# CENTER FOR DRUG EVALUATION AND RESEARCH

*APPLICATION NUMBER:*

**21-813**

# MEDICAL REVIEW(S)

# CLINICAL REVIEW

| | |
|---|---|
| Application Type | NDA |
| Submission Number | 21-813 |
| Submission Code | S-000 |
| Letter Date | February 10, 2006 |
| Stamp Date | February 16, 2006 |
| PDUFA Goal Date | December 16, 2006 |
| Reviewer Name | Theresa H. van der Vlugt, MD |
| Review Completion Date | October 16, 2006 |
| Established Name | Estradiol Gel |
| (Proposed) Trade Name | Bio-E-Gel |
| Therapeutic Class | Estrogen |
| Applicant | BioSante Pharmaceuticals, Inc. |
| Priority Designation | Standard |
| Formulation | Estradiol Gel |
| Dosing Regimen | Daily |
| Indications | • Treatment of moderate to severe vasomotor symptoms associated with the menopause. [redacted] |
| Intended Population | Postmenopausal Women |

## Table of Contents

## Table of Tables



# 1 EXECUTIVE SUMMARY

## 1.1 Recommendation on Regulatory Action

This reviewer recommends approval of the 0.87 gram per day estradiol gel dose containing 0.52 mg of estradiol providing an estimated mean systemic delivery rate of 0.0125 mg of estradiol per day for the treatment of moderate to severe vasomotor symptoms associated with the menopause. This reviewer recommends approval of the 1.7 gram per day estradiol gel dose containing 1.02 mg of estradiol providing an estimated mean systemic delivery of 0.0375 mg of estradiol per day for the treatment of moderate to severe vasomotor symptoms associated with the menopause. These recommendations are based upon the reported findings in single, 12-week, Phase 3 Study EST005 conducted to support the safety and efficacy of estradiol gel for this indication.

For the treatment of moderate to severe vasomotor symptoms associated with the menopause, the Agency's 2003 draft Guidance for Industry entitled "Estrogen and Estrogen/Progestin Products to Treat Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms – Recommendations for Clinical Evaluation" recommends that one or more 12-week, randomized, double-blind, placebo-controlled clinical trials be conducted that:

1) have appropriate inclusion and exclusion criteria;
2) conduct appropriate study analyses; and
3) evaluate the following four co-primary endpoints:

- Mean change in frequency of moderate to severe vasomotor symptoms from baseline to week 4.
- Mean change in frequency of moderate to severe vasomotor symptoms from baseline to week 12.
- Mean change in severity of moderate to severe vasomotor symptoms from baseline to week 4.
- Mean change in severity of moderate to severe vasomotor symptoms from baseline to week 12.

For study inclusion, study participants should have a minimum of 7 to 8 moderate to severe hot flushes per day at baseline, or 50 to 60 moderate to severe hot flushes per week at baseline. The primary efficacy analysis should show a statistically significant reduction in hot flush frequency and severity within 4 weeks of initiation of treatment and maintained throughout 12 weeks of treatment in the treated group compared to the placebo group. The primary efficacy analysis should also show a clinically significant reduction in frequency identified as a reduction of at least two moderate to severe hot flushes above placebo at week 4 and week 12.

The 1.7 gram per day estradiol gel dose achieved a clinically and statistically significant difference compared to placebo in reducing the frequency and severity of hot flushes at week 4 that was maintained through week 12 ($p<0.0001$ at both time points). The 0.87 gram per day

estradiol gel dose achieved a clinically and statistically significant difference compared to placebo in reducing the frequency and severity of hot flushes at week 5 that was maintained through week 12 ($p<0.001$ and $p<0.0001$, respectively). Although the 0.87 gram per day estradiol gel dose did not meet the Agency's 2003 draft clinical evaluation guidance recommended efficacy endpoints at week 4, a significant proportion of subjects in the 0.87 gram per day estradiol gel treatment group experienced a reduction in the frequency and severity of hot flushes at week 4 (56% experienced $\geq$ 50% reduction and 25% experienced $\geq$ 80% reduction in hot flush frequency; 15% experienced $\geq$ 50% reduction and 8% experienced $\geq$ 80% reduction in hot flush severity). The delay to reaching clinical and statistical significance of the 0.87 gram per day estradiol gel dose compared to placebo will be reflected in labeling.

[redacted]

For the treatment of moderate to severe symptoms of vulvar and vaginal atrophy associated with the menopause, the Agency's 2003 draft clinical evaluation guidance document recommends the following three co-primary endpoints:

- The mean change from baseline to week 12 in the vaginal maturation index (superficial and parabasal cells). For study inclusion, study participants would have no greater than 5 percent superficial cells on a vaginal smear at baseline. The primary efficacy analysis should show a statistically significant increase in superficial cells and a statistically significant decrease in parabasal cells.

