

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

022247Orig1s000

PHARMACOLOGY REVIEW(S)

NDA 22247 Duavee (conjugated estrogens/bazedoxifene) tablets

Comments from A. Jacobs, AD

Date Oct 2, 2013

1. I concur that there are no outstanding pharm/tox issues.
2. I concur that the pregnancy labeling is appropriate
3. I have previously conveyed comments to the pharm/tox reviewer on the pharm/tox review and labeling, and they have been addressed as appropriate.

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/s/

ABIGAIL C JACOBS
10/02/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22-247
Supporting document/s:	SDN #1
Applicant's letter date:	October 3, 2012
CDER stamp date:	October 3, 2012
Product:	Bazedoxifene and conjugated estrogens
Indication:	Vasomotor symptoms, vulvar vaginal atrophy and prevention of osteoporosis in postmenopausal women
Applicant:	Wyeth Pharmaceuticals Inc. Wholly owned subsidiary of Pfizer Inc.
Review Division:	Division of Reproductive and Urologic Drugs
Reviewer:	Leslie McKinney, PhD
Supervisor/Team Leader:	Alex Jordan, PhD
Division Director:	Hylton Joffe, MD
Project Manager:	Samantha Bell, BS, BA, RAC

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1 Executive Summary

1.1 Recommendations

1.1.3 Labeling

Pharmacological class: estrogen with an estrogen agonist / antagonist

General comment: Pharm Tox concurs that the ter [REDACTED] (b) (4) [REDACTED] should be deleted throughout the label. Pharm Tox was asked to edit Section 12.1, Mechanism of Action.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

[REDACTED] (b) (4)

The following paragraph replaces the sponsor's text:

TRADENAME pairs conjugated estrogens with bazedoxifene. Conjugated estrogens and bazedoxifene function by binding to and activating estrogen receptors (ER) α and β , which vary in proportion from tissue to tissue. Conjugated estrogens are composed of multiple estrogens and are agonists of ER- α and β . Bazedoxifene is an estrogen agonist/antagonist that acts as an agonist in some estrogen-sensitive tissues and an antagonist in others. The [REDACTED] (b) (4) of conjugated estrogens and bazedoxifene produces a [REDACTED] (b) (4) effect that is specific to the target tissue.

The *nonclinical* sections of the sponsor's proposed labeling are shown below with suggested edits. Suggested deletions are shown as strikethroughs, and

suggested additions are highlighted in yellow. A comment was sent to the sponsor to verify the multiples of exposure by systemic exposure (AUC).

8 USE IN SPECIFIC POPULATIONS

Reviewer's note: There are no nonclinical data contained in the label for conjugated estrogens for this section.

8.1 Pregnancy

Pregnancy Category X

TRADENAME must not be used in women who are or may become pregnant [see [Contraindications \(4\)](#)].

(b) (4)

Administration of bazedoxifene to (b) (4) rats at maternally toxic dosages ≥ 1 mg/kg/day (\geq (b) (4) 0.3 times the human (b) (4) AUC) resulted in reduced numbers of live fetuses and/or reductions in fetal body weights. No fetal developmental anomalies were observed. In studies conducted with pregnant rabbits (b) (4) treated with bazedoxifene, abortion and an increased incidence of heart (ventricular septal defect) and skeletal system (ossification delays, misshapen or misaligned bone, primarily of the spine and skull) anomalies in the fetuses were present at maternally toxic dosages of ≥ 0.5 mg/kg/day ((b) (4) times the human (b) (4) AUC).

(b) (4)

13 NONCLINICAL TOXICOLOGY

Reviewer's note: There are no nonclinical data contained in the label for conjugated estrogens for this section.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Carcinogenicity studies with CE/ bazedoxifene have not been conducted.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, uterus, cervix, vagina, testis, and liver.

In 6-month oral gavage carcinogenicity studies of bazedoxifene in transgenic Tg.RasH2 mice, there was a drug-related increased incidence of benign,

ovarian granulosa-cell tumors in female mice given 150 or 500 mg/kg/day. ,
(b) (4)

In a two-year (b) (4) -dietary carcinogenicity study of bazedoxifene in rats (administered at 0.003%, 0.01%, 0.03%, or 0.1%), a drug-related marked increased incidence of benign, ovarian granulosa-cell tumors was observed in female rats at (b) (4) concentrations of 0.03% and 0.1%. Systemic exposure (AUC) of bazedoxifene in these groups was (b) (4) 3 and (b) (4) times that observed in postmenopausal women administered 20 mg/day. (b) (4)

(b) (4)

(b) (4)

Mutagenesis

Mutagenicity studies with CE/bazedoxifene have not been conducted. Bazedoxifene was not genotoxic or mutagenic in a battery of tests, including *in vitro* bacterial reverse mutation assay, *in vitro* mammalian cell forward mutation assay at the thymidine kinase (TK⁻) locus in L5178Y mouse lymphoma cells, *in vitro* chromosome aberration assay in Chinese hamster ovary (CHO) cells, and *in vivo* mouse micronucleus assay

Impairment of Fertility

Impairment of fertility studies with CE / bazedoxifene have not been conducted.

Female rats were administered daily dosages of 0.3 to 30 mg/kg

bazedoxifene (b) (4)

(b) (4) (0.03 to (b) (4)
human (b) (4) (AUC) at the 20 mg (b) (4) dose) (b) (4)
prior to and during mating with untreated males. Estrous cycles and fertility
were adversely affected in all bazedoxifene-treated female groups.

13.2 Animal Toxicology and/or Pharmacology

Reviewer's comment: As per PLR guidance, this section should only contain animal findings that have not been clinically documented or that indicate potential relevant clinical toxicity. Therefore, only the section on bone is retained.

(b) (4)

(b) (4)

(b) (4)

In a 12-month study in ovariectomized rats, coadministration of CE (2.5 mg/kg/d) and bazedoxifene (0.1, 0.3, or 1 mg/kg/d) (b) (4)

[Redacted text block]

Reviewer's note: For reference, relevant sections of the labeling from Dr. Kuiper's review of [Redacted] (b) (4) are reprinted below.

[Redacted text block]

(b) (4)

References

Komm, BS, F Vlasseros, R Samadfam, L Chouinard, SY Smith. Skeletal effects of bazedoxifene paired with conjugated estrogens in ovariectomized rats. *Bone* 49:376-386, 2011.

Perry, R, CA Thompson, JN Earnhardt, DJ Wright, S Bailey, B Komm, MA Cukierski. Renal tumors in male rats following long-term administration of bazedoxifene, a tissue-selective estrogen receptor modulator. *Toxicologic Pathology*, 00: 1-10, 2013

Wright, DJ, JN Earnhardt, R Perry, S Bailey, B Komm, DR Minck, MA Cukierski. Carcinogenicity and hormone studies with the tissue-selective estrogen receptor modulator bazadoxifene. *J Cell. Physiol.* 228: 724-733, 2013

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/s/

LESLIE C MCKINNEY
06/14/2013

ALEXANDER W JORDAN
06/14/2013

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PUBLIC HEALTH SERVICE
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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22-247
Supporting document/s:	SDN #1
Applicant's letter date:	October 3, 2012
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Product:	Conjugated estrogens plus bazedoxifene (Duavee®)
Proposed Indications:	Vasomotor symptoms, vulvar vaginal atrophy and prevention of osteoporosis in postmenopausal women
Applicant:	Wyeth Pharmaceuticals Inc. Wholly owned subsidiary of Pfizer Inc.
Review Division:	Division of Reproductive and Urologic Drugs
Reviewer:	Leslie McKinney, PhD
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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

Nonclinical data support approval of the combination of conjugated estrogens (CE; 0.45 or 0.625 mg daily dosage) plus bazedoxifene (BZA; 20 mg daily dosage), for treatment of osteoporosis, vasomotor symptoms (VMS), and vulvovaginal atrophy (VVA) in postmenopausal women. CEs are already approved for these indications. BZA is a new molecular entity (NME) that was previously evaluated as a single use agent for prevention or treatment of osteoporosis (b) (4). PharmTox found BZA approvable (b) (4).

The sponsor's rationale for pairing BZA with CE as a single product (Duavee®) was to (b) (4). Nonclinical evaluation of the combination product was limited to pivotal repeat-dose toxicology studies and additional mechanistic and efficacy pharmacology studies. No new nonclinical safety concerns were identified. From a PharmTox perspective, the application is approvable.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

Labeling will be the subject of a separate review.

1.2 Brief Discussion of Nonclinical Findings

Nearly all of the nonclinical studies submitted to NDA 22-247 were submitted in 2007 (b) (4).

In addition, much of the data from the nonclinical development program has been published in the open scientific literature and is referenced throughout. Nonclinical studies of the combination CE + BZA were conducted to determine whether coadministration of the two estrogenic compounds would lead to toxicity different from that observed with each alone.

Pharmacology:

Bazedoxifene is a selective estrogen agonist/antagonist that binds to both the alpha and beta forms of the estrogen receptor (ER- α and ER- β). It acts as an estrogen agonist in bone and for venous thrombotic events

(VTEs) and an antagonist in the uterus, breast, and against vasomotor symptoms. Potency relative to estrogen in each tissue is variable. It is effective at protecting rat and monkey from bone loss following ovariectomy.

Pharmacological characterization of the combination CE + BZA included in vitro mechanistic studies on gene expression as well as in vivo studies designed to address clinical efficacy and risk/benefit issues.

- A 1-year study in the rat demonstrated efficacy of CE + BZA in protecting bone strength following ovariectomy (OVX).
- CE + BZA showed weak efficacy against vasomotor instability in the rat using a surrogate endpoint of provoked changes in tail skin temperature, which modestly supports the sponsor's claim for treatment of hot flush.
- Two studies examined the ability of BZA to oppose the estrogen-induced increase in uterine wet weight in the three-day immature rat uterine model. Both studies established that BZA antagonized the estrogen-induced increase in uterine wet weight in a dose-dependent way and had little agonist effect when tested alone. BZA also prevented estrogen-induced myometrial and luminal cell hypertrophy
- To explore the effect of CE + BZA on venous thrombus formation, an in vivo study was conducted using a mouse model of femoral vein thrombosis. Data were equivocal as to whether BZA could protect against a very weak enhancement of thrombus formation by CE in this model.

Safety pharmacology:

Bazedoxifene alone was negative in safety pharmacology studies and had no significant off-target activity at other steroid receptors; weak cross-reactivity was reported at the sigma opioid receptor, the only positive finding from screening assays against a standard panel of receptors. No safety pharmacology studies were conducted with the combination CE + BZA.

PK / ADME:

PK/ADME of BZA was characterized in mouse, rat, dog, and monkey (b) (4) BZA was well absorbed and its bioavailability was limited by first pass metabolism. Glucuronidation represented the major metabolic pathway. The primary route of excretion was biliary / fecal. There was no significant induction of hepatic enzymes and the potential for drug-drug interaction was deemed low.

For the combination CE + BZA, one in vitro study was conducted to examine potential metabolic interactions between the two compounds. None were found. In vivo, TK data from 1 and 6-month rat, and 1 and 9-

month monkey repeat-dose studies demonstrated that absorption and metabolism of either BZA or CE are not altered by administration of the two compounds together.

General Toxicology:

BZA alone: General toxicology studies were conducted with BZA alone in mouse, rat, and monkey at high multiples of exposure. Text from the Executive Summary of (b) (4) is given below:

“In 1-month, 6-month, and 9-month studies in the monkey, at doses ranging from 4x-26x (10-200 mg/kg/day, 1-month), 0.3x-7x (1-15 mg/kg/day, 6-month), or 4x-39x (10-300 mg/kg/day, 9-month, females only) the proposed human dose of 20 mg/day based on AUC comparison, effects included decreases in body weight and food consumption, increase in liver weight in females (after 9 months), and increased ovary weight, decreased uterine weight, ovarian cysts/cystic follicles, ovary mineralization, atrophy of uterus, cervix and vagina. Most effects were seen at all doses in all studies and were completely reversed after a 3-month recovery period.

Body weight, clinical chemistry and reproductive organ effects were most likely due to the pharmacologic effect of bazedoxifene at the estrogen receptor. The effects on uterus, cervix and vagina (weight decrease and atrophy) were probably the result of a direct anti-estrogenic action on these organs. The ovarian effects (weight increase, cystic follicles) were probably the result of a suppression of estrogen's negative feedback on the HPO axis due to a central anti-estrogenic action leading to pituitary gonadotropin release and ovarian stimulation. Body weight reduction due to reduced food intake and changes in hepatic protein and lipid metabolism are most likely due to an estrogenic effect of bazedoxifene. Kidney mineralization and other renal pathology in male rats have been observed with other SERMs and are believed to represent a male rat-specific ER-mediated effect. The myocardial degeneration in female rats is of unclear significance.

Assuming all effects were pharmacologically mediated, the NOAEL in the rat was the highest dose evaluated of 100 mg/kg/day, i.e. 16x human AUC at 20 mg/day, and the NOAEL in the monkey was the highest dose evaluated of 300 mg/kg/day, i.e. 39x human AUC at 20 mg/day.”

CE + BZA: General toxicology studies were conducted with the combination CE + BZA in rat (1- and 6-month repeat dose) and cynomolgus monkey (1- and 9-month repeat dose) at acceptable multiples of exposures for each compound. Studies were conducted in females only. No new toxicities were observed. BZA-associated effects on the

uterus, cervix, vagina, and ovary were still present. Benign granulosa cell tumors were observed in the rat.

Genetic toxicology

BZA alone was negative for genotoxicity in the standard battery of in vitro and in vivo genotox assays. Genotoxicity of the combination BZA + CE was not evaluated.

Carcinogenicity

Carcinogenicity of BZA was evaluated in a 2-year rat study and in two 6-month Tg.rasH2 studies (b) (4). Carcinogenicity for the combination CE + BZA was not evaluated.

In both the rat and mouse study, there were dose-related increases in benign granulosa cell tumors of the ovary. These tumors may be the result of hyperstimulation of the ovary by pituitary gonadotropins (LH, FSH) due to a central anti-estrogenic action of BZA. However, data from rodent studies on gonadotropin levels in response to BZA treatment were not available, and a direct effect of BZA on the ovary can not be excluded.

In the rat, survival was increased due to a reduced incidence of pituitary tumors in males and of pituitary and mammary tumors in females in all dose groups.

Reproductive Toxicity

Fertility (Segment 1) and embryofetal (Segment 2) toxicity studies were conducted in the rat and rabbit with BZA alone (b) (4). Reproductive toxicity for the combination CE + BZA was not evaluated.

In the rat, BZA had no effect on male fertility but interfered with estrous cyclicity, fertility, and ability to maintain pregnancy in females. In both the rat and rabbit, maternal toxicity (reduced body weight and consumption) was observed in pregnant dams at all doses, along with reduced implantation (rat) or increased abortion (rabbit) and fetal survival. Major malformations were not observed, but vascular abnormalities, delayed ossification, and enlarged thyroid were reported. NOAELs for all of the reprotox studies were at or below the lowest administered dose, and were less than 1x the human AUC at the proposed therapeutic dose.

2 Drug Information

2.1 Drug **Bazedoxifene (BZA) + Conjugated estrogens (CE)**

The following section will cover bazedoxifene only. Conjugated Estrogens is a USP monograph material. Refer to approved NDA 04-782 for PREMARIN® (conjugated estrogens tablets, USP) for all drug information.

2.1.1 CAS Registry Number (Optional)

198481-33-3 (acetate salt)

2.1.2 Generic Name

INN: Bazedoxifene

USAN: Bazedoxifene Acetate

2.1.3 Code Name

WAY-140424-B; TSE-424 Acetate, PF-05208749-14

2.1.4 Chemical Name

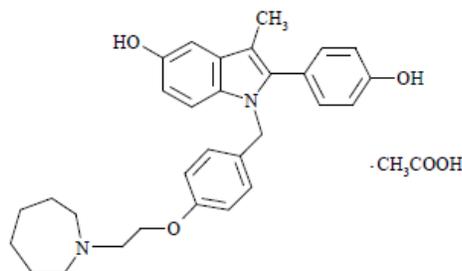
2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-hexamethyleneimine-1-yl-ethoxy)benzyl]-1*H*-indol-5-ol acetate (CAS Index name)

1*H*-Indol-5-ol, 1-[[4-[2-(hexahydro-1*H*-azepin-1-yl)ethoxy]phenyl]methyl]-2-(4-hydroxyphenyl)-3-methyl-, monoacetate (salt) (CAS Index Name)

2.1.5 Molecular Formula/Molecular Weight

C₃₂H₃₈N₂O₅ or C₃₀H₃₄N₂O₃ · C₂H₄O₂ / 530.65 (salt), 470.60 (free base)

2.1.6 Structure



2.1.7 Pharmacologic class

Bazedoxifene is a benzothiophene-derived selective estrogen receptor modulator (SERM) with mixed estrogen agonist/antagonist activity. It is structurally related to raloxifene, arzoxifene, and lasofoxifene.

