3 month	50	NOEL*	6260/4940	1x	8x
	M/F	3 month	300	--	12600/18000	2.5x	49x
	M/F	3 month	2000	NOAEL#	13900/16700	3x	324x
	M/F	Carci	10	LOAEL	1500/1470	28%	2x
	M/F	Carci	50	--	5440/5400	100%	8x
	M/F	Carci	300	--	7170/6470	125%	48x
	F	EFT	0.1	--	81	1%	2%
	F	EFT	1	--	332	8%	16%
	F	EFT	4	NOAEL	725	13%	65%
	F	PPND	0.01	NOAEL	≤LLOQ	ND	0.2%
	F	PPND	0.05	LOAEL	≤LLOQ	ND	0.8%
	F	PPND	0.25	--	≤LLOQ	ND	4%
Rabbit	F	EFT	3	NOAEL	ND	ND	1x
	F	EFT	10	LOAEL	ND	ND	3x
	F	EFT	30	--	ND	ND	10x
Mouse	F	Carci	100	LOAEL	11400	2x	8x
	F	Carci	400	--	21700	4x	32x
	F	Carci	1500	--	25400	5x	120x
Monkey	M/F	9 month	15	NOEL*	808/618	13%	5x
	M/F	9 month	50	--	1355/1312	25%	16x
	M/F	9 month	150	NOAEL#	3145/3499	62%	49x
Human	F	15-50927	60 mg		5448 (3204 - 7219)	--	

M, Male; F, Female; Cmax, Maximal Concentration; AUC_{0-inf}, Area Under the Curve for 0-infinity; EFT, Embryo-fetal Toxicity; PPND, Pre- and Post natal Development; LLOQ, Lower Limit of Quantification; ND, Not Done

*The NOEL is < the lowest dose tested

The NOAEL is ≥ the highest dose tested

Reproductive toxicology: No fertility and early embryonic development study was conducted or necessary for the indicated population of post-menopausal women. It is likely that ospemifene is embryotoxic due to the uterine antagonism pharmacology and adverse reproductive effects noted below.

Embryofetal toxicity (EFT) studies for ospemifene were conducted with rats and rabbits. For rats, the EFT study conducted where pregnant animals were treated daily with oral ospemifene from GD 6 through LD 16 at 0.1, 1 and 4 mg/kg. For rabbits, pregnant

animals were treated daily with oral ospemifene from GD 6 through GD 18 at 3, 10, and 30 mg/kg.

Reviewed by Dr. Wafa Harrouk (DARRTS, 9/28/2005):

In rats, an increase in placental weight and an increased number of testicular displacements among pups was noted. Maternal and fetal NOAEL was 4 mg/kg/day.

In rabbits, a dose-related increase in total resorptions correlated with a decrease in the number of live fetuses and an increase in post-implantation loss was seen. The maternal NOAEL was <3 mg/kg/day and fetal NOAEL was 30 mg/kg/day. This reviewer concurs with Dr. Harrouk's conclusions.

A definitive pre-and post-natal development study was conducted where pregnant rats were treated daily with oral ospemifene from GD 6 through LD 21 at 0.01, 0.05, and 0.25 mg/kg. There was increased mortality at 0.25 mg/kg preceded by clinical signs of difficult parturition such as dystocia, vaginal bleeding, ruffled fur, lethargy, hypothermia, and/or uterine prolapse in 6/21 dams. At 0.25 mg/kg, there were 3 dams with total litter loss compared to none in all other groups. There was also a significant gestational length increase at ≥ 0.05 mg/kg, consistent with mortality, prolapse, and dystocia.

At ≥ 0.05 mg/kg, there was a significant decrease mean viable pups born and increased post-implantation loss (total and %), and non-significant increase in number of litters with dead pups compared to control. The maternal NOAEL was set 0.01 mg/kg. In the offspring, there was significantly early incisor eruption and opening of eyes at ≥ 0.05 mg/kg, and pinna unfolding and coat development onset at 0.05 mg/kg only. The F₁ NOAEL was set at 0.01 mg/kg due to sooner manifestation of developmental signs and reduced live pups at 1st evaluation. Findings in a pilot study were very similar.