- The mean change from baseline to week 12 in vaginal pH. For study inclusion, study participants should have a vaginal pH > 5.0 at baseline. The primary efficacy analysis should show a statistically significant lowering of vaginal pH.

- The mean change from baseline to week 12 in the moderate to severe self-assessed symptom identified by the subject as being the most bothersome to her. For study inclusion, study participants would have self-identified at least one moderate to severe most bothersome vulvar and vaginal atrophy symptom. The primary efficacy analysis should show statistically significant improvement in the moderate to severe symptom identified by the subject as most bothersome. The recommended subject self-assessed symptoms of vulvar and vaginal atrophy include:

    1. Vaginal dryness (categorized as none, mild, moderate or severe).
    2. Vaginal and/or vulvar irritation/itching (categorized as none, mild, moderate or severe).
    3. Dysuria (categorized as none, mild, moderate or severe).
    4. Vaginal pain associated with sexual activity (categorized as none, mild, moderate or severe).
    5. Vaginal bleeding associated with sexual activity (categorized as none, mild, moderate or severe).

Study EST005 was initiated on September 9, 2003 prior to receipt of the Agency's November 12, 2003 letter recommending specific inclusion criteria for single, 12-week, Phase 3 Study EST005. Per the submission, the Applicant elected not to amend the protocol for Study EST005 to include the Agency's recommended inclusion criteria for a treatment of moderate to severe symptoms of vulvar and vaginal atrophy [redacted]

BioSante Pharmaceuticals, Inc., 111 Barclay Boulevard, Suite 280, Lincolnshire, IL 60069 is the Applicant for NDA 21-813/S-000.

## 1.2 Recommendation on Postmarketing Actions

### 1.2.1 Risk Management Activity

No postmarketing risk management activities are recommended.

### 1.2.2 Required Phase 4 Commitments

No Phase 4 clinical study commitment is proposed.

### 1.2.3 Other Phase 4 Requests

There are no other Phase 4 requests.

## 1.3 Summary of Clinical Findings

### 1.3.1 Brief Overview of Clinical Program

Estradiol gel is a transdermal formulation composed of 0.06% estradiol in a hydroalcoholic gel proposed for use as a treatment for moderate to severe vasomotor symptoms (VMS) associated with the menopause [redacted]

The primary source of efficacy data submitted in support of a VMS indication [ ] [ ] is single, 12-week, Phase 3 Study EST005. Study EST004, a Phase 2 dose-ranging study was conducted prior to primary Phase 3 Study EST005. Study EST004 was only 4 weeks duration and is considered supportive of the proposed indications.

The primary sources of safety data are Phase 3 Study EST005 and Phase 2 Study EST004. A total of 645 treated subjects are represented in these two studies (484 subjects in Study EST005 and 161 subjects in Study EST004). Adverse event data was pooled across Studies EST005 and EST004 for the 2.5 gram per day estradiol gel treatment group in Study EST004 and the 2.6 gram per day estradiol gel treatment group in Study EST005 and for the placebo treatment groups in these two studies. Adverse event data were presented across all additional doses in both studies (0.625 gram per day estradiol gel and 1.25 gram per day estradiol gel in Study EST004 and 0.87 gram per day estradiol gel and 1.7 gram per day estradiol gel in Study EST005). Laboratory data collected at baseline and at the end of the studies were not pooled due to the difference in double-blind treatment duration between Studies EST004 and EST005 (4 weeks and 12 weeks, respectively).

[ ] Phase 1 investigations were conducted in postmenopausal women in order to evaluate the pharmacokinetic (PK) characteristics of estradiol gel. [ ] of these [ ] PK studies used the [ ] [ ] formulation and not the [ ] formulation planned for marketing (Studies [ ] [ ] The remaining four PK studies used the [ ] formulation (Studies EST003, EST006, EST007, and EST008).

Data from the two placebo-controlled clinical studies and [ ] of the [ ] PK studies are included in the Integrated Summary of Safety (Study [ ] [ ]. The Integrated Summary of Safety (ISS) summarizes data on a total of 756 subjects (645 subjects in Studies EST004 and EST005 and 111 subjects in Studies [ ] EST003, EST006, EST007, and EST008 including 24 male partners in Study EST006).

### 1.3.2 Efficacy

**Moderate to Severe Vasomotor Symptoms:**

The results from 12-week, primary, Phase 3 Study EST005 demonstrate the effectiveness of the 0.87 gram per day estradiol gel treatment group and the 1.7 gram per day estradiol gel treatment group in producing a statistically significant reduction compared with placebo in the frequency and severity of hot flushes. A third treatment group in Study EST005 also demonstrated efficacy for a VMS indication (2.6 gram per day estradiol gel). [ ]

[ ]

In Study EST005, a statistically significant reduction in daily moderate to severe hot flush frequency compared to placebo was observed at week 5 for the 0.87 gram per day estradiol gel treatment group ($p<0.001$) and at week 4 for the 1.7 gram per day estradiol gel treatment group