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

This application is cross-referenced to the following:

(b) (4)
IND 62288 (conjugated estrogens and bazedoxifene acetate),
(b) (4)
NDA 04782 (conjugated estrogen tablets - Premarin®).

(b) (4)

Letters of authorization for DMFs for bazedoxifene acetate drug substance, noncompensial excipients and container/closure were also provided.

2.3 Clinical Formulation

2.3.1 Drug Formulation

The drug product is formulated in two dosages in tablet form: BZA 20 mg / CE 0.45 mg and BZA 20 mg / CE 0.625 mg (3.2.P.1 Tables 1-1 and 1-2). The active coating contains BZA and the following ingredients:

Ingredient	Reference to Standards	Function	BZA 20 mg / CE 0.45 mg tablet (mg)
Sucrose	NF/Ph.Eur.	(b) (4)	(b) (4)
Hypromellose (b) (4)	USP/Ph.Eur.		
(b) (4)	USP/Ph.Eur.		
Sucrose monopalmitate (Sucrose palmitic acid esters)	USP/Ph.Eur.		
Ascorbic Acid	USP/Ph.Eur.		
Bazedoxifene acetate (b) (4)	In house	Active	22.56 (20 mg free base)
(b) (4)			
Lactose monohydrate	NF/Ph.Eur.	(D) (4)	
Microcrystalline cellulose	NF/Ph.Eur.		
Hypromellose (b) (4)	USP/Ph.Eur.		
Magnesium stearate	NF/Ph.Eur.		
(b) (4)	USP/Ph.Eur.		
Total			

Other inert ingredients: hydroxypropyl cellulose, polyethylene glycol (b) (4)
(b) (4) isopropyl alcohol.

2.3.2 Comments on Novel Excipients

None

2.3.3 Comments on Impurities/Degradants of Concern

There are four potential impurities (b) (4)
(structures from 2.3.S.3 p 8). None have structural alerts. Two have been qualified and two were characterized but did not require qualification.



(b) (4)
was stated by the sponsor to have modest antagonistic effects in a transactivation assay in MCF-7 cells in the presence of 17β -E₂ (4.2.1.1: RPT-49441). (b) (4) is also a minor metabolite in postmenopausal women and Tg.rasH2 mice (2.6.4 Pharmacokinetics Written Summary, Section 2.6.4.5.1.1.3 and Section 2.6.4.5.1.4).

Both impurities were previously qualified to (b) (4) respectively with repeat-dose 1-month tox studies using drug substance spiked with (b) (4) of each impurity (4.2.3.7.6 RPT #s 45685). Genotoxicity of these two impurities was assessed in an Ames test and a chromosome aberration assay in human peripheral blood lymphocytes, with negative results (4.2.3.7.6 RPT #s 46133, 46134, 46229, 46326).

(b) (4) are controlled to specification levels of NMT (b) (4) respectively, in the drug product. In the batches tested to date they were present at NMT (b) (4) respectively.

Two additional impurities, (b) (4) were present in the drug substance at (b) (4), which is below the reporting threshold. The only assessment for potential genotoxicity of these two compounds was a DNA adduct test (RPT #78224 submitted to (b) (4) SDN 728). HPLC purified samples of each compound were tested at 20 or 5 uM, respectively, with or without metabolic activation, and found negative for DNA adduct formation. These two impurities are controlled to specification levels of NMT (b) (4) in the drug product. In the batches tested to date, they have each been below the reporting limit (b) (4) per tablet.

(b) (4)

2.4 Proposed Clinical Population and Dosing Regimen

This NDA proposes to treat postmenopausal women for any of three conditions that result from low estrogen: (1) prevention of osteoporosis, (2) moderate to severe vasomotor symptoms (VMS), or (3) moderate to severe symptoms of vulvar vaginal atrophy (VVA). Two dose regimens were investigated in clinical trials:

BZA 20 mg / CE 0.45 mg
BZA 20 mg / CE 0.625 mg

2.5 Regulatory Background

BZA monotherapy is approved in the EU and Japan but not the US for the treatment of postmenopausal osteoporosis. (b) (4)

(b) (4)

Investigation of the combination BZA and CE for postmenopausal indications was conducted under INDs 62288 (b) (4), opened in 2001. The nonclinical development program was completed prior to the submission of the BZA

monotherapy (b) (4) No nonclinical comments were transmitted to the sponsor for the submission of this NDA.

Use of conjugated estrogens (PREMARIN®) for treatment of postmenopausal indications was approved in 1942 under NDA 04-782.

3 Studies Submitted

The full nonclinical development program for bazedoxifene (BZA) was previously submitted to (b) (4) or to the supporting INDs to which this application is cross-referenced. Studies previously reviewed (b) (4) will not be re-reviewed here. Listed below are all the studies that were not previously submitted to Wyeth (b) (4) and (b) (4). This review will focus on those studies that investigate the combined effects of BZA and CE.

3.1 Studies Reviewed

Primary Pharmacology

- GTR-34887: Premarin and TSE-424 (WAY140424) in the Rat Hot Flash Model (1998)
- RPT-68443: Expression Profiling Studies of SERMs Alone and When Paired with CE in the Immature Rat Uterus (2007)
- RPT-80571: Venous Thromboembolism: Development of a rat model and a preliminary evaluation of bazedoxifene, conjugated estrogens, and bazedoxifene + conjugated estrogens. (2010)
- RPT-58143: Bazedoxifene/CE: A 12-month study to determine the effects on bone mass, strength and architecture in the ovariectomized Sprague-Dawley rat (2007)

Secondary Pharmacology

- RPT-75759: Ancillary Pharmacology Profile of WAY-140424 (Bazedoxifene) in a Rat Model for Glucocorticoid/Antiglucocorticoid Activity (2008)

Pharmacokinetics

Drug/drug interaction studies:

- GTR-37100: In vitro evaluation of potential metabolic interactions between TSE-424 and conjugated estrogens (1999)

Toxicology

- RPT-39409: TSE-424/Conjugated Estrogens: 30-day oral (gavage) safety study in female rats (with accompanying TK RPTs-40031 and -39930) (2001)
- RPT-50335: BZE/CE: Multiple (6-month) oral (gavage) toxicity study with a 3-month recovery in female rats (with accompanying TK RPTs-50444 and 50748) (2004)
- RPT-39419: TSE-424/Conjugated Estrogens: 30-day oral (gavage) safety study in female monkeys (with accompanying TK RPTs-39969 and -39941) (2001)
- RPT-50336: BZE/CE: Multiple (39 weeks) oral (gavage) toxicity study in the female cynomolgus monkey (with accompanying TK RPTs-50834 and 51302) (2004)

3.2 Studies Not Reviewed

Primary Pharmacology

- RPT-71396: Binding Affinity (IC₅₀) of WAY-138923 and WAY-140424 for the Ligand Binding Domains of Human Estrogen Receptors - α and - β (2007)
- RPT-70486: Characterization of Premarin Estranes and Selective Estrogen Receptor Modulators in a Multiplexed ER α / Cofactor Assay (2007)
- RPT-68444: Expression Profiling Studies of SERMs Alone and When Paired With Conjugated Estrogens (CE) in MCF-7 Breast Cancer Cells (2007)
- RPT-59139: The Effect of WAY-140424 on the Mouse Mammary Gland and Uterus (2005)
- RPT-73139: Effect of Bazedoxifene Acetate on the Mammary Gland of Ovariectomized Mice (2008)
- GTR-35197: Effects of TSE-424 Administered with Premarin or with Ethinyl Estradiol on Bone Parameters, Uterine Weight and Serum Cholesterol in Ovariectomized Rats (1999) (6-week study)
- RPT-44690: Dose response Evaluation of Premarin on Skeletal Parameters, Uterine/Body Weights and Total Cholesterol in Ovariectomized Rats (2003) (6-week study)
- RPT-46970: Effects of Conjugated Estrogens, Alone and in Combination with Bazedoxifene, Raloxifene and Lasofoxifene or on Bone Parameters, Uterine Weight and Serum Cholesterol in Ovariectomized Rats (2007) (6-week study)
- RPT-66674: Effects of TSE-424 on fracture repair in the rat (2006) (20-wk study)

Pharmacokinetics

Analytical methods were not reviewed

Absorption:

- RPT-80098: Bazedoxifene: transport and inhibition of P-glycoprotein activity in Caco-2 cell monolayers (2010)
- RPT-58001: TSE-424: Determination of effective intestinal permeability in rat intestinal in situ perfusion model (2006)

3.3 Previous Reviews Referenced

(b) (4)
Review of IND 62288 by Dr. Wafa Harrouk.
(b) (4)

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action

The physiological effects of conjugated estrogens (CE) and bazedoxifene (BZA) are accomplished by binding to estrogen alpha and beta receptors (ER- α and ER- β) with nM affinity (4.2.1.1: GTR-29784, RPT-71396). CEs are agonists of ER- α and ER- β . BZA has mixed estrogen agonist/antagonist properties, which result from the altered pattern of recruitment of coactivator and corepressor molecules to ER- α and ER- β receptors when bound to BZA.

In vitro characterization utilizing various gene expression, cofactor interaction, and functional assays in a number of different cell types support the conclusion that the combination of CE + BZA will result in tissue responses that are different from either CE or BZA alone and different for each tissue type (Berrodin et al., 2009; Chang et al., 2010)

In vivo studies related to the proposed indications for the combination BZA + CE

Osteoporosis: 1 year rat bone study

The effect of the combination BZA / CE on bone parameters was studied in a 1-year ovariectomized (OVX) rat study. The results of this study have been published (Komm et al., 2011). The study was conducted using doses of BZA similar to those used in a 1-year OVX rat study conducted with BZA alone, (b) (4)

Study title: Bazedoxifene/CE: : A 12-month study to determine the effects on bone mass, strength and architecture in the ovariectomized Sprague-Dawley rat	
Study no.:	RPT-58143
Study report location:	4.2.1.1
Conducting laboratory and location:	Wyeth-Ayerst Research 641 Ridge Road, Chazy, NY 12921
Date of study initiation:	8 Feb 2005
GLP compliance and QA statement:	Yes and Yes
Drug, lot #, and % purity:	BZA (TSE-424), Lot # RB2660, 87-89% purity (b) (4), Lot #s A32055, B02652, A72142, (b) (4)

Key study findings:

In the rat, loss of estrogen due to OVX produces well-understood changes in bone properties (decreased bone mineral density (BMD) loss of bone volume, decreased bone strength), which are prevented by administration of exogenous

estrogen. Previously, under (b) (4), the effect of BZA alone on bone parameters was characterized in a 1 year rat study. BZA was shown to partially prevent the OVX-induced bone changes, but without clear dose-dependence over the range 0.15 – 1.5 mg/kg/d.

The purpose of this study was to determine whether BZA administered in combination with CE to OVX rats would enhance or reduce the protective effect of CE on bone parameters. Overall, BZA did not reduce the effectiveness of CE in maintaining bone strength and tissue integrity following OVX when administered in combination with a fixed dose of CE at any of the BZA doses tested.

In addition, the combination BZA / CE also mitigated the changes in uterine weight and body weight induced by OVX.

Methods	
Doses*: (based on active moiety and adjusted for strength)	BZA : 0.1, 0.3, 1 mg/kg/d CE: held constant at 2.5 mg/kg/d
Frequency of dosing:	daily
Route of administration:	oral gavage
Dose volume:	5 mL/kg each; BZA preceded CE
Formulation/Vehicle:	The vehicle-control article for BZA consisted of final concentrations of 1.0% polysorbate 80 and 0.5% methylcellulose (4000 cps) in purified water. The vehicle-control article for CE consisted of a final concentration of 0.5% Methocel, A15C in purified water.
Stability and homogeneity:	Acceptable (Appendix 5).
Species/Strain:	(b) (4) SD CrI:CD® (SD)BR) rats
Number/Sex/Group:	24/gp except baseline control N=10
Age:	6 mo at start of treatment
Weight:	261-354 g
Satellite groups:	No TK
Unique study design: (See p 34 for group designations and animal numbers)	Group 1: sham control Group 2: OVX vehicle control Group 3: OVX CE 2.5 mg/kg/d Group 4: OVX BZA 0.3 mg/kg/d Group 5: OVX BZA 0.1 mg/kg/d / CE 2.5 mg/kg/d Group 6: OVX BZA 0.3 mg/kg/d / CE 2.5 mg/kg/d Group 7: OVX BZA 1.0 mg/kg/d / CE 2.5 mg/kg/d Group 8 Baseline control (euthanized at the end of the acclimation period for histomorphometry and biomechanical tests.
Deviation from study protocol:	none that affect study outcome

* Sponsor's dose justification: "The dosages of BZA selected for this study (0.1, 0.3, and 1 mg/kg) are multiples of the efficacious dosage (0.3 mg/kg) in the rat osteopenia model. The dosage of CE (2.5 mg/kg) being used produces a maximal effect on bone parameters in rats. Since the study goal is to evaluate the effect of BZA on CE-enhanced bone quality in OVX rats, CE was maintained at one constant dosage while 3 dosages of BZA were evaluated."

Observations and Results

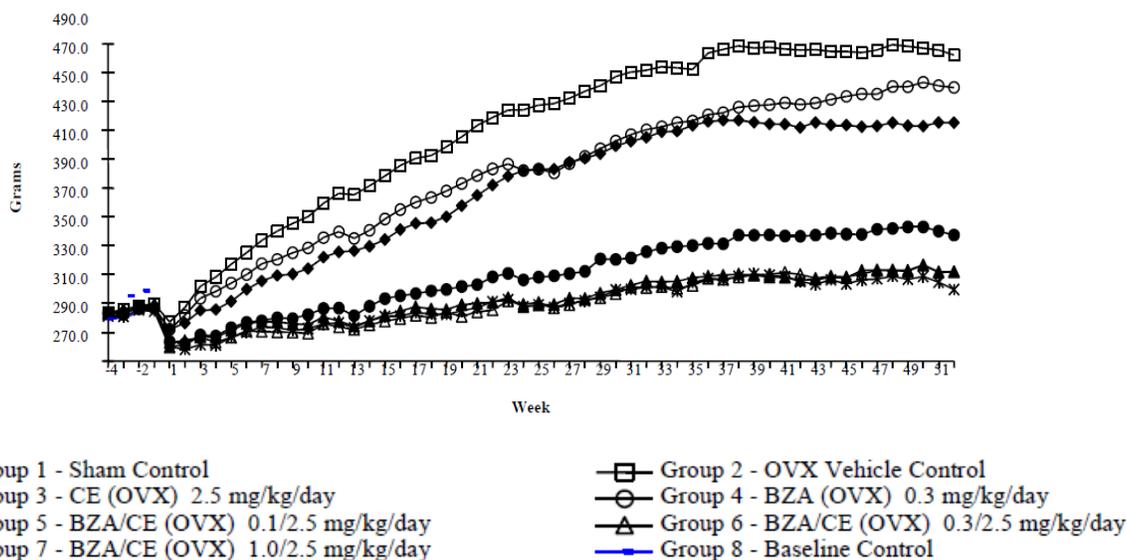
Mortality: twice daily

Results: Sixteen rats (of 178 total) were euthanized or found dead prior to study termination: 4 sham or vehicle control, 5 CE treated, 1 BZA treated (mid-dose), 6 BZA/CE treated (3 low, 3 mid-dose). Most occurred at the end of study. 10/16 were found to have neoplastic changes at necropsy deemed by the sponsor to be age-related. The remainder were unexplained or occurred following blood collection or bone scan procedures.

Clinical Signs: twice daily; detailed exams weekly throughout dosing. Results: No treatment-related adverse signs reported.

Body Weights: weekly and just prior to necropsy

Results: OVX animals had the highest body weight of all groups. Sham control or BZA alone (Groups 1 & 4) had similar body weights. Combination BZA/CE groups were close to CE alone and to baseline, and fairly similar to each other.