Carcinogenicity: Ospemifene is carcinogenic to rodents based on the findings from the rat and mouse 2-year carcinogenicity studies. In general, there was no dose-relationship in the tumor findings, probably as a consequence of exposure approaching saturation. Except in the skin, neoplasm differences compared to control groups were consistent with the established pharmacology/toxicology of ospemifene and profiles of other mixed estrogen agonist/antagonists. Both neoplastic and non-neoplastic treatment-related effects were related to exaggerated pharmacology in estrogen target organs, including testes, epididymides, ovary, uterus, mammary gland, bone, liver, adrenal, pituitary, spleen, thymus and thyroid. Neoplasms could be related to the pharmacologic effects on cell types that express the estrogen receptor, and/or disruption of the normal physiological endocrine/steroidogenic feed-back loop. Exposure in mice and rats was saturated in the studies, likely explaining the lack of dose-relationship for neoplastic findings.

Mice: Male mice did not tolerate ospemifene and were terminated early without evaluation. The cause of morbidity was urogenital swelling was determined to be inguinal hernias. An additional study showed that younger mice were more sensitive to the ospemifene-induced urogenital swelling than older males.

Female mice were given oral ospemifene at 100, 400, and 1500 mg/kg/day (8x, 32x, and 120x human exposure based on mg/mm²) for 104 weeks with dual controls. Female mice survival to study termination was 41-51% in treated groups compared to 41% of controls. At termination, body weight gain in the MD and HD was reduced by 13% and 8%, respectively, compared to controls. The most relevant macroscopic finding was an increased incidence of large ovary and ovarian masses correlated with the neoplastic findings. Exposure multiples at the LD, MD, and HD were 2x, 4x, and 5x the proposed clinical dose, respectively.

There were statistically significant increases in adrenal, liver, pituitary and ovarian neoplasms. The incidences of adrenal and ovarian neoplasms were greater than concurrent and maximum historical control (HC) rates for female CD-1 mice. Liver and pituitary neoplasms showed a statistically significant increase compared to concurrent control, but were within HC incidence range. Also, there were significant decreases in mammary gland, uterus, and blood vessel neoplasms.

Adrenal- Both benign adrenal subcapsular and benign and malignant cortical tumors were significantly increased for overall response and at HD when compared to concurrent and historical controls. There was a dose-response for subcapsular tumors; the incidence was 0%, 2% (1 female), and 7.8% (4 females) compared to 0% in concurrent controls and a maximal HC incidence of 5.8%. There was significant increase in cortical tumors with an incidence of 2% in all-treated animals and 5.8% (3 females) in the HD group. Two of the three neoplasms were malignant at the HD. There were no tumors found in controls, LD, and MD animals. While adrenal cortical neoplasms are considered common, there were none present in the HC. The increase in adrenal findings is likely related to the estrogen agonist effect of ospemifene on the hypothalamo-pituitary-adrenal axis.

Ovary- There was significant treatment-related increase in neoplastic findings in the ovary. There was an increase in benign and malignant tubulostromal tumors, sex-cord stromal tumors, granulosa cell tumors, and luteal tumors, compared to controls. Tubulostromal adenomas occurred in all dose groups, while both tubulostromal carcinomas were noted at the MD. Benign and malignant sex-cord stromal tumors occurred at increased incidence in all dose groups, compared to controls; there were no malignant tumors observed in controls. Benign luteomas showed a treatment-related increase incidence in all dose groups (12%) compared to control groups (5%), but the numbers were higher for LD (7) than MD and HD (4 each). Malignant luteomas were present in 5 MD and 1 HD animal compared to 0 control and LD animals. Benign granulosa cell tumors were present in 1 LD and 3 HD animals, and malignant granulosa cell tumors were found in 3 MD and 1 HD animals, compared to none in control animals. Additionally, two thecoma (both at MD), three cystadenoma (1 LD and 2 HD), and two cystadenocarcinoma (1 LD and 1 HD) were noted in treated groups, but not in controls. For sex chord stromal tumor, the incidence in all treated groups was 4-6x greater than the maximal HC incidence with 53.6%, 41.5% and 66.7% compared to 10.8%. The concurrent control incidence was 6.8%. There were also 16 malignant sex chord

stromal tumors combined in treated groups (1, 11, 4) compared to none in concurrent controls. Except for benign sex chord stromal tumors (2 total, 1 each control group) and benign luteomas (5 total), no tumors were noted in control animals. Ovary adenoma/carcinoma findings would be more relevant to humans than sex chord stromal tumors since most human ovarian tumors are of epithelial origin. However, the relevance of these findings to humans based on lifetime treatment of sexually immature rats to postmenopausal women is probably low.