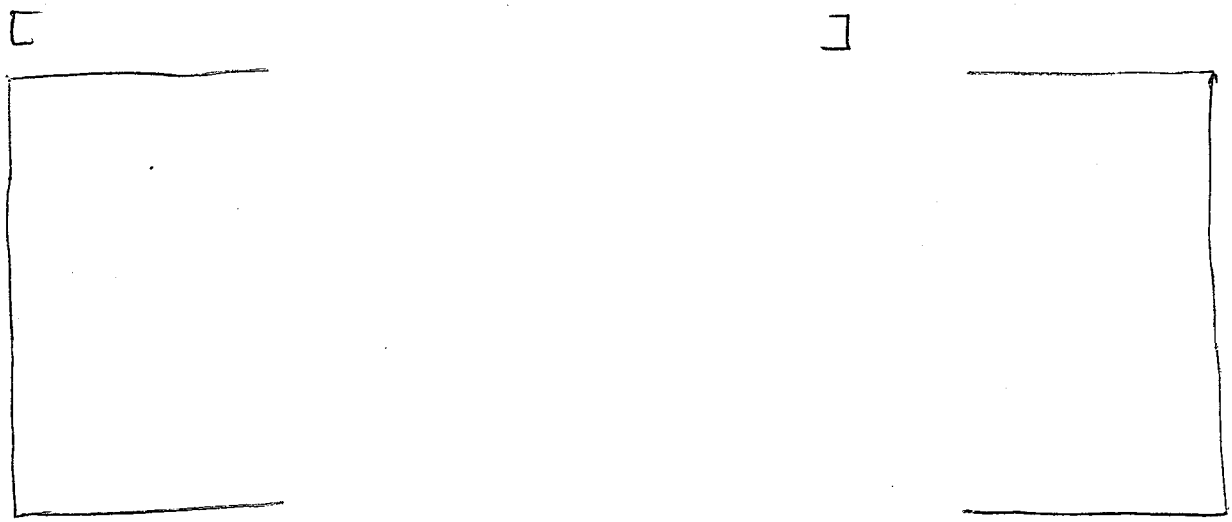
($p<0.0001$). The 0.87 gram per day estradiol gel treatment group did not demonstrate statistical significance compared to placebo at week 4 in Study EST005 ($p=0.0965$). Statistically significant reductions in daily moderate to severe hot flush frequency compared to placebo were demonstrated at week 12 for both doses ($p<0.0001$ for the 0.87 gram per day estradiol gel and 1.7 gram per day estradiol gel dosage strengths).

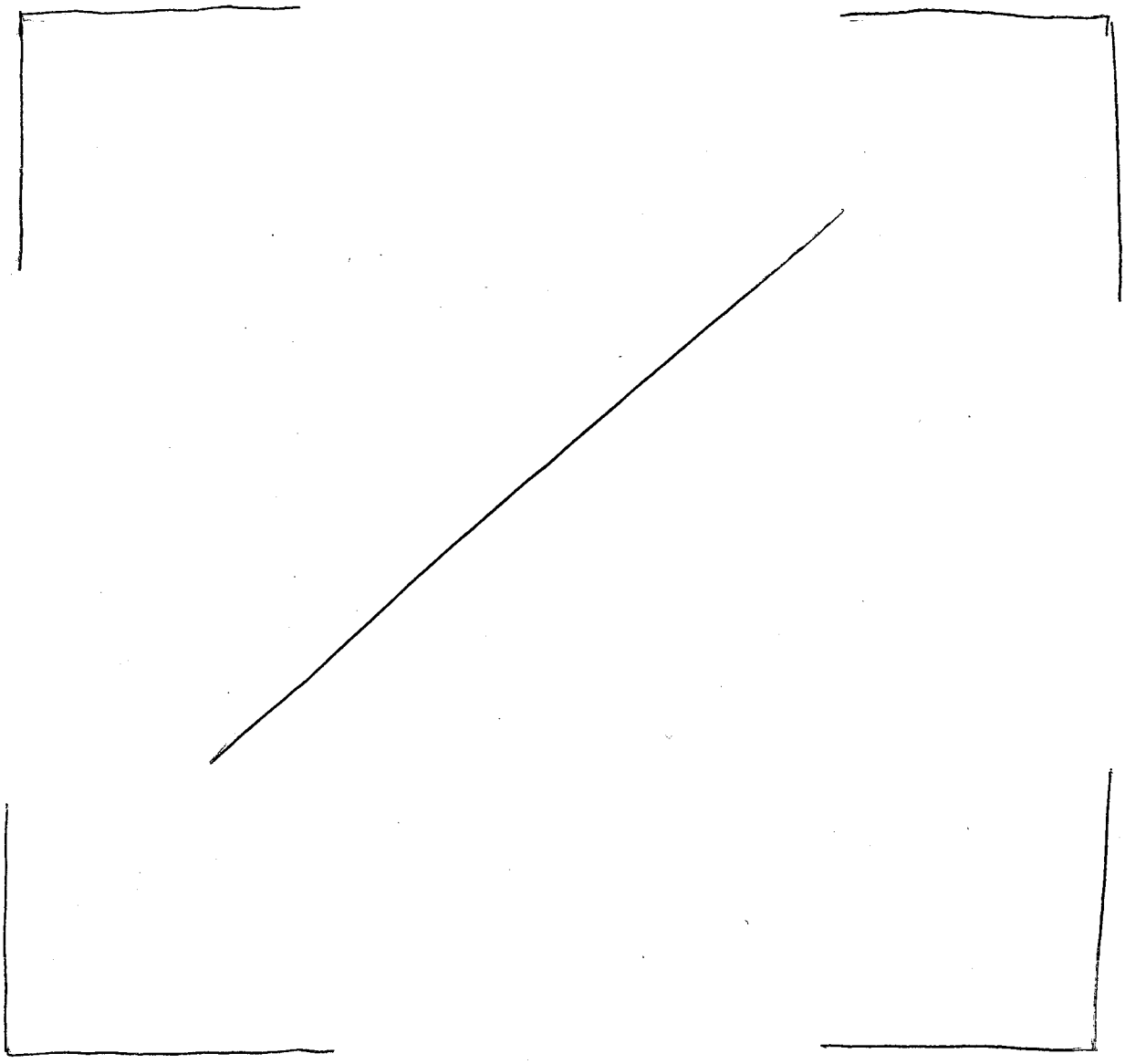
A clinically meaningful reduction in daily hot flush frequency compared with placebo was observed for the 0.87 gram per day estradiol gel treatment group at week 5 (> 2 difference in the number of moderate to severe hot flushes per day over placebo) and at week 4 for the 1.7 gram per day estradiol gel treatment group (> 2 difference in the number of moderate to severe hot flushes per day over placebo). The reduction in the number of hot flushes over placebo was not clinically meaningful for the 0.87 gram per day estradiol gel treatment group at week four (1.2 difference in the number of moderate to severe hot flushes per day compared with placebo at week 4).