Feed Consumption: daily by recording the number of pellets remaining; no treatment-related effects.

Ophthalmoscopy: not done

ECG: not done

Hematology and coagulation parameters: not done

Clinical Chemistry:

Blood and urine samples were collected for selected clinical chemistry, urinalysis, biochemical markers of bone turnover, and hormone parameters. Blood and overnight urine samples were collected from 10 animals in Groups 1 to 7 at the end of the acclimation period (prior to surgery) and at 1, 3, 6 and 12 months during the treatment period.

Parameters examined; cholesterol, creatinine, inorganic phosphorus, total serum calcium, triglycerides. Hormones: 17-beta estradiol.

Results:

- Cholesterol: OVX increased serum cholesterol 15% compared to sham controls. Treatment with BZA, CE, or BZA/CE significantly reduced (~50%) serum cholesterol compared to sham or OVX controls, but values remained within normal range (data plotted in Fig 3 of Komm et al., 2011). There was no effect of OVX or treatment on triglycerides.
- Serum calcium levels were within the expected physiological ranges for all groups. However, OVX resulted in a statistically significant decrease in serum calcium levels. CE alone, or in combination with low and mid doses of BZA slightly prevented the effect of OVX on serum calcium levels.
- There were no treatment-related effects on serum phosphorus.
- 17-beta estradiol decreased with OVX or increased with CE or CE/BZA treatment, as expected. BZA alone was comparable to OVX animals.

Urinalysis: Parameters examined: calcium, creatinine, inorganic phosphorus, volume.

Results: No treatment related differences reported.

Gross Pathology: organ weights and macroscopic findings:

At necropsy, uteri were weighed to confirm OVX procedures. Tissues retained were bones, pituitary, uterus, and vagina. Bones retained were femurs (L/R), lumbar vertebra (1-4), tibia (R/L).

Uterine findings: Consistent with the known physiological effects of estrogen on the uterus, uterine weights were significantly reduced in OVX animals. Also as expected, treatment with CE alone restored uterine weight but with BZA alone did not. Treatment with the combination BZA/CE yielded reduced uterine weights at the mid and high BZA dose, but not the low BZA dose. The data are shown below in figure form taken from the publication by Komm et al., 2011, Fig. 2.



Other macroscopic findings: Findings of pituitary or adrenal enlargement also tracked with the presence (non-OVX or CE treated) or absence (OVX) of estrogen. BZA/CE at the mid or high BZA dose reduced the incidence of pituitary but not adrenal enlargement. CE-associated macroscopic findings that appeared to be mitigated by the presence of BZA were mammary gland thickening, and small thymus size.

Bone turnover markers:

Bone formation markers (serum): total alkaline phosphatase (ALP), osteocalcin (OC).

Bone resorption markers (urine): free deoxypyridinoline (DPD), C-telopeptide (CTX).

Results:

- OVX (estrogen depletion) produced the expected increase in biochemical markers of bone turnover, which was effectively prevented (except for total-ALP) by treatment with CE.
- BZA alone at 0.3 mg/kg/day partially prevented the OVX-induced rise in resorption markers, DPD and CTX, by approximately 41% and 20%, respectively. Bone formation markers, OC and total-ALP, were unaffected.
- Combination treatment with CE + low- or mid-dose BZA inhibited the OVX-induced increases in markers of bone turnover (OC, DPD and CTX).
- Combination treatment with CE + high dose BZA had a more mixed effect

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Fig 4A and B. Komm et al., 2011. Mean (SD) serum osteocalcin (a) and urinary free deoxypyridinoline (b).

Bone densitometry: Bone density scans, using dual energy x-ray absorption (DXA) and peripheral quantitative computed tomography (pQCT) were performed once during the acclimation period (prior to surgery) and at Weeks 5-6, 13-14, 24-25 and 51-52 of treatment. (Note: ex vivo scanning was also carried out on excised femurs (DEXA and pQCT) and vertebrae (pQCT) prior to mechanical testing to provide area, BMD and BMC data for correlation analyses with bone strength parameters.)

In vivo scanning:

DXA scans were taken of the whole body, right femur and A/P lumbar spine of each animal. The in vivo DXA scans were used to measure bone mineral density (BMD) and bone mineral content (BMC) and area of the whole body, whole right femur (with region of interest at the proximal, mid and distal femur) and lumbar spine (L1-L4).

pQCT scans were acquired at the proximal tibia metaphysis and diaphysis (single slice of all surviving animals). The proximal tibia metaphysis scans were evaluated for area, BMC and BMD of the total slice, trabecular and cortical/subcortical regions. Diaphysis scans were evaluated for total area, cortical area, cortical BMC, cortical BMD, cortical thickness and periosteal and endosteal circumference.

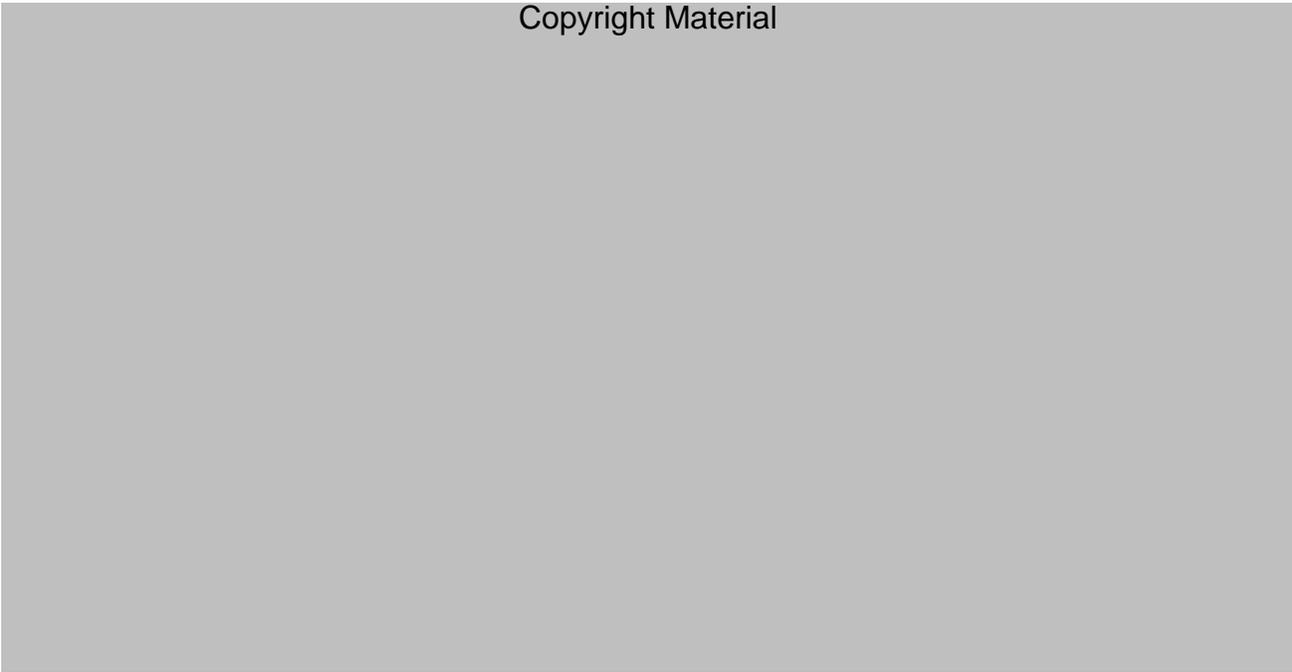
Results (DEXA scans):

- Whole body BMD:
 - OVX resulted in the expected reductions in whole body BMD relative to Sham controls.
 - Treatment of OVX rats with BZA alone at 0.3 mg/kg/day prevented this response and maintained whole body BMD at sham levels.
 - Treatment with CE or BZA/CE also prevented the OVX-induced effects on whole body BMD.

- Lumbar spine and femur
 - OVX (▲) resulted in a progressive loss of bone mass at the lumbar spine and femur. BMC and BMD were decreased 17% and 19%, respectively in the lumbar spine and 10% and 12% in the femur.
 - Treatment with CE alone (□) completely prevented the OVX-induced decreases in lumbar spine bone mass.
 - Treatment of OVX rats with BZA alone (○) at 0.3 mg/kg/day partially prevented the OVX-induced decrease in bone mass. This finding is consistent with the previously characterized weak estrogenic effect of BZA on bone mass.
 - Treatment with the combination CE + BZA (all doses) completely prevented the OVX-induced decreases in BMD and BMC, that is, BZA did not decrease the efficacy of CE in preventing OVX-induced bone loss.

Mean (SE) percent change from baseline in BMD (g/cm²) by DEXA. at the lumbar spine (Fig. 5A) and right proximal femur (Fig 5B, Komm et al., 2011).

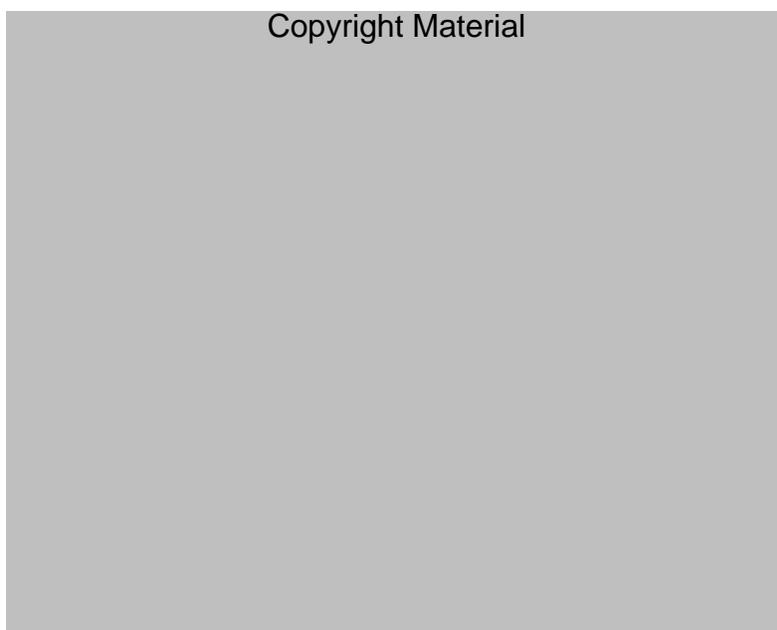
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Results (pQCT scans):

- Tibia metaphysis (trabecular bone)
 - OVX (▲) resulted in the expected progressive loss of bone mass at the tibia metaphysis
 - Treatment of OVX rats with CE alone (□) completely prevented the OVX-induced decreases in bone mass
 - Treatment of OVX rats with BZA alone (○) at 0.3 mg/kg/day partially prevented the OVX-induced decreases in bone mass
 - Treatment of OVX rats with CE + BZA (all doses) completely prevented the OVX-induced decreases in total slice BMC and BMD, that is, BZA did not decrease the efficacy of CE in preventing OVX-induced bone loss.

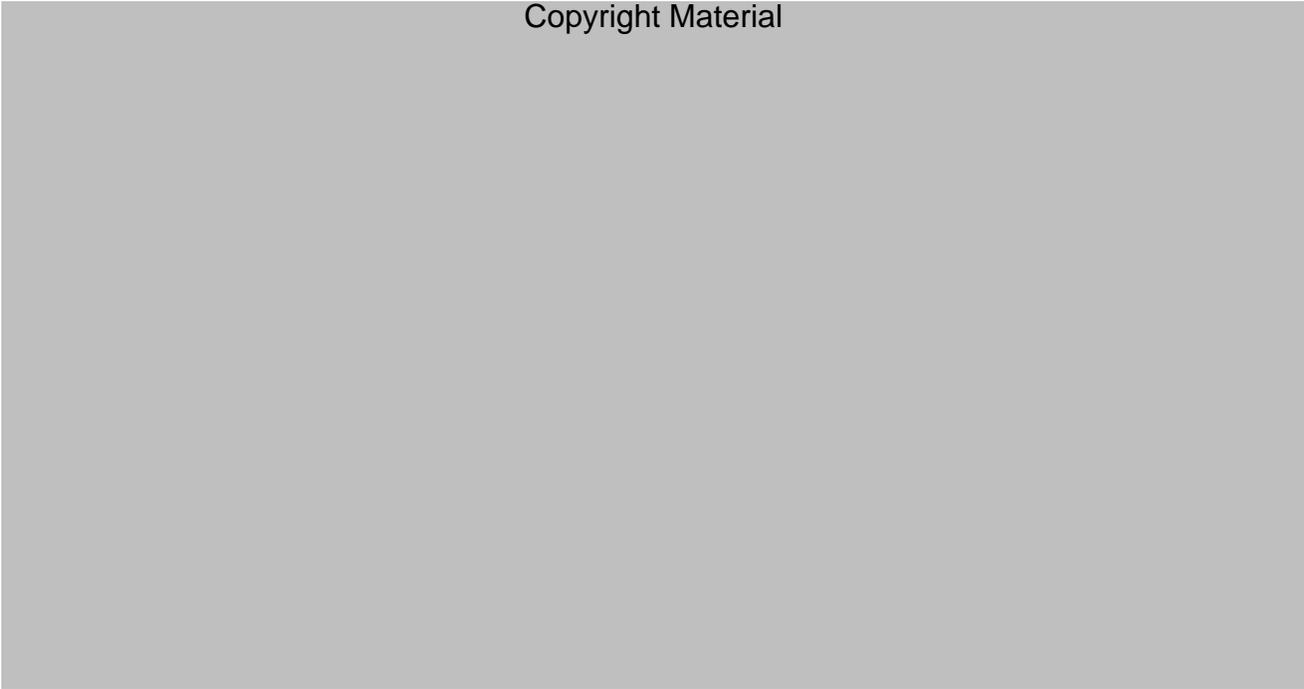
Mean percent changes from baseline in trabecular BMD (mg/cm²) of proximal tibia metaphysis. Fig 6 from Komm et al., 2011.



- Tibia diaphysis (cortical bone)
 - OVX (▲) resulted in increased periosteal circumference, cortical thinning and decreased cortical BMD at the proximal tibia diaphysis, but only late in the study.
 - Treatment of OVX rats with CE alone (□) completely prevented the OVX-induced loss in cortical BMD and increases in periosteal circumference
 - Treatment of OVX rats with BZA alone (○) at 0.3 mg/kg/day prevented the OVX-induced decrease in cortical BMD but not the increase in periosteal circumference
 - Treatment of OVX rats with CE + BZA completely prevented the OVX-induced loss in cortical BMD (all BZA doses) and increased periosteal circumference (low and mid-dose BZA only). Overall, BZA did not decrease the efficacy of CE in preventing OVX-induced bone loss.

Mean (SE) percent changes from baseline in proximal tibia diaphysis cortical BMC (mg/cm³) and periosteal circumference (mm). Fig 7A and B from Komm et al., 2011.

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Fluorescent bone labeling: 10 days and again 3 days prior to the scheduled terminal necropsy, each animal (Groups 1 to 7) was injected SC with a bicarbonate buffered calcein solution (8 mg/kg, 1 mL/kg). Baseline controls (Group 8) were IV injected, instead of SC as prescribed by protocol, with a bicarbonate buffered calcein solution (8 mg/kg, 1 mL/kg). This deviation was considered to have had no impact on the study.

Histomorphometry: The diaphysis and proximal metaphysis of the right whole tibia and the 1st and 2nd lumbar vertebrae were prepared for histomorphometric processing and analysis at PCS-MTL. Bones were processed and evaluated for the first 10 surviving animals in each group (Groups 1 to 7) and for all animals in Group 8. Animals presenting major necropsy findings that could alter bone metabolism, such as advanced renal disease or large or advanced tumoral process, were excluded from bone processing and evaluation at the discretion of the pathologist and replaced by the following surviving animal in the same group. Histomorphometric evaluation was conducted for static and dynamic bone parameters.

Results: Histomorphometry data will not be shown but are available in Tables 2 and 3 of Komm et al., 2011. Sponsor indicated that histomorphometry data was consistent with parameters derived from other methodologies at each of the sites that were analyzed.