Liver- There was a significant increase in overall response and at HD for liver neoplasms with only HD having tumors. The HD incidence was 5.8% compared to 0% in concurrent controls and 0% in LD and MD groups. The increase in liver neoplasms may be the result of enzymatic induction since exposure from Day 1 to Week 52 decreased by >60% for all treated groups. Although an estrogen-like effect is plausible, the HD incidence was within the HC incidence range of 0-10.8%.

For further discussion, see **Clinical Risk of Carcinogenicity** below.

Pituitary- There was a significant increase in overall response and at HD for pituitary neoplasms. The incidence was 0%, 1.9%, and 5.8% compared to 1.9% in concurrent controls. However, pituitary tumors are common in rodents, and the overall incidence in treated groups of 2.6% and the HD incidence of 5.8% are within the HC range of 1.9-12.6%. The increase in pituitary findings, like the adrenal, may be related to the estrogen agonist effect of ospemifene on hypothalamo-pituitary signaling.

Rats: Rats were given oral ospemifene at 10, 50, and 300 mg/kg/day (2x, 8x, and 48x human exposure based on mg/mm²) for 104 weeks with dual controls. Rat survival at study termination was significantly higher for all treated groups compared to controls. There were 68%, 74%, 96%, 86%, and 94% surviving in Control 1, Control 2, LD, MD, and HD groups of males, respectively. Female survival was 72%, 58%, 92%, 82%, and 88% in Control 1, Control 2, LD, MD, and HD groups, respectively. Body weight gain for males at termination was significantly and markedly reduced by 56%, 60%, and 64%, respectively, with reduction occurring over the entire dosing period. For females, the body weight gains were significantly reduced but slightly less marked than males with reductions of 49%, 47%, and 48%. Macroscopic findings generally correlated with the neoplastic findings. Combined male and female rat exposure multiples at the LD, MD, and HD were 28%, 100%, and 125% the proposed clinical dose, respectively.

There were significant increases in liver and thymus neoplasms, and non-significant increases in ovary neoplasms. The incidences of adrenal and ovarian neoplasms were greater than concurrent and maximum historical control (HC) rates for Han/Wistar rats. Additionally, there were significantly decreased lymphocytic leukemia, and mammary gland, pituitary, skin, testes, thyroid, and uterus neoplasms.

Liver- There was a significant increase in hepatocellular tumors in overall response, MD and HD of both sexes. The incidence was 4%, 6%, and 6% in males and 2%, 12%, and 8% in females with 0% in the concurrent controls. The incidence rates in all treated

groups exceeded the HC rate of 1.7% for males and 2.0% for females. Additionally, there was 1 HD male and 2 MD females with malignant tumors compared to no concurrent or HC animals. In a separate study, ospemifene did not induce DNA adducts in livers female rats, while adducts were induced by tamoxifen.

For further discussion, see **Clinical Risk of Carcinogenicity** below.

Thymus- There was a significant increase of benign thymoma at all doses in both sexes. The incidence of benign thymoma was sex-dependent with twice as many treated female animals/group with 28% of treated females compared to 15% of treated males having this finding. There was a 0% and 2.5% incidence rate for males and female controls. HC data confirms this finding showing that females have about 2.5-fold background incidence compared to males; the maximal HC rate for males was 8.5% and 12.5% for females. Malignant thymoma was present in 2 MD females, but in no other group of either sex. Oddly, minimal or no thymus effects were noted in any repeat dose toxicity study, even if animals were peri-pubertal at study initiation. The finding of increased thymoma is unique for this SERM, and probably due to a higher estrogen antagonism that blocked the function of estrogen in thymic involution at puberty.

The role of sex steroids and thymic involution at puberty is well described, if not entirely understood from a mechanistic view. Since these rats were 6 weeks old at the start of the study, they were not likely sexually mature, and thus this may be an artifact of the experimental design. The thymoma findings are not likely relevant for the indicated population of post-menopausal women.