Reduction in hot flush severity was statistically significantly different from placebo treatment by week 5 for the 0.87 gram per day estradiol gel treatment group ($p<0.01$ at week five, $p=0.714$ at week 4), and by week 4 for the 1.7 gram per day estradiol gel treatment group ($p<0.0001$). Statistically significant reductions in daily moderate to severe hot flush severity compared to placebo were demonstrated at week 12 for both doses ($p<0.0001$ for 0.87 gram per day estradiol gel and 1.7 gram per day estradiol gel dosage strengths).

Based on effectiveness analyses presented in the NDA 21-813/S-000 submission, this reviewer recommends approval of the 0.87 gram per day estradiol gel dose and the 1.7 gram per day estradiol gel dose for the treatment of moderate to severe vasomotor symptoms associated with the menopause.

Product labeling will clearly delineate that in the clinical trial the 0.87 gram per day estradiol gel dose demonstrated delay until week 5 in achieving a clinically and statistically significant reduction in the frequency and severity of hot flushes.





### 1.3.3 Safety

The safety data presented in the submission shows that the overall safety profile of the 0.87 gram per day estradiol gel dose and the 1.7 gram per day estradiol gel dose is acceptable. Both dosing regimens of estradiol gel were well tolerated, although more subjects in the 1.7 gram per day estradiol gel treatment group discontinued (6.3%, nine of 142 subjects) than in the 0.87 gram per day estradiol gel treatment group (2.9%, four of 136 subjects). In addition, more subjects in the 1.7 gram per day estradiol gel treatment group discontinued due to adverse events (3.5%, five of 142 subjects) than in the 0.87 gram per day estradiol gel treatment group (0.7%, 1 of 136

subjects). These rates of discontinuation due to adverse events are not unexpected and pose no safety concerns for these two dosage strengths.

No deaths occurred during or following the conduct of Phase 3 Study EST005, Phase 2 Study EST004, or during any of the PK studies submitted.

A total of three subjects experienced a serious adverse event (SAE) among the 484 treated subjects in Study EST005. One SAE occurred during the single-blind placebo lead-in period. The remaining two SAEs occurred during the 12-week double-blind treatment period. Subject 261 (2.6 gram per day estradiol gel treatment group, 54 years of age) experienced a worsening of a cervical cyst noted at study entry and an increase in endometrial thickness at end-of-study (4 mm at screening at baseline, 6 mm at end-of-study). She required hospitalization approximately three months after the last dose of study medication and underwent a transabdominal hysterectomy and bilateral salpingoophorectomy. The event was considered possibly related to study drug. Subject 106 (1.7 gram per day estradiol gel treatment group, 50 years of age) experienced a severe staphylococcal infection in her left thumb at a site of previous surgery which required hospitalization. Medication was discontinued. The event was not considered related to study drug.