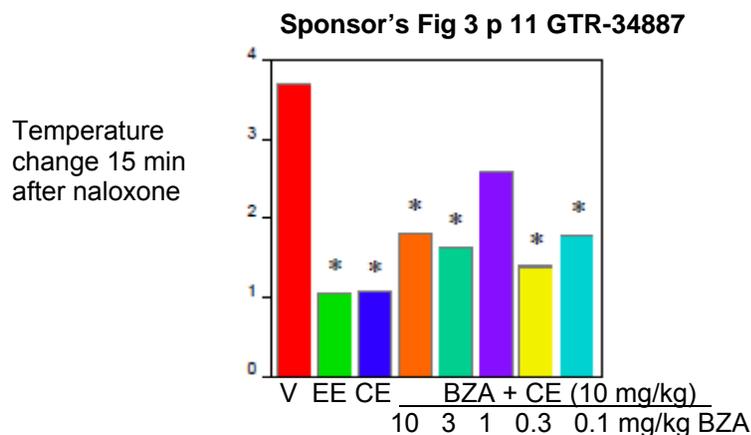
Biomechanical testing: Specimens were processed and evaluated for the first 10 animals per group (Groups 1 to 7) and all animals in Group 8, and corresponded to those selected for histomorphometry. Parameters evaluated were 3-point bending (femur), shear (femoral neck) and compression testing (4th lumbar vertebrae).

Results: Biomechanical data were published in Komm et al., 2011 in Table form for the femur (3-point bending) and the L4 vertebral body (compression) but will not be reproduced here. Briefly, OVX-induced decreases in bone mass were associated with loss of biomechanical competency. Treatment with CE alone maintained bone mass and strength of the femoral shaft and lumbar vertebrae. Treatment with BZA alone partially prevented the OVX-induced effects on bone strength; values trended toward increased bone strength but did not reach significance. The combination BZA/CE at all BZA doses prevented the OVX effects at the femur shaft and lumbar vertebrae, though not always significantly at all doses.

Vasomotor symptoms:

GTR-34887. Premarin and TSE-424 (WAY140424) in the Rat Hot Flash Model.

BZA with and without CE was tested for efficacy in a rat model of vasomotor instability (hot flush) (4.2.1.1. GTR-34887 and GTR-30836-not reviewed). Briefly, in the rat hot flush model, OVX, morphine-addicted rats are given a bolus injection of naloxone that induces a rapid thermoregulatory response detected as an increase in tail skin temperature. Either ethinyl estradiol (EE; 0.3 mg/kg) or CE (10 mg/kg) is effective in suppressing the change in tail skin temperature. BZA alone had no agonistic effect in this model, but partially opposed the effect estrogen when tested over the range 0.1-10 mg/kg. The antagonistic effect of BZE was not dose-dependent, however.



Uterine (endometrial) safety:

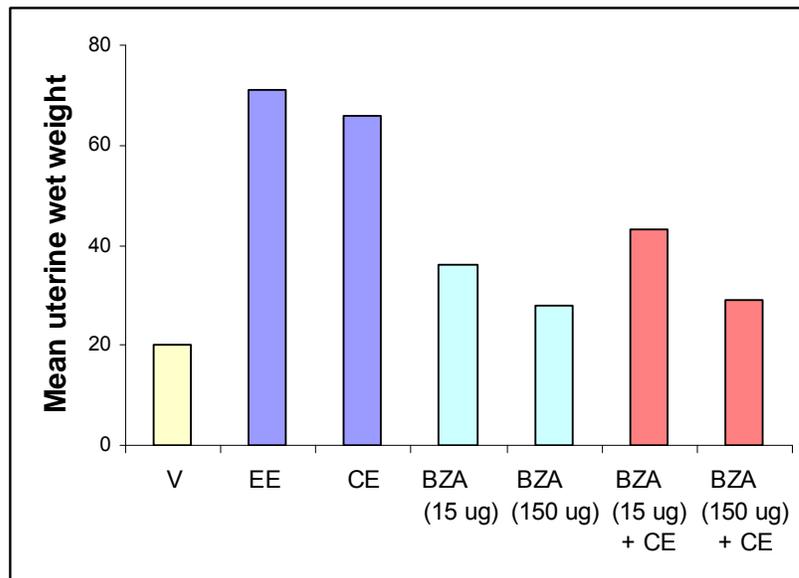
GTR-30822: Evaluation of uterine wet weight, histology and complement component 3 (C3) expression in the immature rat following treatment with WAY-138923, WAY-140424 and WAY-13186

RPT-68443: Expression Profiling Studies of SERMs Alone and When Paired with CE in the Immature Rat Uterus.

Two studies examined the ability of BZA to oppose the estrogen-induced increase in uterine wet weight in the three-day immature rat uterine model. The first (GTR-30822, reviewed under (b) (4)) used EE as the estrogenic agonist and contained a histological evaluation. The second study (RPT-68443) used CE as the estrogenic agonist and included gene expression analysis of uterine tissue.

Both studies established that BZA antagonized the estrogen-induced increase in uterine wet weight in a dose-dependent way and had little agonist effect when tested alone (see figure below). BZA also prevented EE induced myometrial and luminal cell hypertrophy (histology data given in Table 3 p 12 RPT GTR-30882).

Effect of BZA + CE on uterine wet weight in the immature rat uterine model.



Reviewer's figure using data from RPT-68443 Figure 5-1 p 13
Rats were orally dosed for 3 days with 0.6 ug EE/rat or 35 ug CE/rat.
BZA dose/rat shown in the figure. N=6/dose.

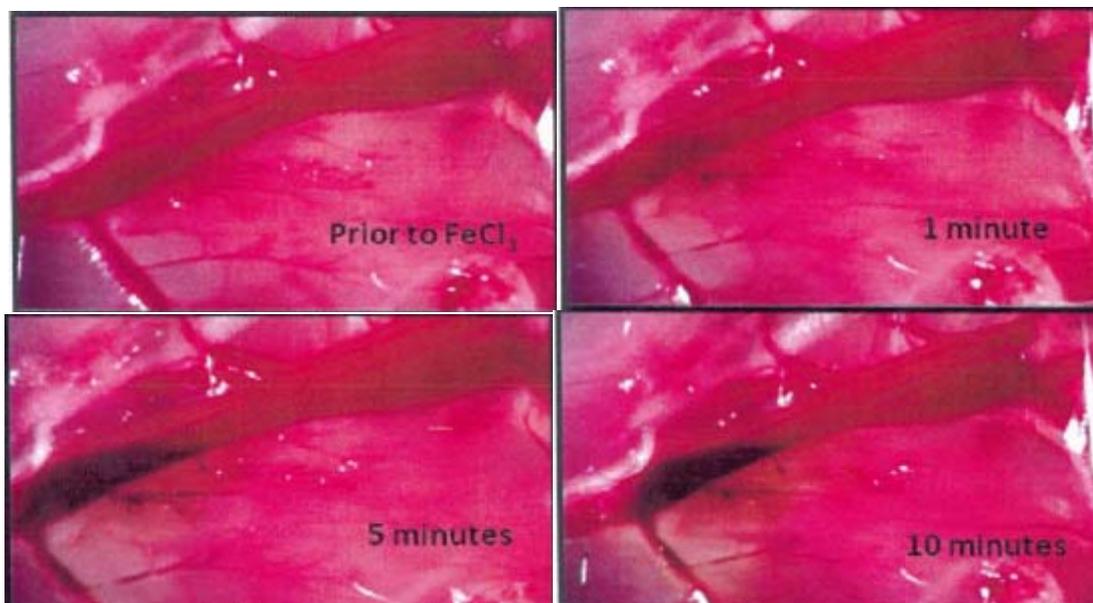
Venous thromboembolism (VTE):

RPT-80571: Venous thromboembolism: Development of a rat [sic]* model and preliminary evaluation of bazedoxifene, conjugated estrogens, and bazedoxifene + conjugated estrogens. *The study was conducted in the mouse.

One of the defined risks of estrogen therapy is venous thromboembolism (VTE; Tchaikovski and Rosing, 2010). The sponsor conducted a study to evaluate the effect of BZA alone and in combination with CE on the time course of formation of VTE in mouse model of femoral vein thrombosis.

Study design:

Ovariectomized (OVX) mice were treated with test article for 7 days prior to thrombus induction. Thrombus induction was accomplished by the well established method (Wang and Xu, 2005) of applying 15% ferric chloride solution (FeCl₃) to the adventitial surface of the surgically exposed femoral vein. Femoral vein occlusion occurs within 10-20 minutes and was assessed using intravital video microscopy in conjunction with a laser Doppler flow probe. Thrombus formation was evaluated in a blinded manner to determine the time to complete occlusion and the extent of blood flow through the vessel at several time points using the Thrombolysis in Myocardial Infarction (TIMI) scoring scale.



**Visualization of thrombus formation in a control animal.
Panels selected from Figure 1 p 17**

The study endpoints were: time to complete occlusion, TIMI grade, and Doppler flow. Uterine weight and body weight were monitored as pharmacodynamic endpoints. Study drugs were BZA, CE, and medroxyprogesterone acetate (MPA). The negative control was saline and the positive control was the anticoagulant Lovenox® (enoxaparin sodium). C57/B6 mice were OVX at 8-10 weeks of age and allowed to recover for at least 1 wk prior to testing. Animals were treated for 7-days as follows:

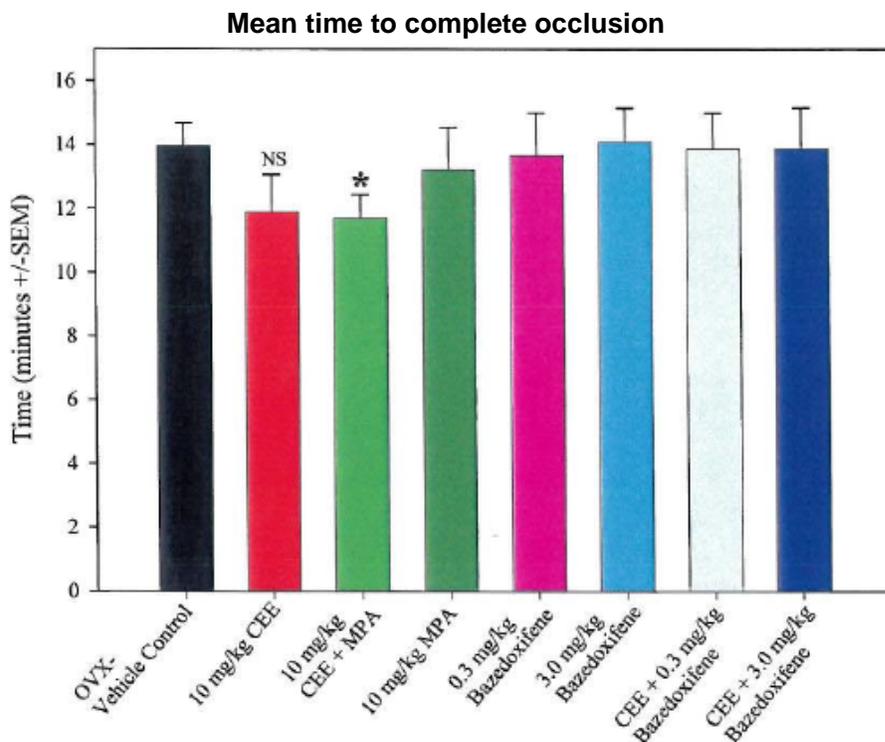
Treatment:

1	Saline control	(N=30)
2	10 mg/kg CE	(N=10)
3	10 mg/kg CE + 10 mg/kg MPA	(N=20)
4	10 mg/kg MPA	(N=10)
5	0.3 mg/kg BZA	(N=10)
6	3 mg/kg BZA	(N=10)
7	10 mg/kg CE + 0.3 mg/kg BZA	(N=10)
8	10 mg/kg CE + 3 mg/kg BZA	(N=10)

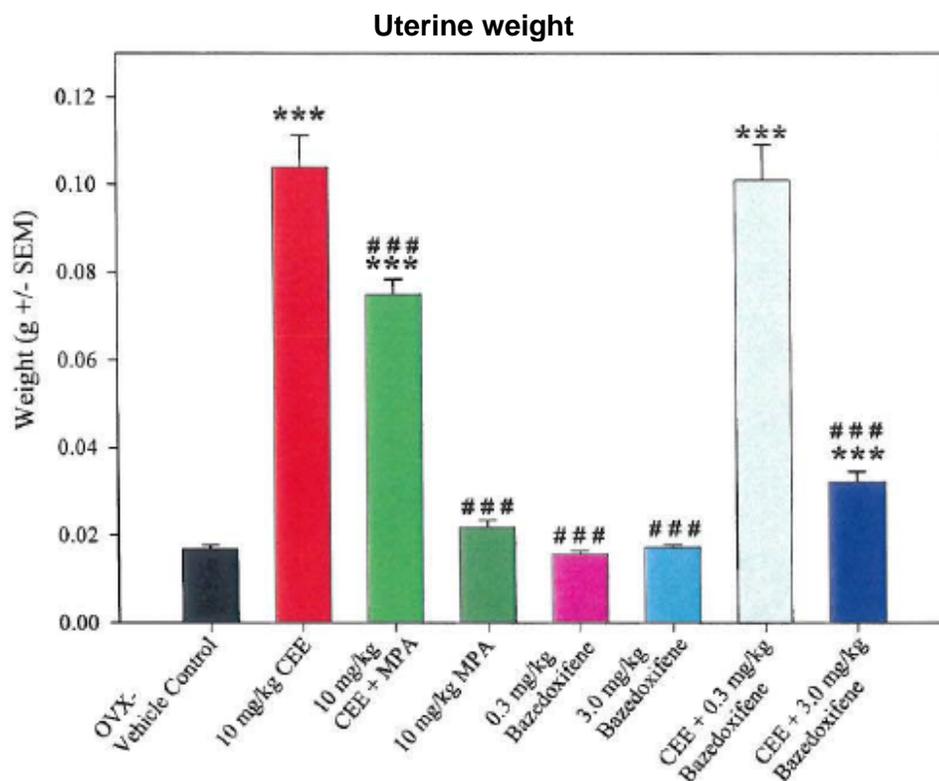
A positive control was run as a separate experiment using Lovenox at 20 mg/kg administered once, 2-3 hrs prior to thrombus induction.

Results and conclusions for VTEs:

BZA alone did not affect the mean time to occlusion. CE and CE + MPA did reduce the time to occlusion, but only slightly, and only significantly for the combination CE + MPA. BZA appeared to prevent the CE-induced reduction in time to occlusion, but since the CE effect was itself very small, it is difficult to interpret this finding (Figure 8 p 26). TIMI data is not shown but followed a similar pattern. As a comparison, the mean time to complete occlusion was more than doubled by Lovenox® (Figure 2 Appendix 5 p 47).



The pharmacodynamic effect of the test articles was verified by measuring uterine weights. Of interest is the finding that BZA alone was not estrogenic in its actions on uterine weight. Instead, BZA showed dose-dependent antagonism of the CE-induced increase in uterine weight (Fig 7 p 25).



4.2 Secondary Pharmacology

Off target receptor binding:

In vitro: GTR-32432: NovaScreen evaluation of TSE-424 for cross-reactivity on non-target receptors. Cross reactivity of bazedoxifene binding was observed at the sigma opioid receptor (binding at concentrations >100-fold binding affinity at the estrogen receptor)

In vivo: RPT-75759: Ancillary Pharmacology Profile of BZA in a Rat Model for Glucocorticoid/Antiglucocorticoid Activity.

The effect of BZA was tested in an *in vivo* model of glucocorticoid activity, the adrenalectomized male rat. Administration of glucocorticoids leads to decreased thymus weight. When administered alone (Figure 5-1), BZA at 0.3 or 3 mg/kg, had no effect on thymus weight, indicating no agonist activity at the glucocorticoid receptor. (Dexamethasone was the positive control.) BZA was also ineffective as an antagonist, showing no effect on thymus weight when coadministered with dexamethasone (Figure 5-2). RU486 was the positive control.