Ovary- Incidence of benign ovarian adenoma was 0%, 0%, 2%, and 2% for control, LD, MD, and HD, with a maximal HC incidence of 1.6%. The overall incidence in treated groups was 1.3%. Sex cord stromal tumor incidence was 0%, 4%, 0%, and 2%, with a HC incidence of 2%.

Executive CAC and Nonclinical Statistical Evaluations

A nonclinical statistical evaluation of the data by Atair M. Rahman (see Appendix) was conducted and generally concurred with the applicant's findings. Hemangiomas of the spleen were also deemed significantly increased in rats, differing from the applicant's analysis. The applicant grouped all hemangiomas, as is usual based on Ex-CAC guidance, whereas Dr. Rahman evaluated the hemangiomas separated by tissue. Hemangiomas of the spleen have been noted for 2 unapproved SERMs: lasofoxifene (rat) and bazedoxifene (mouse), as treatment related, but not statistically significant.

The Executive CAC met on November 27, 2012, and considered the following neoplasms to be drug related in female mice:

- **Ovary**- benign and malignant tubulostromal tumors, sex-cord stromal tumors, granulosa-cell tumors, and luteal tumors
- **Adrenal**- subcapsular adenomas at the mid dose and high dose, cortical adenomas and carcinomas in females at the high dose.

The Executive CAC considered the following neoplasms to be drug related in rats:

- **Liver** – benign and malignant hepatocellular neoplasms in females.
- **Thymus** – benign thymoma in both sexes and malignant thymoma in females.

Clinical Risk of Carcinogenicity: While the findings from these carcinogenicity studies are valid for rodents, they are not entirely relevant for humans. Treating rodents from a sexually immature age through the entire reproductive lifetime does not accurately inform on the risk to the proposed population and duration. Ospemifene is indicated for postmenopausal women that will likely take this drug only until the symptoms of vulvar vaginal atrophy recede. Most likely, the rodents significantly overstate the risk. No treatment-related neoplasms were detected in eight Phase 2 and 3 clinical trials where 1476 patients were exposed to 60 mg daily ospemifene for up to 52 weeks.

Except for tamoxifen, the triphenylethylene-derived, mixed estrogen agonist/antagonist (SERM) drug class (that include ospemifene, clomiphene, toremifene, and tamoxifen) has not shown clear neoplastic signals in pre-marketing clinical trials or in the post-marketing setting. Tamoxifen, the exception, causes uterine tumors. While the exact mechanism is not elucidated, it is thought that tamoxifen has a high level estrogenic activity in the uterus compared to other SERMs and for the benzothiophene-derived SERM, raloxifene (ospemifene appears to have reduced estrogenic activity in humans and is an antagonist in rodents). The primary and pharmacologically active metabolite, 4OH-tamoxifen is genotoxic, which may contribute to the uterine cancer signal. An increase in lung cancers was observed for lasofoxifene (a benzothiophene) in clinical trials, but lung neoplasm increases were not observed in rodents.

In contrast, estrogens increase the incidence of breast, uterus, cervix, vagina, and liver cancer in both rats and humans. All SERMs have increased incidences of ovarian tumors (predominantly sex chord stromal tumors) in at least one rodent species at clinically relevant exposures, but this type is not relevant to humans since most human tumors are epithelial in origin. However, there were ovary epithelial neoplasms observed in raloxifene at exposures lower than the clinical dose, which has not resulted in increased ovarian cancers in women taking raloxifene for osteoporosis. Additionally in rodents, there have been increased neoplastic incidences in adrenal (mouse: lasofoxifene), bone (mouse: toremifene), kidney (rat: raloxifene, lasofoxifene), liver (rat: tamoxifen), mammary gland (rat: arzoxifene), prostate (mouse: raloxifene), and testes (rat: tamoxifen; mouse: raloxifene, lasofoxifene, toremifene). From NDA review of raloxifene, an increase in liver neoplasms was noted, but not a statistically significant level. Previous clinical experience is markedly different from the rodent carcinogenicity findings in studies conducted to support marketing applications for SERMs.

12 Appendix/Attachments

Executive CAC

Date of Meeting: November 27, 2012

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Lynnda Reid, Ph.D., DRUP, Alternate Member
Jeffrey Bray, Ph.D., DRUP, Reviewer
Alex Jordan, Ph.D., DRUP Team Leader

Author of Draft: Jeffrey Bray, Ph.D.