Per the Integrated Summary of Safety, the reproductive disorders class was observed to be most affected by estradiol gel treatment, and the incidence in this class overall and individually (breast tenderness, metrorrhagia, vaginal discharge, endometrial hyperplasia, nipple pain) increased in a time and dose-dependent manner. There was a higher incidence of overall treatment-emergent adverse events (TEAEs) of this class in the estradiol gel all doses group than in the all placebo group and the difference was statistically significant (110 subjects [23.6%] versus 15 subjects [8.4%], respectively). These reported TEAEs may be considered expected, and are generally similar to adverse events known to occur during treatment with estrogens.

There was an increased incidence of changes from a normal endometrium at baseline as determined by transvaginal ultrasound (TVUS) or endometrial biopsy, to an abnormal endometrial thickness of greater than 4 mm by TVUS and/or abnormal hyperplastic endometrial biopsy results at the final visit in 12-week Study EST005 with the 1.7 gram per day estradiol gel and the 2.6 gram per day estradiol gel dosage strengths. Endometrial hyperplasia with atypia was reported for one subject receiving 1.7 gram per day estradiol gel (incidence rate of 1.05%, one case per 95 subjects with a uterus), and endometrial hyperplasia without atypia for five subjects receiving 2.6 gram per day estradiol gel (incidence rate of 11.1%, five cases per 45 subjects with a uterus).

Endometrial hyperplasia with and without atypia, while not unexpected with unopposed estrogen therapy, is infrequently observed in 12-week clinical trials of unopposed estrogen therapy. The reported 1% endometrial hyperplasia incidence rate in the 1.7 gram per day estradiol gel treatment group has been observed in other controlled 12-week clinical trials of unopposed estrogen therapy. However, the reported 11.1% endometrial hyperplasia incidence rate in the 2.6 gram per day estradiol gel treatment group exceeds the endometrial hyperplasia rate observed in other controlled 12-week clinical trials of unopposed estrogen therapy.

[redacted]

### 1.3.4 Dosing Regimen and Administration

During the clinical development program of estradiol gel, multiple dosage regimens of estradiol gel have been investigated including 0.625 gram per day, 0.87 gram per day, 1.25 gram per day, 1.7 gram per day, 2.5 gram per day, and 2.6 gram per day. The dosing regimens requested by the Applicant for approval for the treatment of moderate to severe vasomotor symptoms [redacted] are 0.87 gram per day estradiol gel containing 0.52 mg of estradiol providing an estimated mean systemic delivery rate of 0.0125 mg of estradiol per day, 1.7 gram per day estradiol gel containing 1.02 mg of estradiol providing an estimated mean systemic delivery of 0.0375 mg of estradiol per day. [redacted]

This reviewer recommends approval of the 0.87 gram per day estradiol gel and the 1.7 gram per day estradiol gel dosage strengths for the treatment of moderate to severe vasomotor symptoms associated with the menopause. [redacted]

In 12-week, Phase 3, primary safety and efficacy Study EST005, subjects applied estradiol gel to the entire upper arm and shoulder area from a metered-dose pump that delivered 0.87 grams of gel each time the pump is depressed. Subjects participating in Study EST005 were instructed to prime the metered-dose pump before first use by fully depressing the pump spout four times and discarding the expressed content, after which the pump was ready to use for approximately 100 pump depressions. Study subjects were cautioned to apply the daily gel dose to clean, dry, unbroken skin of the upper arm and shoulder after a bath, shower, or sauna, to never apply the estradiol gel to the breast, to wash hands with soap and water after applying the gel to reduce the chance of spreading the gel to others from the hands, and to allow the gel to dry for five minutes or more before dressing.

Patient information labeling includes the following cautions:

**Important things to remember when using [redacted]**

**Wash your hands with soap and water after applying the gel to reduce the chance that the medicine will spread from your hands to other people.**

Allow the gel to dry for five minutes or more before dressing.

Always move the spout into the locked position and place the cap over the top of the pump after each use.

**Never apply estradiol gel to the breast. Never apply [ ] in or around the vagina.**

Do not allow others to [ ]

**Do not apply sunscreen to the area where the gel was applied for at least 25 minutes.**

**Do not apply sunscreen to the area where the gel was applied for 7 or more consecutive days.**

[ ] **Avoid fire, flame or smoking until the gel has dried [ ] are flammable.**

Estradiol, given alone, is approved for use in a variety of delivery systems that include two oral tablets (Estrace® and Femtrace®), a vaginal tablet (Vagifem®), a vaginal cream (Estrace® Cream 0.01%), two vaginal rings (Estring® IVR and Femring®), six transdermal patch systems (Estraderm®, Vivelle®, Vivelle-Dot®, Climara®, Alora®, and Esclim®), one transdermal emulsion (Estrasorb®), and one transdermal gel (Estrogel®). There are four approved combination (containing estradiol plus a progestin) delivery systems, Activella® and Prefest® oral tablets, and Combipatch™ and ClimaraPro® transdermal patch systems.