Figure 5-1: Effects of BZA on Thymus Weight in Male SD Adrenalectomized Rats Compared to Dexamethasone (Dex)

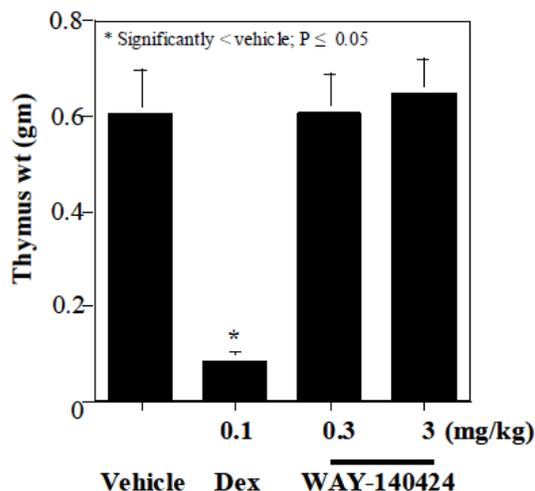
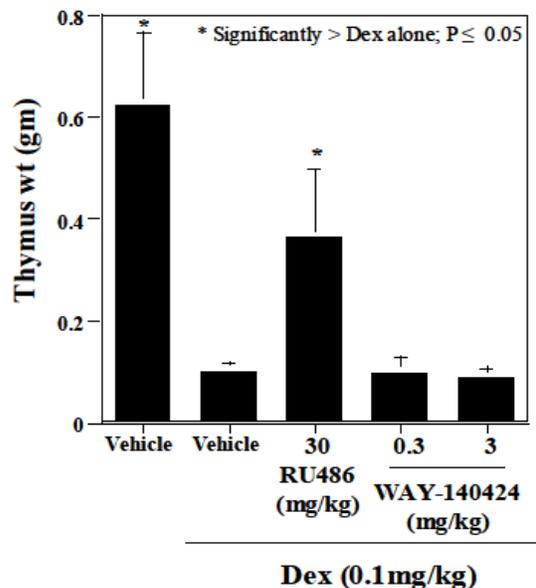


Figure 5-2: Effects of the Combination of BZA and Dexamethasone (Dex) on Thymus Weight in Male SD Adrenalectomized Rats



4.3 Safety Pharmacology

Safety pharmacology studies were conducted for BZA alone. (b) (4)
 Safety pharmacology studies were all negative.

Neurological and respiratory effects: RPTs 53745 and 53744: Female SD rats (N=8/grp) were given single oral doses of 0, 10, 100, 1000 mg/kg. CNS function (assessed by functional observational battery) and respiratory function were assessed at predose, 4, 8, and 24h. There were no treatment-related effects. C_{max} at 1000 mg/kg (estimate) = 335 ng/mL which is 54x C_{max} in humans at 20 mg/day (6.2 ng/mL).

Cardiovascular effects:

RPT-57815: Effects on cloned hERG channels expressed in mammalian cells. BZA inhibited hERG channel potassium current with IC₅₀ = 1.2 uM (565 ng/mL). Ratio IC₅₀/C_{max} (non-protein bound parent drug) = 565/0.043 = 13,140x (0.7% protein binding, total C_{max}=6.2 ng/mL). Data suggest no effect on hERG channels expected at clinical doses.

RPT-58489: Effects on action potential in isolated rabbit cardiac Purkinje fibers. Female rabbit Purkinje fibers were studied at 0, 0.01-10 uM. BZA had no significant effect on action potential duration, APD₆₀ and APD₉₀ (measures of Ca ion channel current and rapid I_{Kr}). BZA caused 33-55% reduction in V_{max} at 10 uM (Na channel). Ratio of 10 uM (4710 ng/mL) to human C_{max} at 20 mg/day is 760x (total) and 109,535x (unbound). Data do not give a signal for QTc prolongation of arrhythmia at clinical doses.

GTR-31030: An oral single dose cardiovascular study in female rats. Female rats were given a single oral administration of vehicle, followed 48 hrs later by a single oral dose of BZA at 0.5 or 5 mg/kg (N=5/group). Arterial blood pressure (systolic, diastolic, and mean), heart rate, spontaneous gross motor activity, and core body temperature were monitored via telemetry for 20-sec periods at 15-min intervals over 24 hrs before and after administration of the vehicle and BZA. There were no compound-related changes. C_{max} at 5 mg/kg = 5.5 ng/mL (1-mo repeat dose study data). This is 0.9x C_{max} at human dose of 20 mg/day.

GTR-31738: An oral single dose cardiovascular study in male and female cynomolgus monkeys. Monkeys (3/sex) were given BZA at single oral (gavage) dosages of 0, 10, and 50 mg/kg according to a Latin square crossover paradigm. Each animal received every treatment in a randomized order with a 2-day or 5-day dose-free period between treatments. Arterial BP (systolic, diastolic, and mean), heart rate, spontaneous gross motor activity, temperature, and ECG were recorded via telemetry for 20-sec periods at 30-min intervals over 24 hrs before and after administration of the test compound. There were no treatment-related changes. C_{max} at 50 mg/kg = 54.2 ng/mL (1-mo repeat dose study data). This is 8.7x C_{max} at human dose of 20 mg/day. Note: ECG's were not evaluated in repeat dose monkey toxicity studies of up to 9 months.

Renal effects: No studies

Gastrointestinal effects: No studies

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

PK/ADME for bazedoxifene alone was characterized in mouse, rat, dog, and monkey and was previously reviewed under (b) (4). ADME data from the rat were published in 2010 (see Chandrasekaran et al.). The summary text from section (b) (4) is reproduced below.

“In rats and monkeys, absorption of bazedoxifene by IV or oral route was rapid to moderate (Tmax 0.35h-6h). T1/2 was 3.8h-5.5h (parent), or 15-29h (total radioactivity). Bioavailability was 16% in rats, 11.3% in monkeys and 7% in dogs. PK profile suggested first pass metabolism and enterohepatic recirculation. Unchanged bazedoxifene was a minor part of circulating plasma radioactivity.

Radioactivity was rapidly distributed to tissues, with high initial levels in GI tract and liver. Accumulation upon repeat dosing occurred in thyroid gland, bone, bone marrow, and spleen (>5x). Accumulation was also observed in uveal tract and skin in pigmented rats. This may represent melanin binding but has an unclear relation to ocular toxicity. Bazedoxifene radioactivity was mostly distributed to the placenta but did not readily cross this barrier in pregnant rats. Protein binding in plasma was high, i.e., 99.77% (mouse), 98% (Tg.Ras mouse) 99.79% (rat), 99.89% (monkey), 99.94% (human). Binding to SHBG was not remarkable.

Bazedoxifene is highly metabolized in mice, rats and monkeys to the phenyl and indole glucuronides (4'- and 5-glucuronides). The relative levels of these two metabolites were different among species, with both 4'- and 5-glucuronides present in mouse and the 5-glucuronide predominant in rats, monkeys, human. The metabolites are pharmacologically active and antagonized the effect of estrogen in the rat uterus. Comparison of in vitro and in vivo metabolism data suggested that the bazedoxifene-4'-glucuronide is formed in monkeys and humans but preferentially excreted in the bile. Minor metabolites included the diglucuronide (monkey, human) and N-oxide (Tg.Ras mouse urine, human feces). CYP enzymes played a minor role in metabolism. In vitro studies showed that human UGT1A1 and UGT1A10 were active in glucuronidating parent compound.

The primary route of excretion was biliary/fecal in mice, rats, monkeys, and humans. Urinary excretion was minor. In mice, >50% of an oral dose was recovered within 24 hours. In rats, recovery of radioactivity was >97% and in monkeys and women it was ca. 85% within 7 days. Less than 1% of dose was recovered in urine. In feces, unchanged drug was the predominant form (>90%). The major urinary metabolite in rats was bazedoxifene-5-glucuronide in rats and bazedoxifene-4'-glucuronide in monkeys.

There was no significant induction of hepatic drug metabolizing microsomal enzymes. Drug interactions with compounds metabolized by CYP enzymes 3A4, 2D6, 2C9, 2C19, or 1A2 by bazedoxifene or its glucuronides are unlikely. The

potential for a drug-drug interaction between bazedoxifene and co-administered drugs due to alterations of protein binding is also low.”

One in vitro study was conducted to examine the potential metabolic interactions between BZA and CE (4.2.2.6: GTR-37100). Briefly, ¹⁴C-labeled BZA was incubated in vitro with the two major components of CE, estrone sulfate and equilin sulfate, in preparations of either human (female) liver hepatocytes, or human (female) liver microsomes and S-9 fractions. Dose selection for the test compounds was based on the estimated exposure to the liver in women following administration of the therapeutic dose. Metabolite profiles were characterized by HPLC. Coadministration of BZA and CE did not alter the metabolism of each other.

5.2 Toxicokinetics

For BZA alone, from the (b) (4) review: “Toxicokinetic analysis in pivotal toxicology studies showed less than dose-proportional increases in exposure (C_{max}, AUC) and no significant gender differences.”

For the combination CE + BZA, see TK data for 1 and 6-month rat and 9-month monkey studies under Section 6. Coadministration of BZA and CE did not alter the pharmacokinetics of each other.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies were not conducted with the combination BZA + CE. Single dose studies with BZA were reviewed under (b) (4). Very high oral doses (up to 4000 mg/kg) produced no mortality in rodents.

6.2 Repeat-Dose Toxicity

Study title: TSE-424/Conjugated Estrogens: 30-day oral (gavage) safety study in female rats	
Study no.:	RPT-39409
Study report location:	4.2.3.2.1
Conducting laboratory and location:	Wyeth-Ayerst Research 641 Ridge Road, Chazy, NY 12921
Date of study initiation:	15 Feb 2000
GLP compliance and QA statement:	Yes and Yes
Drug, lot #, and % purity:	BZA (TSE-424), Lot # 0C8617, 81% purity (b) (4) ID # R009266, $\geq 38.6\%$ $\leq 47.2\%$ Estradiol, lot #3056098, 99.4%

(b) (4)

Key Study Findings

- There was no mortality and there were no significant clinical signs.
- Modest (~10%) decreases in body weight were observed in all treated groups and were similar between the TSE-424/CE and TSE-424/E2 groups.
- Expected estrogenic changes in clinical chemistry (increased T4 and decreased cholesterol) occurred similarly between the two groups and were not considered adverse.
- Expected weight decreases occurred in the pituitary and adrenal glands, without microscopic findings. Decreased pituitary and adrenal gland weights occurred in previous studies in rats with TSE-424. Thus, the addition of CE or E2 did not negate the effects of TSE-424 on the pituitary or adrenal glands.
- Uterine weight decreased in all treatment groups, and correlated microscopically with uterine atrophy. Thus, TSE-424 in combination with CE or E2 negated the expected hypertrophic effect of estrogen on the uterus. TSE-424 alone also causes reduced uterine weight and atrophy.
- Effects on the ovary were mixed. Ovarian weight was generally decreased across all groups, which is consistent with estrogenic stimulation by CE or E2. However, cystic follicles were also present in all treatment groups, and in the high dose TSE-424/CE group correlated with increased ovarian weight. In previous rat studies, TSE-424 alone caused increased ovarian weights and cystic ovarian follicles. (Ovarian atrophy and decreased ovarian weight are known effects of estrogen.)

- Mammary gland lobular hyperplasia occurred in a few animals in the mid- and high-dose TSE-424/E2 group.

Conclusions: The administration of TSE-424 in combination with CE or E2 resulted in a similar toxicological profile between the two groups. With the exception of mammary gland lobular hyperplasia, which was not observed in studies with TSE-424 alone, the toxicological profile of TSE-424 in combination with either CE or E2 was similar to that of TSE-424 alone.

Methods	
Doses: (adjusted for purity of the active moiety)	BZA/CE: 3/0.33, 12/1, 60/3 mg/kg/d BZA/E ₂ : 3/1, 12/3, 60/10 mg/kg/d
Frequency of dosing:	daily, adjusted weekly based on body weight
Route of administration:	oral gavage
Dose volume:	Each dosing formulation was administered at a constant dose volume of 5 mL/kg, for a total daily dose volume of 10 mL/kg. Group 1 controls were given 5 mL/kg of the vehicle for TSE-424 and 5 mL/kg of the vehicle for CE and E2 sequentially.
Formulation/Vehicle:	The vehicle-control article for TSE-424 consisted of final concentrations of 1.0% polysorbate 80 and 0.5% methylcellulose (4000 cps) in purified water. The vehicle-control article for E2 and CE consisted of a final concentration of 0.5% Methocel, A15C in purified water.
Species/Strain:	(b) (4) CD VAF rats
Number/Sex/Group:	15 F/group; Total 159
Age:	Approximately 7.5 weeks old on the day of dose initiation
Weight:	150 to 193 g on the day prior to dose initiation
Satellite groups:	9/group for TK 3 animals per gp per timepoint Samples taken on day 29 at 0, 2, 4, 6, 9, and 12 hrs.
Unique study design: (See p 11 for group designations and animal numbers)	Group 1: vehicle control Groups 2-4: low, mid, high dose BZA / E2 Groups 5-7: low, mid high dose BZA / CE Groups 8-10: TK for BZA / E2 Groups 11-13: TK for BZA / CE
Deviation from study protocol:	None stated

Study design

Sponsor's text on dose selection:

"The dosages of TSE-424 were selected based on estimated exposure ratios of E2. Based on the toxicokinetic data of TSE-424, a dosage of 60 mg/kg/day would provide an AUC of approximately 773 ng·hr/mL (based on data from the 30-day study, a 100 mg/kg/day dosage gave an AUC of 1289 ng·hr/mL), which would provide a 9x multiple over the human TSE-424 dose at 20 mg (AUC = 88.5 ng·hr/mL). The low and middle dosages were selected proportionally (at a ratio of approximately 4 or 5) as was done for E2.

The dosages of CE were selected based on the clinical dosage of Premarin®, which at 0.625 mg/day, is approximately 3x lower than the clinical dosage of E2; therefore the CE dosages selected were 1/3 of the dosages for E2."

Observations and Results

Mortality (at least twice daily starting on day -11): Results: No unscheduled deaths

Clinical Signs (at least twice daily starting d-8; detailed exam once per wk starting d 8) Results: Noted at low incidence in treated groups, generally not dose-related: alopecia, rough hair coat, red pigment around eye/nose/mouth, salivation, dyspnea, rales, and yellow discharge in the perineal area.

The sponsor indicated that all of these signs (except yellow discharge) have been observed in previous studies with TSE-424 in rats and are TSE-424 related. Respiratory signs, salivation, and red pigment around eye/nose/mouse were attributed to the dosing formulation. Alopecia was attributed to estrogenic action of the test compounds.

Body Weights (once per week starting day -8):

Results: Modest (~10%) treatment-related decreases in body weights were observed in all treated groups. For animals given the TSE-424/CE combination, the final body weights were 91%, 89%, and 90% of controls at the low, middle, and high combinations, respectively. Body weight gains were 68%, 60%, and 65% of control. Similar values were reported for animals in the TSE-424/E2 treatment groups.

Feed Consumption (cage-based consumption once per week, starting day -7):

Results: Treatment-related effects on food consumption in animals given either combination paralleled the effects on body weight.

Ophthalmoscopy (weeks -1 and 4): No treatment-related findings.

ECG: not done

Hematology (weeks 2 and 4): Results: Slight increases in red cell parameters (8% max) and fibrinogen (16% max) observed in all treated groups were not considered toxicologically significant.

Coagulation parameters (weeks 2 and 4): No treatment-related findings.

Clinical Chemistry (weeks 2 and 4):

Results: T4 increased (16% to 60%) in all treated groups. The increase was attributed to the estrogenic action of the study drugs. Because estrogen has been shown to cause enlargement of the thyroid gland in rats (Gibson et al., 1967) the increase in T4 was not considered toxicologically important.

Changes in lipid parameters were also attributed to the estrogenic action of the study drugs. Cholesterol was decreased (26% to 37%) in all treated groups, and triglycerides were decreased (17% to 19%) in all groups given the TSE-424/CE combination.

AP activity was modestly increased (22% to 52%) in all treated groups, but was not associated with liver weight increases or microscopic alterations and was not considered toxicologically important. Also noted was a modest decrease in Fe concentration (22%) and total iron binding capacity (5-10%) but was not considered significant since there were no adverse changes in other hematology parameters.

Urinalysis: not done

Gross Pathology: organ weights and macroscopic findings

Pituitary gland mean weights (absolute and relative) were decreased non-dose-dependently (22-38%) at all dosages for both treated groups. The sponsor attributed the decreased weight to a possible negative feedback mechanism of the estrogenic study drugs. No microscopic findings were associated with the decreased pituitary gland weights.

Adrenal gland mean weights (absolute and relative) were decreased non-dose-dependently to the roughly the same extent (~15% absolute, ~5% relative) in both treatment groups. No microscopic correlate was observed for the decreased adrenal gland weight.

Uterine mean weights (absolute and relative) were similarly decreased in all treatment groups, with animals in the TSE-424/CE groups showing slightly greater maximal decreases (60% vs 53%). The decreased uterine weights correlated microscopically with uterine atrophy.