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

NDA #203-505

Drug Name: Ospemifene

Sponsor: Shionogi, Inc.

Background: Ospemifene is a mixed estrogen agonist/antagonist (SERM) developed for treatment of vulvar and vaginal atrophy in postmenopausal women. The carcinogenicity study protocols were concurred with by eCAC on October 19, 2006. Ospemifene was considered to be non-genotoxic based on a battery of in vitro and in vivo studies.

Rat Carcinogenicity Study

Han Wistar rats (50/sex/group) were dosed with 10, 50, and 300 mg/kg/d in corn oil, with the high dose based on MFD. Dual control groups were used, each with 50 /sex/group. Markedly lower body weight was observed in all treated groups relative to controls for males and for females. Survival was significantly increased in all treated groups compared to control ranging from 86% to 96% for males compared to 68% and 74% for controls and 82% to 92% for females compared to 72% and 58% for controls. Exposure based on AUC at termination did not achieve very high multiples of the clinical exposure at the proposed dose (30%, 60%, and 125%), but this was expected.

There were significantly increased incidences of neoplasms in liver and thymus compared to control and above historical control incidence rates. In general, neoplastic findings are consistent with the known pharmacologic effects of a mixed estrogen agonist/antagonist on cell types that express the estrogen receptor.

Liver and Thymus Neoplastic Findings in Rats at Necropsy during the 2-Year Oral Ospemifene Carcinogenicity Study

		Incidence of liver tumours: liver									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Liver	No. examined:	50	50	50	50	50	50	50	50	50	50
hepatocellular adenoma	Finding present	0	2	3	2	0	0	1	4	4	0
hepatocellular carcinoma	Finding present	0	0	0	1	0	0	0	2	0	0
hepatocellular tumours combined		0	2	3*	3*	0	0	1	6**	4*	0

Key: Statistical analysis: * = P<0.05, ** = P<0.01

		Incidence of thymic tumours: thymus									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Thymus	No. examined:	50	50	48	49	48	49	49	49	49	50
benign thymoma	Finding present	0	5	8	9	0	3	10	21	11	2
malignant thymoma	Finding present	0	0	0	0	0	0	0	0	2	0
thymus epithelial tumours combined		0	5**	8***	9***	0	3	10*	21***	13**	2

Key: Statistical analysis: * = P<0.05, ** = P<0.01, *** = P<0.001

(Excerpted from Applicant's package)

Mouse Carcinogenicity Study

CD-1 mice (51/sex/group) were dosed with 100, 400, and 1500 mg/kg/d in corn oil, with the high dose based on MFD. Dual control groups were used, each with 51/sex/group. No significant treatment-related effect on body weight or survival was noted in females, except a lower relative body weight at the mid dose. The exposure based on AUC at termination did not achieve very high multiples of the clinical exposure at the proposed dose (2x, 4x, and 5x). However, this appears to be caused by a time-dependent decrease in exposure at all doses between weeks 13 and 52.

Female mice had significant treatment-related increases in adrenal and ovary neoplasms; the ovary neoplasms were without a dose relationship. The incidences of adrenal and ovarian neoplasms were above maximum historical control rates for female CD-1 mice. Liver and pituitary neoplasms showed a statistical increase compared to concurrent controls, but were within historical control incident rates. In general, neoplastic findings are consistent with the known pharmacologic effects of a mixed estrogen agonist/antagonist on cell types that express the estrogen receptor.

Males were terminated early (by week 24) with eCAC concurrence. Treatment-related morbidity due to urogenital swelling (inguinal hernias) was observed at all dose groups. This was determined to be an age-related phenomenon with younger males more susceptible to this effect.

Table - Adrenal and Ovary Neoplastic Findings in Female Mice at Necropsy during a 2-Year Oral Ospemifene Carcinogenicity Study

		Incidence of neoplastic lesions: adrenal gland									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
		0	100	400	1500	0	0	100	400	1500	0
Adrenal gland subcapsular cell tumour	No. examined:	-	-	-	-	-	51	51	51	51	51
	Grade	-	-	-	-	-	51	51	50	47	51
	+	-	-	-	-	-	0	0	1	4	0
cortical adenoma	Grade	-	-	-	-	-	51	51	51	50	51
	+	-	-	-	-	-	0	0	0	1	0
cortical carcinoma	Grade	-	-	-	-	-	51	51	51	49	51
	+	-	-	-	-	-	0	0	0	2	0