### 1.3.5 Drug-Drug Interactions

In Phase 1 Study EST008, when sunscreen was applied 10 minutes before the application of the 2.6 gram per day estradiol gel dose for seven days $C_{ave}$ and $AUC_{0-24}$ for estradiol, estrone, and estrone sulfate increased by 55%, 34%, and 36%, respectively compared to when estradiol gel was applied alone. When the sunscreen was applied 25 minutes after estradiol gel application for seven days, no significant change in $C_{ave}$ and $AUC_{0-24}$ for estradiol or its metabolites were observed. These findings will be reflected in labeling.

In Phase 1 Study EST008, estradiol $AUC_{0-24}$ was observed to be [ ]-fold higher on day 37 compared to day 15. Study EST008 was conducted as follows: 2.6 gram per day estradiol gel dose was applied alone on days 1-15, sunscreen was applied either before or after the application of estradiol gel on days 16-22, 2.6 gram per day estradiol gel was applied alone on days 23-37; sunscreen was applied either before or after the application of estradiol gel on days 38-42. The Agency's Clinical Pharmacology Reviewer agrees with the Applicant that sex hormone binding globulin (SHBG) can account for $\leq$ 15% of the observed 110% increase in estradiol $AUC_{0-24}$ on

day 37. However, it remains unclear why there is a [ ]-fold increase in estradiol $AUC_{0-24}$ on day 37 relative to day 15 in Study EST008. These findings will be reflected in labeling.

In vitro and in vivo studies have shown that estrogens are metabolized partially by cytochrome P450 3A4 (CYP3A4). Therefore, inducers or inhibitors of CYP3A4 may affect estrogen drug metabolism. Inducers of CYP3A4 such as St. John's Wort preparations (Hypericum perforatum), phenobarbital, carbamazepine, and rifampin may reduce plasma concentrations of estrogens, possibly resulting in a decrease in therapeutic effects and/or changes in uterine bleeding profile. Inhibitors of CYP3A4 such as erythromycin, clarithromycin, ketoconazole, itraconazole, ritonavir and grapefruit juice may increase plasma concentrations of estrogens and may result in side effects. This information will be provided in labeling.

### 1.3.6 Special Populations

Estradiol gel is only indicated for use in postmenopausal women. There were insufficient number of geriatric subjects in primary, Phase 3 Study EST005 and secondary, Phase 2 Study EST004 to determine if those over 65 years of age differ from younger subjects in their response to estradiol gel.

Estradiol gel was not studied in women with liver disease or renal impairment. Estradiol gel should not be used in pregnant women.

# 2 INTRODUCTION AND BACKGROUND

## 2.1 Product Information

Estradiol gel is a transdermal formulation composed of 0.06% estradiol in a hydroalcoholic gel proposed for use as a treatment for moderate to severe vasomotor symptoms (VMS) associated with the menopause [redacted]

Estradiol is a lipophilic molecule that is readily absorbed across cellular membranes including skin, vaginal mucosa, and the gastrointestinal tract. Estradiol is highly lipid-soluble, will accumulate in fatty tissue, and is distributed throughout the body. Estradiol binds extensively to albumin, sex hormone binding globulin (SHBG), cortisol binding globulin (CBG), and α-glycoproteins. The main site of estradiol metabolism is in the liver although estradiol is metabolized in the intestine wall. The plasma elimination half-life of estradiol is approximately one hour, independent of the route of administration.

BioSante Pharmaceuticals, Inc. first submitted IND 51,229/S-000 on November 7, 2001. The initial submission for IND 51,229 included a [redacted] However, the Division of Reproductive and Urologic Products (DRUP) recommended that the Applicant consider conducting a Phase 2 dose-ranging, placebo-controlled clinical trial of four weeks duration to determine the lowest effective dose of estradiol gel for the proposed indications.

Phase 2 Study EST004 entitled, "A Phase II/III, Multi-Center, Double-Blind Study of the Safety and Efficacy of Bio-E-Gel (Topical Estradiol Gel) Versus Placebo for Treatment of Vasomotor Symptoms in Postmenopausal Females" randomized 167 postmenopausal women to four treatment groups for a four-week study duration: placebo (n=42), 0.625 gram per day estradiol gel containing 0.375 mg estradiol (n=41), 1.25 gram per day estradiol gel containing 0.75 mg estradiol (n=40), and 2.5 gram per day estradiol gel containing 1.5 mg estradiol (n=38).

Study EST004 was not sufficiently powered to demonstrate statistical significance. However, the reported results indicated that the 2.5 gram per day dose of estradiol gel demonstrated the strongest reduction in both the frequency and severity of postmenopausal hot flushes. The 1.25 gram per day dose of estradiol gel showed similar results to the 2.5 gram per day estradiol gel dose in reducing the severity of hot flushes, but it did not produce a similar reduction in the frequency of hot flushes. The 0.625 gram per day estradiol gel dose either performed the same as or was inferior to placebo in Study EST004.