Ovarian mean and absolute weights decreased dose-dependently (max 25%) at the low and mid-dose in both treatment groups. There was no microscopic correlate for the decreased weight. At the high dose of either combination, ovarian weight was not decreased and follicular cysts were present.

Mean absolute heart, kidney, and liver weights were modestly decreased (~10%) in both treated groups secondary to the effects of study drugs on total body weight.

Histopathology Findings: Adequate Battery - Yes Peer Review - Yes

Mammary gland lobular hyperplasia at the mid (2/14) and high (3/15) doses of the TSE-424/E2 treated groups, slight to mild in severity. The sponsor made some specific comments (p 32-33) about differences observed in the microscopic findings of TSE-424/E2 treated rats as compared to findings from previous studies with TSE-424 alone:

“Lobular hyperplasia observed in these rats differed from lobuloalveolar change described in the mammary gland of female rats in previous TSE-424 rat studies (GTR-34756, 1999, 26-Week oral toxicity study in rats, and GTR-30847, 1997, 30 day oral toxicity study in rats). In the present study, mammary tissue did not undergo lobuloalveolar change as seen with TSE-424 alone but rather consisted of increased numbers of glands. Mammary gland lobular hyperplasia has been associated with the administration of estrogens. Lobular hyperplasia of the mammary gland did not occur in rats given TSE-424/CE.”

Uterine and cervical atrophy, dose-dependent in severity, at all doses of the treated groups. Increased vaginal mucification at all doses of the treated groups.

Ovarian cystic follicles were present at all doses of the treated groups.

	Placebo	TSE-424/E2			TSE-424/CE		
Dose (mg/kg/d)	0	3/1	12/3	60/10	3/0.33	12/1	60/3
Incidence	0	9/15	14/15	12/15	7/15	13/15	15/15
Severity	0	0.9	2.1	1.9	0.7	1.7	2.7

Data from sponsor's table section 5.9.3 p 31

Special Evaluation: noneToxicokinetics

Plasma from animals given TSE-424/CE combination was analyzed for TSE-424 and for the 6 components of CE, including estrone, E2, 17 β -dihydroequilin, delta-8,9-dehydroestrone, and 17 β -delta-8,9-dihydroestradiol. Blood samples from animals given TSE-424/E2 were analyzed for TSE-424, estrone, and E2

Results:

Exposure to TSE-424 was similar given either in combination with E2 or CE. With either combination, the AUC values increased with increasing dose, however, the increases were less than dose-proportional (data from sponsor's tables p 35-36).

MEAN (\pm SE) PHARMACOKINETIC PARAMETERS FOR TSE-424 IN FEMALE RATS – DAY 29

Combination	Dosage (mg/kg/day)	C _{max} (ng/mL)	t _{max} (hr)	AUC ₀₋₂₄ (ng·hr/mL)	AUC/Dose	t _{1/2} (hr)
TSE-424/CE	3/0.33	9.58 \pm 0.72	4	74.5 \pm 9.0	24.8 \pm 3.0	ND
	12/1	53.1 \pm 11.3	2	396 \pm 20	33.0 \pm 1.7	6.0
	60/3	150 \pm 34	4	1121 \pm 142	18.7 \pm 2.4	4.5
TSE-424/E2	3/1	7.40 \pm 3.71	4	48.5 \pm 15.2	16.2 \pm 5.1	ND
	12/3	58.1 \pm 18.2	2	415 \pm 41	34.6 \pm 3.4	ND
	60/10	212 \pm 4	2	1411 \pm 73	23.5 \pm 1.2	ND

It was noted that AUC normalized to dose was significantly lower for the high dose TSE-424/CE dosing group, and significantly higher for the mid-dose TSE-424/E2 mid-dose group.

Exposure to estrone and 17 β -estradiol increased with increasing dose of TSE-424/CE combination, in an approximately dose proportional manner.

MEAN (\pm SE) PHARMACOKINETIC PARAMETERS FOR TSE-424/CE IN FEMALE RATS – DAY 29

TSE-424/CE (mg/kg/day)	Analyte	C _{max} (pg/mL)	t _{max} (hr)	AUC ₀₋₂₄ (pg·hr/mL)	AUC/Dose	t _{1/2} (hr)
3/0.33	Estrone	103 \pm 51	9	747 \pm 207	2265 \pm 626	ND
	Equilin	ND	ND	ND	ND	ND
	17 β -estradiol	33.1 \pm 33.1	4	215 \pm 129	651 \pm 391	ND
	17 β -dihydroequilin	16.7 \pm 16.7	9	ND	ND	ND
12/1	Estrone	297 \pm 58	2	1186 \pm 114	1186 \pm 114	ND
	Equilin	18.8 \pm 18.8	2	ND	ND	ND
	17 β -estradiol	118 \pm 25	2	558 \pm 150	558 \pm 150	ND
	17 β -dihydroequilin	53.4 \pm 31.8	2	ND	ND	ND
60/3	Estrone	382 \pm 68	2	3333 \pm 410	1111 \pm 137	ND
	Equilin	69.7 \pm 3.1	6	397 \pm 117	132 \pm 39	ND
	17 β -estradiol	126 \pm 15	2	1681 \pm 399	560 \pm 133	ND
	17 β -dihydroequilin	118 \pm 29	2	1111 \pm 141	370 \pm 47	ND

Exposure to estrone and 17 β -estradiol increased in with increasing dose of TSE-424/E2 combination, although in an approximately greater than proportional manner.

MEAN (\pm SE) PHARMACOKINETIC PARAMETERS FOR TSE-424/E₂ IN FEMALE RATS – DAY 29

Combination	Dosage (mg/kg/day)	Analyte	C _{max} (ng/mL)	t _{max} (hr)	AUC ₀₋₂₄ (ng•hr/mL)	AUC/Dose	t _{1/2} (hr)
TSE-424/E2	3/1	Estrone	223 \pm 37	2	633 \pm 90	633 \pm 90	ND
		17 β -estradiol	58.7 \pm 29.6	2	ND	ND	ND
	12/3	Estrone	2259 \pm 1229	2	9401 \pm 3355	3134* \pm 1118	ND
		17 β -estradiol	492 \pm 175	2	2102 \pm 443	701 \pm 148	ND
	60/10	Estrone	7519 \pm 4277	2	28352 \pm 8880	2835* \pm 888	
		17 β -estradiol	1802 \pm 1111	2	9297 \pm 2258	930 \pm 226	ND

*Significantly different than corresponding value in the low TSE-424/E₂ dosage group.

Stability and Homogeneity

TSE-424; verified p 275-276; (b) (4) verified p 278; E2: verified p 277

Study title: BZA/CE: Multiple (6-month) oral (gavage) toxicity study with a 3-month recovery in female rats	
Study no.:	RPT-50335
Study report location:	4.2.3.2.1
Conducting laboratory and location:	Wyeth European Drug Safety & Metabolism Research Center, Catania, Italy
Date of study initiation:	May 14, 2002
GLP compliance and QA statement:	Yes and Yes
Drug, lot #, and % purity:	BZA (TSE-424), Lot #RB1618, 88.68% (b) (4) (CE), Lot # R029246

Key Study Findings

- There were 15 unscheduled deaths, 6 of which were attributed to gavage error. The rest were undetermined.
- There were no significant clinical signs but similar to the 1 month-study, body weight decreased ~10% in all drug-treated groups.
- BZA/CE related organ changes were: increased adrenal and kidney weight and decreased pituitary and uterine weight.
- No microscopic findings were reported for adrenal, kidney, and pituitary. Microscopic uterine findings included dilatation, squamous metaplasia and atrophy that did not resolve after the recovery period.
- Ovarian weight was unchanged but cystic follicles were observed that resolved after the recovery period.
- Lobular hyperplasia of the mammary gland was found in a majority of the animals at all doses at the recovery necropsy.

All findings were considered expected exaggerated pharmacology on the target organs.

Methods	
Doses:	BZA/CE: 3/0.33, 12/1, 60/3 mg/kg/d (adjusted for purity of the active moiety) same doses used in the 1 month study
Frequency of dosing:	daily, adjusted weekly based on body weight
Route of administration:	oral gavage
Dose volume:	Each dosing formulation was administered at a constant dose volume of 5 mL/kg, for a total daily dose volume of 10 mL/kg. Group 1 control animals were given 5 mL/kg of the vehicle for TSE-424 and 5 mL/kg of the vehicle for CE sequentially.
Formulation/Vehicle:	The vehicle-control article consisted of final concentrations of 1.0% polysorbate 80 and 0.5% methylcellulose (4000 cps) in purified water. The vehicle-control article for E2 and CE consisted of a final concentration of 0.5% Methocel, A15C in purified water.
Species/Strain:	CrI:CD(SD) IGS BR rats
Number/Sex/Group:	30 F/group; Total 147
Age:	Approximately 8 weeks old on the day of dose initiation
Weight:	188.9 to 258.1 grams on the first day of dosing
Satellite groups:	9/group for TK 3 animals per gp per timepoint Blood for TK was collected twice from each rat. On d177 and 178, non-terminal samples were collected at 2, 4, and 6 hrs. Terminal samples were taken at 9, 12, and 24 hrs. Two animals from Group 4 were found dead prior to blood collection; blood was collected from the remaining 2 rats from that group at the appropriate 4, 6, 12, and 24 hour time points.
Unique study design: (See p 15 for group designations and animal numbers)	Group 1: vehicle control Groups 2-4: low, mid, high dose BZA / CE 20 F/group were necropsied at the end of the 26-week dosing phase and 10 F/group were retained for a 3-month recovery period.
Deviation from study protocol:	none reported to affect study outcome

Observations and Results

Mortality (twice daily including pretest): There were 13 unscheduled deaths, which occurred between days 95 and 166 in control and BZA/CE treated animals. Two additional deaths occurred on days 73 and 94 in TK animals given 60/3 mg/kg/day. 5/13 deaths in the toxicology groups were attributed to gavage error. Cause of death was not established for the remaining 8 animals. Of the two deaths in the TK groups, 1 was attributed to gavage error and 1 was undetermined.

Clinical Signs (at least once daily including pretest; detailed clinical examination, once per week, including pretest): Alopecia was noted at the mid and high doses that partially resolved during recovery. This was an expected finding.

Body Weights (once per week including twice pretest 1 and 7 days prior to dosing): The mean body weight gain was decreased 25% to 27% in all drug-treated groups, which resulted in a slight decrease (8-10%) in mean body weight at all doses at week 27. Changes in body weight were reversed during the recovery period.

Feed Consumption (Individual food consumption was recorded on all animals weekly during pretest and during the dosing and recovery periods):

Results: Treatment-related effects on food consumption in animals paralleled the effects on body weight and were reversible during the recovery period.

Ophthalmoscopy (pretest and weeks 14 and 26): No treatment-related findings.

ECG: not done

Hematology (During weeks 13 (days 86 or 87) and 26 (day 179 or 182) of treatment period and week 13 (day 91) of the recovery period):

Results: No treatment-related findings.

Coagulation parameters (During weeks 13 (days 86 or 87) and 26 (day 179 or 182) of treatment period and week 13 (day 91) of the recovery period):

Results: No treatment-related findings.

Clinical Chemistry (During weeks 13 (days 86 or 87) and 26 (day 179 or 182) of treatment period and week 13 (day 91) of the recovery period):

Results: Slight decreases in mean cholesterol, triglycerides, total protein, albumin, and A/G ratio and increases in alkaline phosphatase (AP) and phosphorus (IP) were detected in some BZA/CE-treated groups. None of these changes was considered toxicologically significant. At the end of the recovery phase, these differences were no longer present, except for TRIG. The alterations were consistent with those seen in the previously conducted 1-month study with BZA/CE.

Urinalysis: not done

Gross Pathology: organ weights and macroscopic findings

Pituitary gland mean weights (absolute and relative) decreased similarly at the mid and high dose (23% max) and were attributed by the sponsor to treatment. A smaller (~10%) absolute (but not relative to BW) decrease was observed in the low dose group. The sponsor attributed the decreased weight to a possible negative feedback mechanism of the estrogenic study drugs. No microscopic findings were associated with the decreased pituitary gland weights.

Adrenal gland mean weights (absolute and relative) were increased (35% max) at all doses in a non-dose-dependent manner at all doses. There were no BZA/CE-related microscopic changes in the adrenal gland in animals surviving to final necropsy, and increased adrenal weights were not considered adverse.

Uterine mean weights (absolute and relative) were decreased similarly at the mid and high doses (24% max). Decreased mean uterine weights were associated with microscopic dilatation, metaplasia, and atrophy of the uterus.

Ovarian weight: No changes reported

Kidney mean weights (absolute and relative) were increased similarly at the mid and high doses (17% maximum increase at the high dose, relative to body weight). There were no microscopic findings and the increased weight was not considered adverse.

Mean absolute and/or relative heart and liver weights were modestly decreased at the mid and high dose secondary to decreased body weight and attributed to the effects of study drugs on total body weight.

Recovery Necropsy

There were no BZA/CE-related organ weight changes at the recovery necropsy (week 39). Complete recovery of organ weight changes seen at final necropsy occurred in adrenal gland, pituitary gland, kidney, uterus, and terminal body weights. Slight increases (2% to 9%) in kidney and decreases (11% to 21%) in uterus weights were detected at recovery necropsy, but they were not statistically significant and did not occur with dose-related trends.

Histopathology Findings: Adequate Battery - Yes Peer Review - Yes

BZA/CE-related microscopic observations at final necropsy (week 26) consisted of cystic follicular arrest of the ovaries, dilatation, squamous metaplasia, and atrophy of the uterus, and increased incidence of estrus based on vaginal morphology.

Ovarian cystic follicles were present at all doses of the treated groups. Cystic follicular arrest of the ovary consisted of greatly reduced numbers of corpora lutea and Graffian follicles with increased numbers of interstitial cells.

	Placebo	TSE-424/E2			TSE-424/CE		
Dose (mg/kg/d)	0	3/1	12/3	60/10	3/0.33	12/1	60/3
Incidence	0	9/15	14/15	12/15	7/15	13/15	15/15
Severity	0	0.9	2.1	1.9	0.7	1.7	2.7

Data from sponsor's table section 5.9.3 p 31

Uterus: Uterine dilatation, atrophy and squamous metaplasia (dose-related) were present in all BZA/CE groups and deemed related to BZA/CE pharmacology.

Recovery necropsy (p 40):

BZA/CE-related microscopic observations at the recovery necropsy consisted of dilatation, squamous metaplasia, and atrophy of the uterus, and lobular hyperplasia of the mammary gland. Cystic follicular arrest of the ovaries was considered to have resolved. Diestrus was observed more frequently in females given BZA/CE.

Toxicokinetics

BZA was rapidly absorbed with peak concentrations observed in the first samples collected (2 hrs). Total exposure increased with dosage, but did not appear to be dose proportional. The concentration data and AUC values were highly variable.

Table 5.10-1: BZA Mean (\pm SE) Pharmacokinetic Parameters Week 26

BZA/CE dose mg/kg/day	C _{max} (ng/mL)	T _{max} (hr)	AUC ₀₋₂₄ (ng.hr/mL)	C _{max} / Dose	AUC/ Dose
3/0.33	20.3 \pm 9.5	2	97.8 \pm 24.5	6.78 \pm 3.17	32.6 \pm 8.2
12/1	25.0 \pm 9.0	2	299 \pm 51	2.08 \pm 0.75	24.9 \pm 4.3
60/3	111 \pm 23	2	966 \pm 166	1.85 \pm 0.38	16.1 \pm 2.8

The concentrations of CE-related components (unconjugated estrone, equilin, 17 β -estradiol, and 17 β -dihydroequilin) were determined. Concentrations of these analytes

were below the LOQ in most or all samples. CE was rapidly absorbed and exposure appeared to increase with dosage. Dose proportionality could only be evaluated for unconjugated estrone, for which the change in exposure was approximately proportional to the administered dose of CE.