Key: "-" = finding not present, "+" = finding present

		Incidence of neoplastic lesions: ovary									
		Males					Females				
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
		0	100	400	1500	0	0	100	400	1500	0
Ovary Benign sex cord stromal tumour	No. examined:	-	-	-	-	-	51	51	51	51	51
	Grade	-	-	-	-	-	50	43	38	38	50
	+	-	-	-	-	-	1	8	13	13	1
malignant sex cord stromal tumour	Grade	-	-	-	-	-	51	50	50	49	51
	+	-	-	-	-	-	0	1	1	2	0
tubulostromal adenoma	Grade	-	-	-	-	-	51	49	45	49	51
	+	-	-	-	-	-	0	2	6	2	0
tubulostromal carcinoma	Grade	-	-	-	-	-	51	51	49	51	51
	+	-	-	-	-	-	0	0	2	0	0
Benign granulosa cell tumour	Grade	-	-	-	-	-	51	50	51	48	51
	+	-	-	-	-	-	0	1	0	3	0
malignant granulosa cell Tumour	Grade	-	-	-	-	-	51	51	48	50	51
	+	-	-	-	-	-	0	0	3	1	0
Benign luteoma	Grade	-	-	-	-	-	49	41	47	47	48
	+	-	-	-	-	-	2	10	4	4	3
malignant luteoma	Grade	-	-	-	-	-	51	51	46	50	51
	+	-	-	-	-	-	0	0	5	1	0

Key: "-" = finding not present, "+" = present

(Excerpted from Applicant's package)

Executive CAC Recommendations and Conclusions:

Rat:

The Committee agreed that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

The following were considered to be drug-related neoplasms in rats:

- **Liver** – benign and malignant hepatocellular neoplasms in females.
- **Thymus** – benign thymoma in both sexes and malignant thymoma in females.

Mouse:

The Committee agreed that the study in females was adequate, noting prior Exec CAC agreement with the protocol.

The following neoplasms were considered to be drug related in female mice:

- **Ovary**- benign and malignant tubulostromal tumors, sex-cord stromal tumors, granulosa cell tumors, and luteal tumors
- **Adrenal**- subcapsular adenomas at the mid dose and high dose, cortical adenomas and carcinomas in females at the high dose.

David Jacobson-Kram, Ph.D.

Chair, Executive CAC

cc:\

/Division File, DRUP

/Alex Jordan, DRUP

/Jeffrey Bray, DRUP

/George Lyght, DRUP

/ASeifried, OND IO

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/s/

ADELE S SEIFRIED
11/30/2012
DAVID JACOBSON KRAM
11/30/2012

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/s/

JEFFREY D BRAY
01/15/2013

ALEXANDER W JORDAN
01/15/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

	with 201.57?			Impairment of Fertility and not under Use During Pregnancy. There was no Segment 1 study conducted to assess the effect of ospemifene on fertility.
10	If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.)	X		Acceptance criteria were set for impurities based on exposure levels in the nonclinical batches (Harrouk, 2005 safety review). The sponsor also states that the impurity levels of any single impurity in the batches used in the pivotal toxicological studies was below (b) (4), making impurity specification unnecessary.
11	Has the sponsor addressed any abuse potential issues in the submission?			N/A
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
13	From a pharmacology/toxicology perspective, is the NDA fileable? If “no”, please state below why it is not.	X		

Any Additional Comments:

The supporting IND for this application, 67216, was opened in 2003. The original sponsor was QUATRx. Nonclinical studies, including safety pharmacology, genotoxicity, and single- and repeat-dose toxicity in the rat, dog, and monkey, have been previously reviewed. Carcinogenicity and Segment 3 developmental toxicity studies have been submitted in finalized form and will be reviewed for the first time as part of this NDA.

Correspondence between the Division and Shinogi was reviewed. A pre-NDA meeting was held on September 29, 2009. Pharm Tox expressed no further requirements for nonclinical studies for the NDA submission.

Leslie McKinney June 18, 2012
 Reviewing Pharmacologist Date

Alex Jordan June 19, 2012
 Team Leader/Supervisor Date

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/s/

LESLIE C MCKINNEY
06/19/2012

ALEXANDER W JORDAN
06/19/2012