Combining the VMS frequency and severity findings reported in Study EST004 with trough serum estradiol concentrations reported in the same study [mean (SD) serum estradiol

concentration for the 1.25 gram per day estradiol gel dose = 34.8 ± 33.0 pg/mL; mean (SD) serum estradiol concentration for the 2.5 gram per day estradiol gel dose = 46.8 ± 44.6 pg/mL], the Applicant determined that the 2.5 gram per day estradiol gel dose (containing 1.5 mg of estradiol delivering approximately 50 mcg of estradiol to systemic circulation daily) was the lowest effective dose.

Phase 3 Study EST005, the primary clinical safety and efficacy 12-week study, was submitted on September 17, 2003 with [ ] dosage strengths of estradiol gel versus placebo gel:

- 1.7 gram per day dose of estradiol gel containing 1.02 mg of estradiol (approximate delivery of 0.0375 mg of estradiol per day)

[ ]

The Applicant was advised in a regulatory letter dated November 12, 2003, that the proposed dosage strengths for Study EST005 were acceptable. DRUP expressed concern, however, that should both dosage strengths demonstrate effectiveness in Study EST005, an ineffective lower dose would not be demonstrated in the primary, Phase 3, 12-week study. In Amendment 7 for Protocol EST005 dated April 21, 2004, the Applicant modified the protocol for Study EST005 to add:

- 0.87 gram per day dose of estradiol gel containing 0.52 mg of estradiol (approximate delivery of 0.0125 mg of estradiol per day)

At the end-of-phase 2 meeting on April 24, 2003, Clinical Pharmacology and Biopharmaceutics advised the Applicant to conduct the following studies: partner transfer, effect of sunscreen, and effects of washing. In addition, the Applicant was advised that they may consider using any of these studies to characterize the full PK of the to-be-marketed product in a sufficient number of subjects.

The submitted established name is estradiol transdermal gel. Bio-E-Gel was the only tradename submitted by the Applicant for consideration on February 16, 2006. [ ]

[ ]

On February 16, 2006, the Division of Reproductive and Urologic Products (DRUP) submitted a Request for Consultation to the Division of Medication Errors and Technical Support (DMETS). The following recommendations were provided on September 13, 2006:

"1. DMETS did not identify any look-alike or sound-alike name concerns with the proposed proprietary name, Bio-E-Gel. [ ]

[ ]

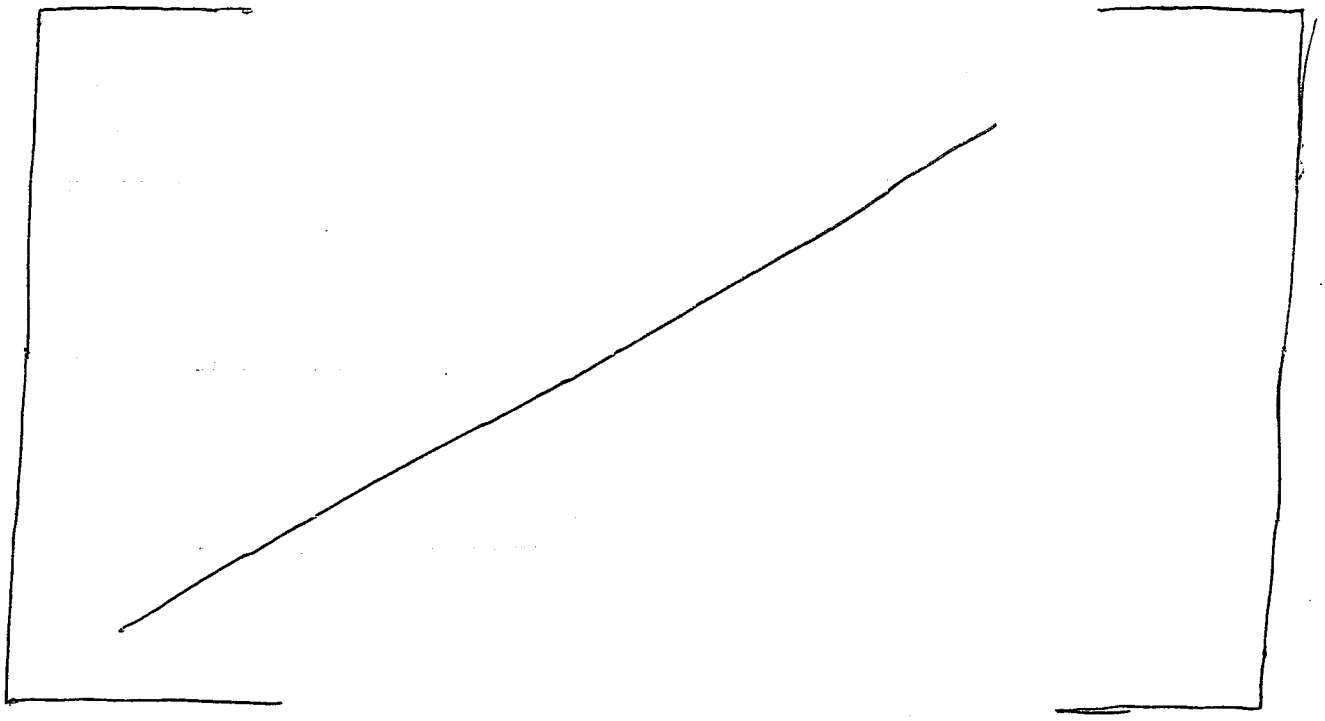
Redacted 1 page(s)