Table 5.10-2: Mean (\pm SE) Pharmacokinetic Parameters of Unconjugated Estrone, Equilin, 17 β -estradiol, and 17 β -Dihydroequilin - Week 26 (p 44)

TSE-424/CE (mg/kg/day)	Analyte	C _{max} (pg/mL)	t _{max} (hr)	AUC ₀₋₂₄ (pg·hr/mL)	C _{max} /Dose	AUC/Dose
3/0.33	Estrone	189 \pm 31	2	660 \pm 108	574 \pm 95	1999 \pm 329
	Equilin	0	NA	0	-	-
	17 β -estradiol	25.5	2	50.9 \pm 50.9	-	-
	17 β -dihydroequilin	29.1 \pm 29.1	2	58.2 \pm 4.9	-	-
12/1	Estrone	277 \pm 52	2	2243 \pm 272	277 \pm 52	2243 \pm 272
	Equilin	0	NA	0	-	-
	17 β -estradiol	31.4	4	162 \pm 94	-	-
	17 β -dihydroequilin	55.6 \pm 30.7	2	251 \pm 133	-	-
60/3	Estrone	822 \pm 82	2	4714 \pm 475	274 \pm 28	1571 \pm 158
	Equilin	159 \pm 15	2	1053 \pm 214	52.9 \pm 4.9	351 \pm 71
	17 β -estradiol	173 \pm 42	2	450 \pm 133	-	-
	17 β -dihydroequilin	240 \pm 42	2	829 \pm 126	80.1 \pm 14.1	276 \pm 42

Stability and Homogeneity

Stability and uniformity of the BZA formulations were previously conducted in the concentration range of 0.6 to 200 mg/mL. Stability of the CE formulations was previously evaluated in the concentration range of 0.066 to 1 mg/mL (p 16). Uniformity of BZA and CE formulations were verified (p 26).

Study title: BZA/CE: 30-day oral gavage safety study in female monkeys	
Study no.:	RPT-39410
Study report location:	4.2.3.2
Conducting laboratory and location:	Wyeth Research Drug Safety, Chazy, NY
Date of study initiation:	1 Feb 2000
GLP compliance and QA statement:	Yes and Yes
Drug, lot #, and % purity:	(b) (4) E2: 43056098 BZA; 0C8617, 99%

Key Study Findings

There was no mortality, no treatment-related clinical findings, and no reported toxicologically significant changes in hematology or clinical chemistry. Mean ovarian weight increased and was associated with cystic follicles. Uterine weight decreased and was associated with atrophy. Cervical and vaginal atrophy were also noted. Decreased adrenal and pituitary weights were not dose-related and did not have associated microscopic findings.

Methods	
Doses: adjusted weekly for most body weight	BZA/E2: 15.0/0.6, 50/2, 150/6 mg/kg/d BZA/CE : 15/0.2, 50/0.66, 150/2 mg/kg/d
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg (2.5 mL/kg BZA and 2.5 mL/CE)
Formulation/Vehicle:	for BZA: 1% Polysorbate 80, NF; 0.5% Methylcellulose 4000 cps Vehicle for CE and E2: Methocel A15C, USO
Stability and Homogeneity:	Acceptable for BZA, CE, and E2 (p18).
Species/Strain:	Macaca fascicularis (cynomolgus monkeys)
Number/Sex/Group:	3/F/gp
Age:	4.4-5 yrs
Weight:	2.7-3.7 kg
Satellite groups:	All animals were sampled for TK at day 30 at 0,1,2,4,8,12, and 24 hrs post dosing.
Unique study design:	Group 1: vehicle control Groups 2-4: low, mid, high dose BZA / E2 Groups 5-7: low, mid high dose BZA / CE The dosages of E2 and TSE-424 were selected based on tolerance seen in previous toxicology studies.
Deviation from study protocol:	None reported to affect outcomes.

Observations and Results

Mortality: Twice daily. There were no unscheduled deaths.

Clinical Signs: Twice daily observations; detailed exams weekly. No treatment related signs.

Body Weights: Prior to treatment and weekly. Slight but not statistically significant body weight losses observed in the treated groups.

Feed Consumption: Daily by visual inspection. No effect on food consumption.

Ophthalmoscopy: Weeks -2 and 4. No treatment-related effects.

ECG: not done

Hematology and coagulation: Twice pretest and at wks 1 and 4. FBGN slightly (10% reduced in mid and high dose BZA/CE groups. Other sporadic differences not considered toxicologically significant.

Clinical Chemistry (plus thyroxine): Twice pretest and at wks 1 and 4. Sporadic differences not considered toxicologically significant.

Urinalysis: Once pretest and at wks 1 and 4. No treatment-related effects.

Gross Pathology: Enlarged and/or cystic ovaries in all groups given TSE-424/CE.

Organ Weights: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, thyroid, parathyroid, uterus.

Findings: increased ovarian and decreased uterine, pituitary, and adrenal gland weights (relative and absolute).

- Ovarian weight increase was larger in the BZA/CE treated group (61-249%) as compared to the BZA/E2 treated groups (3-17%) but was not necessarily dose-dependent. Increased weight in the BZA/CE group was associated with enlarged and/or cystic ovaries.
- Uterine weight decrease was comparable between the CE and E2 groups (30-50 and 30-40%) and was accounted for by uterine atrophy in all animals.
- Adrenal gland weight decrease ranged from 3-14% in the BZA/E2 group and 2-23% in the BZA/CE group (no dose-related trends and no microscopic findings).
- Pituitary gland weight decrease ranged from 5-27% in the BZA/E2 group and 2-24% in the BZA/CE group (no dose-related trends and no microscopic findings).

Histological Findings: Adequate Battery: yes Peer Review: yes

Ovarian cystic follicles:

	Placebo	TSE-424/E2			TSE-424/CE		
Dose (mg/kg/d)	0	15/0.6	50/2	150/6	15/0.2	50/0.66	150/2
Incidence	1/3	0/3	2/3	2/3	3/3	3/3	2/3
Severity	0.7	0	1.7	0.7	4.7	4.3	2.0

Data from sponsor's table section 5.10.3 p 25

Sponsor's further detail: "Ovarian cystic follicles consisted of increased numbers of enlarged follicles with prominent follicular antra. In females given vehicle-control or TSE-424/E2, these cystic follicles were more numerous than expected. In females given TSE-424/CE cystic follicles were large enough to distort the ovarian stroma, were lined by cuboidal epithelium, did not contain cumulated oophora or developing ova, but did not exhibit increased differentiation to either granulosa or luteal cells."

Uterus: Incidence and severity of uterine atrophy was comparable between the TSE-424/E2 or TSE-424/CE groups. Sponsor noted that both the myometrium and endometrium were thinned compared to control.

Vagina / cervix: Slight to moderate atrophy in all dosed animals.

Toxicokinetics:

Plasma from animals given TSE-424/CE combination was analyzed for TSE-424 and for 5 components of CE, including estrone, equilin, E2, 17 β -dihydroequilin, and 17 β -delta-8,9-dihydroestradiol. Blood samples from animals given TSE-424/E2 were analyzed for TSE-424, estrone, and E2.

The pharmacokinetics of TSE-424 were similar in female monkeys given either combination with E2 or CE. With either combination, the AUC values increased with increasing dose, however, the increases were less than dose proportional. Data shown below are from sponsor's tables on p 27-28.

MEAN (\pm SE) PHARMACOKINETIC PARAMETERS FOR TSE-424 IN FEMALE RATS – DAY 29

Combination	Dosage (mg/kg/day)	C _{max} (ng/mL)	t _{max} (hr)	AUC ₀₋₂₄ (ng•hr/mL)	AUC/Dose	t _{1/2} (hr)
TSE-424/CE	15/0.2	42.3 \pm 15.2	5.7 \pm 4.0	640 \pm 125	42.7 \pm 8.3	ND
	50/0.66	53.7 \pm 7.0	10.7 \pm 2.3	976 \pm 149	19.5 \pm 3.0	8.3*
	150/2	87.0 \pm 30.4	8.0 \pm 0.0	1562 \pm 546	10.4 \pm 3.6	ND
TSE-424/E2	15/0.6	40.3 \pm 9.7	3.3 \pm 4.0	524 \pm 26	35.0 \pm 1.7	ND
	50/2	79.2 \pm 35.0	6.7 \pm 2.3	1155 \pm 187	23.1 \pm 3.7	ND
	150/6	99.9 \pm 47.7	9.3 \pm 2.3	1721 \pm 739	11.5 \pm 4.9	ND

It was noted that AUC normalized to dose was significantly lower for the high dose TSE-424/CE dosing group, and significantly higher for the mid-dose TSE-424/E2 mid-dose group.

*N=1

In monkeys receiving the TSE-424/CE combination, exposure to the component estrogens of CE increased with increasing dose but in an apparently less than proportional manner. Data for estrone, equilin, and 17 β -estradiol are shown below.

MEAN PHARMACOKINETIC PARAMETERS FOR TSE-424/CE IN FEMALE RATS – DAY 29

TSE-424/CE (mg/kg/day)	Analyte	C _{max} (pg/mL)	t _{max} (hr)	AUC ₀₋₂₄ (pg•hr/mL)	AUC/Dose	t _{1/2} * (hr)
150/0.2	Estrone	1182	6.7 \pm 2.3	15602	78009	11.1
	Equilin	193	4.3 \pm 3.5	2248	11242	ND
	17 β -estradiol	597	11.3 \pm 11.	10042	50211	9.9
50/0.66	Estrone	2270	6.7 \pm 2.3	36365	55099	ND
	Equilin	324	6.7 \pm 2.3	4651	7047	ND
	17 β -estradiol	61.8	6.7 \pm 2.3	602 (N=1)	912 (N=1)	ND
150/2	Estrone	3686	6.7 \pm 2.3	55892	27946	ND
	Equilin	672	6.7 \pm 2.3	8998	4499	ND
	17 β -estradiol	1282	6.7 \pm 2.3	2034	1017	14.9

Data p 28 *n=1

In monkeys receiving TSE-424/E2 combination, exposure to estrone was similar at the two lower dosages and increased between the middle and high doses, but in a less than proportional manner. Exposure to 17 β -estradiol increased with increasing dose but in a less than proportional manner.

MEAN PHARMACOKINETIC PARAMETERS FOR TSE-424/E₂ IN FEMALE RATS – DAY 29

Combination	Dosage (mg/kg/day)	Analyte	C _{max} (ng/mL)	t _{max} (hr)	AUC ₀₋₂₄ (ng•hr/mL)	AUC/Dose	t _{1/2} (hr)
TSE-424/E2	3/1	Estrone	10990	1.7	133397	222328	11.8*
		17 β -estradiol	2797	3.3	38651	64419	10.0 2.4
	12/3	Estrone	9949	4.3	144450	72225	9.9*
		17 β -estradiol	3195	4.7	47561	23781	12.9*
	60/10	Estrone	13234	6.0	240191	40032	34.4*
		17 β -estradiol	3728	10.7	73171	12195	ND

*N=1 or 2 Sponsor's table p 28

Study title: BZA/CE: Multiple (39 weeks) oral (gavage) toxicity study in the female cynomolgus monkey	
Study no.:	RPT-50336
Study report location:	4.2.3.2
Conducting laboratory and location:	Wyeth Research Drug Safety, (b) (4)
Date of study initiation:	June 14, 2002
GLP compliance and QA statement:	Yes and Yes
Drug, lot #, and % purity:	(b) (4) BZA; RA0615, RB1618; 88%

Key Study Findings

There was no mortality and there were no significant clinical findings. BZA/CE produced increased ovary and decreased uterine weights at all doses that were accompanied by microscopic findings of cystic follicles and atrophy, respectively. Liver weight was modestly increased, without microscopic findings. Changes in the ovary and uterus are expected pharmacologic effects of BZA/CE and are consistent with findings in previous studies in the rat and monkey. Note that dosing in this study was different from dosing in the 30-day monkey study (lower CE and higher BZA except for the high BZA dose, which was the same).

Methods	
Doses:	BZA/CE: 7.5/0.1, 33.5/0.45, 150/2 mg/kg/d
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg (2.5 mL/kg BZA and 2.5 mL/CE)
Formulation/Vehicle:	Polysorbate 80, NF; Methylcellulose 4000 cps Vehicle for CE: Methocel A15C, USO
Species/Strain:	Macaca fascicularis (cynomolgus monkeys)
Number/Sex/Group:	5/F/gp
Age:	~ 3 years old
Weight:	2.5-3.2 kg
Satellite groups:	All animals were sampled for TK at week 39 at 0,1,2,4,8,12, and 24 hrs post dosing.
Unique study design:	Dose selection was based on toxicity observed in prior studies with BZE alone (300 mg/kg/d BZA alone for 9 months) and BZA + CE (150 mkg BZA + 2 mkg CE for 30 days) in the monkey. Doses were adjusted for most recent body weight.
Deviation from study protocol:	none significant

Observations and Results

Mortality: Twice daily. there were no unscheduled deaths.

Clinical Signs: Twice daily observations; detailed exams weekly. No treatment related signs.

Body Weights: Prior to treatment and weekly. No effect on body weight reported.

Feed Consumption: Daily by visual inspection. No effect on appetite.

Ophthalmoscopy: Prior to treatment and at week 39. No treatment-related effects.

ECG: Twice during pretreatment and at weeks 26 and 39. ECGs were evaluate by a consultant board certified veterinary cardiologist. One high dose animal reported to have benign premature ventricular contractions (PVCs) at wk 39. Findings were deemed not treatment related.

Hematology and coagulation: Twice pretest and at wks 4, 13, 26, 39. No treatment related changes.

Clinical Chemistry (plus thyroxine): Twice pretest and at wks 4, 13, 26, 39. No treatment related changes. No effect on T4 levels.

Urinalysis: Once pretest and at week 39. No treatment-related effects.

Gross Pathology: Small uteri noted.

Organ Weights: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, thyroid, parathyroid, uterus. Findings: increased ovary and liver and decreased uterine weights (relative and absolute).

Organ weight	Dose BZA/CE		
	Low 7.5/0.1	Mid 33.5/0.45	High 150/2
ovary	↑18-32%	↑71-78%	↑179-193%
liver	--	--	↑19-20%
uterus	↓75-79%	↓75-79%	↓75-79%

Histological Findings: Adequate Battery: yes Peer Review: yes

Atrophy of the uterus, cervix and vagina were treatment but not dose-related, occurring in all animals with moderate to marked severity. Atrophy of the uterus affected primarily the endometrium. Cystic follicles occurred in 3/5 animals at all doses, and was slightly more severe in the high dose monkeys. Cystic follicles correlated with increased ovarian weight.

Special Evaluation: none

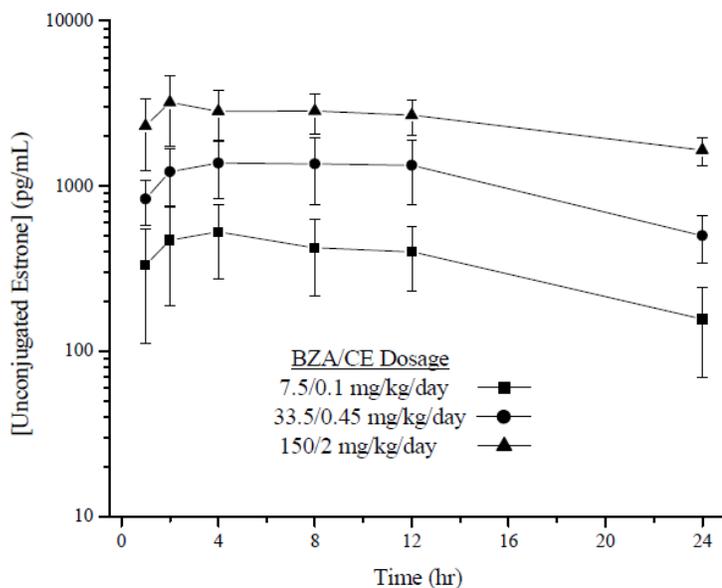
Toxicokinetics:

BZA: BZA was rapidly absorbed with peak concentrations usually observed in the first hour. Many animals showed a secondary rise that represented the C_{max}. Exposure increased with dosage, but was less than proportional between the low and mid-dose.

BZA Mean (±SE) Pharmacokinetic Parameters Week 39 (data from p 32)

BZA/CE dose mg/kg/day	C _{max} (ng/mL)	T _{max} (hr)	AUC ₀₋₂₄ (ng.hr/mL)
7.5/0.1	36.3 ± 17.1	3.2 ± 2.9	477 ± 109
33.5/0.45	92.5 ± 23.1	2.0 ± 1.2	1320 ± 183
150/2	212 ± 56	6.4 ± 2.2	4081 ± 1249

CE: The following analytes of CE were measured: estrone, equilin, $\Delta^{8,9}$ -dehydroestrone, 17β -estradiol, 17β -dihydroequilin, 17β - $\Delta^{8,9}$ -dehydroestradiol. A representative time course of exposure for estrone is shown below. Pharmacokinetic parameters for each of the analytes will not be reproduced here but can be found in text form on p 33-34 and in table form on pp 397 – 398. Qualitatively, for all analytes of CE, exposure increased with dosage, but was generally less than dose proportional over the range of CE dosages used.



Appendix 14, Figure 1 p 399.

Stability and Homogeneity: Appendix 20

BZA – Stability and homogeneity (uniformity) data were generated over the concentration range of 0.6-200 mg/mL and found to be stable and uniform for at least 17 days.

CE – Stability data were generated over the concentration range of 0.066 – 1 mg/mL and found to be stable for 17 days.

7 Genetic Toxicology

Mutagenicity studies with the combination BZA/CE were not conducted. Genetic toxicology studies for BZA alone were reviewed (b) (4). The following text is taken from the Executive Summary of her review.

Bazedoxifene was not genotoxic in the *in vitro* bacterial reverse mutation test, the *in vitro* mammalian cell forward mutation assay in mouse lymphoma cells, the *in vitro* chromosome aberration test in Chinese hamster ovary cells, and the *in vivo* micronucleus assay in CD-1 mice. Data do not indicate a mutagenic potential of bazedoxifene.

8 Carcinogenicity

Carcinogenicity studies with the combination BZA/CE were not conducted.

Carcinogenicity studies for BZA alone were conducted in the Tg.rasH2 mouse and the rat. They were reviewed [REDACTED] (b) (4). The rat study was amended in 2010 to correct for an error in calculating the administered dose (administered dose reduced by 12% at all doses; p 2955). The following text is taken from the Executive Summary of her review.

Mouse

RPT-57589: 26-week oral (gavage) carcinogenicity studies in CB6F1/Jic-TgN (RasH2) @ Tac +/- male and female mice (protocol 04_0493) and female mice (protocol 04_1679). (Note: the second study was conducted due to higher than expected gavage-related mortality in female mice at the high dose.)

In two 26-week carcinogenicity studies, transgenic Tg.rasH2 mice were dosed by oral gavage with 0, 50, 150, 500 mg/kg/day, corresponding to 22X, 37X, 50X (males) and 18X, 35X, 69X (females) the human AUC at the 20 mg/day clinical dose. Survival was reduced in the high dose BZA-treated females due to gavage accidents and this limited the power of the studies.

There was a dose-related statistically significant increase in the incidence of ovary benign granulosa cell tumor in mid- and high-dose females. In a urethane-treated positive control group, there were increased incidences of tumors in lung, harderian gland and spleen (multisystemic) hemangiosarcoma. In a comparator raloxifene (2000 mg/kg/day) group, there was no increase in ovarian or other tumors after the 6 month treatment period.

Ovarian tumors have also been observed in 15- to 24-month carcinogenicity studies in nontransgenic mice with other SERMs (raloxifene, lasofoxifene, tamoxifen) and may be the result of hyperstimulation of the ovary by pituitary gonadotropins (LH, FSH) due to a central anti-estrogenic action of bazedoxifene.

Rat

RPT-49489: Two year oral (diet) carcinogenicity study in rats.

In a 2-year oral carcinogenicity study, SD rats were treated with dietary doses of 0, 0.003%, 0.01%, 0.03%, 0.1%, corresponding to 0.12x, 0.4x, 1.6x, 4.0x (males) and 0.26x, 0.9x, 2.6x, 6.6x (females) the human AUC at the 20 mg/day clinical dose. Survival was increased due to a reduced incidence of pituitary tumors in males and of pituitary and mammary tumors in females in all dose groups.

There was a statistically significant dose-related increase in incidence of ovarian benign granulosa cell tumors in mid-high and high dose females. There was a non-dose-related statistically significant increased incidence of kidney renal

tubule adenoma and carcinoma in all dose groups in males. No other tumor incidences were increased in treated groups.

Ovarian granulosa cell tumors have been observed in rats with other SERMs (raloxifene, lasofoxifene) and may be the result of hyperstimulation of the ovary by pituitary gonadotropins (LH, FSH) due to a central anti-estrogenic action of bazedoxifene. However, data from rodent studies on gonadotropin levels in response to bazedoxifene treatment were not available, and a direct effect of BZA on the ovary can not be excluded. Renal tubule neoplasms in males were associated with increased incidence or severity of renal nonneoplastic histopathology findings. This tumor has also been observed in male rats in parallel with exacerbated nephropathy with other SERMs (raloxifene, lasofoxifene) and appears to be a male rat-specific phenomenon induced by an ER-mediated nongenotoxic mechanism.

Note: Data from the 2-year rat study have recently been published by the sponsor (Wright et al., 2013 and Perry et al., 2013).

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicology studies with the combination BZA/CE were not conducted.

Reprotox studies for BZA alone were conducted in the rat and rabbit. They were reviewed (b) (4). The following text is taken from the Executive Summary of her review.

In a fertility study in male rats at 30, 100, 300 mg/kg/day, corresponding to 11X-26X the human AUC at the 20 mg/day clinical dose, there were no effects on mating or fertility. In a fertility study in female rats at 0, 0.3, 1, 10, 30 mg/kg/day, corresponding to 0.03X-8.4X the human AUC at the 20 mg/day clinical dose, bazedoxifene interfered with estrous cyclicity, fertility and ability to maintain pregnancy at all doses, probably due to central or local anti-estrogenic effects. Animals dosed with ≥ 1 mg/kg/day did not become pregnant and in those that became pregnant at 0.3 mg/kg/day ovulation was suppressed and pre- and postimplantation loss was increased so that the number of live fetuses was reduced. Similar effects have been seen with the SERMs raloxifene and lasofoxifene. NOAEL was <0.3 mg/kg/day.

In a developmental study in rats at 0, 0.3, 1, 10 mg/kg/day, corresponding to 0.03x-3.1x the human AUC at the 20 mg/day clinical dose, bazedoxifene caused maternal toxicity (reduced body weight and food consumption) at all doses and embryo-fetal toxicity at 1 and 10 mg/kg/day. Embryo-fetal toxicity included decreased implantation and survival resulting in decreased litter size at 1 and 10 mg/kg/day, and decreased fetal body weight at 10 mg/kg/day. There were also statistically significant increases in vascular variations at 1 and 10 mg/kg/day and delayed ossification of cranial bones at 10 mg/kg/day. NOAEL was 0.3 mg/kg/day.

In a developmental study in rabbits at 0.05, 0.5, 5 mg/kg/day, corresponding to 0.24X-39X the human AUC at the 20 mg/day clinical dose, bazedoxifene caused slight decreases in maternal food consumption and body weight gain, and increased incidences of visceral abnormalities, including a statistically significant increase in ventricular septal defect at 0.5 and 5 mg/kg/day. Increased incidences of fetal skeletal abnormalities, including statistically significant increases in skull anomalies and vertebral morphological anomalies were also observed at 0.5 and 5 mg/kg/day. The study was repeated since the sponsor suspected that the health of the animals was compromised based on increases in postmortem findings in all groups including controls. NOAEL was 0.05 mg/kg/day.

In another developmental study in rabbits at 0.05, 0.5, 5 mg/kg/day, corresponding to 0.14x-15x the human AUC at the 20 mg/day clinical dose, bazedoxifene caused decreases in maternal body weight gain and/or food consumption, increased abortions and a decreased number of live fetuses at 0.5 and 5 mg/kg/day. Increased incidences of visceral abnormalities, including statistically significant increases in absent innominate artery and enlarged thyroid were seen at 5 mg/kg/day. Increased incidences of skeletal abnormalities in the 5 mg/kg/day group were not statistically significant. NOAEL was 0.05 mg/kg/day.

Data indicate that bazedoxifene poses a risk to women who are or may become pregnant.

10 Special Toxicology Studies

Special toxicology (from [REDACTED] (b) (4))

Bazedoxifene was not antigenic in the passive cutaneous anaphylaxis assay (PCA) test in mice and rats or the PCA or the active systemic anaphylaxis (ASA) test in guinea pigs (GTR-37740 and GTR-37739).

In a hormone study in female monkeys, bazedoxifene at 10 mg/kg (4x human AUC) suppressed menstrual cycles and pre-ovulatory LH surge, decreased levels of progesterone and increased levels of 17B-E2 and LH, supporting a central anti-estrogenic action at the level of the pituitary/hypothalamus (RPT-38690).

11 Integrated Summary and Safety Evaluation

Osteoporosis remains a major cause of morbidity in post-menopausal women. Estrogens remain the most effective treatment for postmenopausal osteoporosis, but prolonged use in this population carries major risks of VTEs and breast cancer. Bazedoxifene is a well-studied member of a class of compounds, known in the literature as selective estrogen receptor modulators (SERMs), which act as estrogen agonists in some tissues and estrogen antagonists in others. The mixed agonist / antagonist effect is brought about by the differential recruitment of promoters and repressors to the ligand-bound nuclear receptor for estrogen (ER- α or ER- β).

No one SERM has an ideal risk / benefit profile for treatment of postmenopausal symptoms. Bazedoxifene was previously found, in both nonclinical and clinical studies, to be moderately effective for treatment of osteoporosis, but to carry an unacceptable risk for VTEs in the postmenopausal population. In its favor, bazedoxifene was characterized nonclinically as having estrogen antagonist effects in the uterus and breast, which could potentially provide some protection to those tissues as compared to estrogen.

The rationale for combining bazedoxifene with conjugated estrogen for treatment of osteoporosis is to gain the benefit of strong estrogenic stimulation to the bone with some tempering of the estrogenic stimulation to the uterus and possibly to the breast. The goal of the nonclinical evaluation of the combination CE + BZA was to determine whether these goals could be met. We relied primarily on in vivo studies using clinically relevant end points to decide whether nonclinical data did or did not support the clinical indications. Gene expression data, while useful for understanding mechanism of action, was not yet considered predictive enough of in vivo changes to contribute to the weight of evidence.

The effect of CE + BZA on bone parameters was addressed in a 1-yr OVX rat study. Doses of CE and BZA were chosen that were known to be effective when administered singly. The conclusion of the study was that BZA neither added to nor diminished the effect of CE on bone strength parameters, which supports the clinical use of the combination.

The effect of CE + BZA on the uterus was addressed in 6- and 9-month toxicology studies in the rat and monkey, respectively, and was also assessed as a pharmacodynamic end point in the 1-yr OVX rat bone study. BZA reproducibly mitigated the effect of CE on uterine tissue, as measured by uterine weight and endometrial histology. These data support a favorable clinical risk/benefit profile for endometrial safety in women.

The effect of CE + BZA on thrombus formation was assessed in a mouse model of femoral vein occlusion. A 7-day drug treatment course preceded the measurement of acute thrombus formation induced by FeCl₂ application to the surgically exposed femoral vein. Results were equivocal as to whether BZA could alter the rate of thrombus formation, in part because the estrogen itself was

a weak inducer. Given the number of variables that contribute to the risk of VTEs (liver function, lipid profiles, vascular physiology), it is unlikely that any one model would yield a clear answer. In addition, VTEs are not endpoints that are evaluable in nonclinical toxicology studies. The nonclinical data are therefore inconclusive as to whether the combination CE + BZA would alter the clinical risk of VTEs.

The effect of CE + BZA on vasomotor instability was investigated in a rat model, and found to be modestly effective in a non-dose-dependent manner. The indirect nature of the measurement (naloxone-induced rise in tail temperature in morphine addicted rats) makes extrapolation to humans somewhat difficult. We conclude that the nonclinical data would only very weakly support any clinical findings of efficacy of the combination for treatment of VMS.

Evaluation of the effect of CE + BZA on breast cancer risk was not addressed in nonclinical studies. However, a number of in vitro and in vivo studies have been conducted examining the effect of CE + BZA on mammary gland proliferation and histology. These studies were not fully reviewed, and no attempt will be made here to determine whether these studies support a more favorable risk / benefit for breast cancer in women. The clinical studies submitted to the NDA were not powered to detect any differences, and nonclinical predictions would not be helpful.

In the other target organs of toxicity, the ovary, vagina and cervix, CE did not appear to overcome BZA-induced changes. Ovarian cystic follicles were prominent in all BZA + CE treated groups, and vaginal and cervical atrophy were not prevented by the presence of CE. It is noteworthy that benign granulosa cell tumors are a finding in rats or mice chronically treated with BZA, and were found in animals chronically treated with the combination as well.

For all other toxicological findings and conclusions regarding BZA, we concur with the assessment (b) (4)
Overall, the combination of CE + BZA was found to have no new nonclinical safety concerns.

12 Appendix/Attachments

Nonclinical

Berrodin, TJ, KCN Chang, BS Komm, LP Freedman, and S Nagpal. Differential biochemical and cellular actions of Premarin estrogens: Distinct pharmacology of bazedoxifene-conjugated estrogens combination. *Mol Endocrinol*, 23(1):74–85. 2009.

Chandrasekaran, A, S Ahmad, L Shen, W Demaio, T Hultin, and J Scatina. Disposition of bazedoxifene in rats. *Xenobiotica* 40(8): 578-585. 2010.

Chang, KCN, Y Wang, PVN Bodine, S Nagpala, BS Komm. Gene expression profiling studies of three SERMs and their conjugated estrogen combinations in human breast cancer cells: Insights into the unique antagonistic effects of bazedoxifene on conjugated estrogens *Journal of Steroid Biochemistry & Molecular Biology* 118:117–124. 2010.

Gibson UP, Newberne JW, Kurn WL, Elsea JR. Comparative chronic toxicity of three oral estrogens in rats. *Toxic Appl Pharmacology*. 11:489-510. 1967.

Komm, BS, F Vlasseros, R Samadfam, L Chouinard, SY Smith. Skeletal effects of bazedoxifene paired with conjugated estrogens in ovariectomized rats. *Bone* 49:376-386, 2011.

Perry, R, CA Thompson, JN Earnhardt, DJ Wright, S Bailey, B Komm, MA Cukierski. Renal tumors in male rats following long-term administration of bazedoxifene, a tissue-selective estrogen receptor modulator. *Toxicologic Pathology* published online 2013. DOI: 10.1177/0192623313477255

Tchikovski, SN and J Rosing. Mechanisms of estrogen-induced venous thromboembolism. *Thrombosis Research* 126:5-11. 2010.

Wang, X, and L Xu. An optimized murine model of ferric chloride-induced arterial thrombosis for thrombosis research. *Thrombosis Research* 115:95-100. 2005.

Wright, DJ, JN Earnhardt, R Perry, S Bailey, B Komm, DR Minck, MA Cukierski. Carcinogenicity and hormone studies with the tissue-selective estrogen receptor modulator bazedoxifene. *J Cell. Physiol*. 228: 724-733, 2013

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/s/

LESLIE C MCKINNEY
06/05/2013

ALEXANDER W JORDAN
06/05/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 22-247	Applicant: Wyeth Pharmaceuticals (subsidiary of Pfizer)	Stamp Date: 10-03-2012
Drug Name: bazedoxifene / conjugated estrogens	NDA Type: 505(b)1 NME	PDUFA date: 10-03-2013

On **initial** overview of the NDA application for RTF:

	Content parameter	Yes	No	Comment
1	On its face, is the pharmacology/toxicology section of the NDA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner allowing substantive review to begin?	X		
3	On its face, is the pharmacology/toxicology section of the NDA legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505b1 and b2 including referenced literature) completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animals studies*, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?	X		
7	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?	X		PharmTox made no new recommendations to the sponsor since the submission (b) (4)
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.)	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

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 full nonclinical development program for bazedoxifene (BZE) was previously reviewed (b) (4) for prevention of postmenopausal osteoporosis and was deemed sufficient to support submission of this NDA. The development program included two pivotal repeat-dose tox studies (6 month rat and 9 month monkey) conducted with the combination BZE + CE, that were not reviewed under (b) (4) and will be reviewed for this NDA.

New nonpivotal studies submitted to this NDA address various aspects of the mechanism of action of the BZE + CE combination and will be reviewed as deemed necessary.

Leslie McKinney, PhD.

November 19, 2012

 Reviewing Pharmacologist

 Date

Alex Jordan, PhD

 Team Leader/Supervisor

 Date

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

/s/

LESLIE C MCKINNEY
11/20/2012

ALEXANDER W JORDAN
11/21/2012