of trade secret and/or

confidential commercial

information from

Medical Review (p.19)



[ ] In the e-mail of a letter dated November 9, 2006, the Applicant proposed [ ] alternate tradenames for Bio-E-Gel: (1) Elestrin [ ] On November 13, 2006, DRUP submitted a Request for Consultation to DMETS for (1) Elestrin [ ]

## 2.2 Currently Available Treatment for Indications

Numerous estrogen alone and estrogen plus progestin drug products are currently approved for both the treatment of moderate to severe vasomotor symptoms and moderate to severe symptoms of vulvar and vaginal atrophy associated with the menopause. These include:

- Oral tablets: Premarin® (conjugated estrogens); Estrace® (estradiol), Femtrace® (estradiol acetate), Prempro™/Premphase® (conjugated estrogens plus medroxyprogesterone acetate), Prefest® (estradiol plus norgestimate), Activella® (estradiol plus norethindrone acetate);

- Transdermal systems: Alora® (estradiol), Climara® (estradiol), Estraderm® (estradiol) Vivelle® (estradiol), Vivelle-Dot® (estradiol), Climara-Pro® (estradiol plus levonorgestrel); and

- Vaginal ring: Femring® (estradiol acetate).

## 2.3 Availability of Proposed Active Ingredient in the United States

Estradiol has been used clinically for estrogen therapy since the mid-1970s. The following products are approved and currently marketed for the treatment of moderate to severe vasomotor symptoms (VMS) associated with the menopause, and/or for the treatment of moderate to severe symptoms of vulvar and vaginal atrophy (VVA) associated with the menopause, and the prevention of postmenopausal osteoporosis:

| Estradiol-alone oral products: | | Nominal daily delivery rate/indication(s): |
|---|---|---|
| **Estrace®** (estradiol tablet) | = | 0.05 mg, 1 mg, or 2 mg daily for VMS, VVA, and prevention of postmenopausal osteoporosis |
| **Femtrace®** (estradiol acetate tablet) | = | 0.45 mg, 0.9 mg, and 1.8 mg daily for VMS |
| Estradiol-alone transdermal products: | | Nominal delivery rate/indication(s): |
| **Estrasorb®** (estradiol topical emulsion) | = | 0.05 mg daily for VMS |
| **Estrogel®** (estradiol gel 0.06%) | = | 0.75 mg daily for VMS and VVA |
| **Alora®** (estradiol transdermal system) | = | 0.025 mg, 0.05 mg, 0.075 mg, or 0.1 mg twice weekly for VMS, VVA, and prevention of postmenopausal osteoporosis |
| **Climara®** (estradiol transdermal system) | = | 0.025 mg, 0.0375 mg, 0.05 mg, 0.060, 0.075 mg, or 0.1 mg for VMS, VVA, and prevention of postmenopausal osteoporosis |
| **Esclim®** (estradiol transdermal system) | = | 0.025 mg, 0.0375 mg, 0.05 mg, 0.075 mg, or 0.1 mg [illegible] for VMS and VVA |
| **Estraderm®** (estradiol transdermal system) | = | 0.05 mg or 0.10 mg twice weekly for VMS, VVA, and prevention of postmenopausal osteoporosis |
| **Vivelle®** (estradiol transdermal system) | = | 0.05 mg or 0.1 mg twice weekly for VMS, VVA, and prevention of postmenopausal osteoporosis |
| **Vivelle-Dot®** (estradiol transdermal system) | = | 0.025 mg, 0.0375 mg, 0.05 mg, 0.075 mg, or 0.1 mg twice weekly for VMS, VVA, and prevention of postmenopausal osteoporosis |
| Estradiol-alone vaginal products: | | Nominal daily delivery rate/indication(s): |
| **Estrace® Cream** 0.01% (estradiol vaginal cream) | = | 0.2 mg or 0.4 mg daily for VVA |

| | | |
|---|---|---|
| **Vagifem®** (estradiol vaginal tablet) | = | 25 mcg daily for VVA |
| **Estring®** IVR (estradiol vaginal ring) | = | [ ] mcg worn continuously for three months for VVA |
| **Femring®**(estradiol vaginal ring) | = | 0.05 mg or 0.10 mg worn continuously for three months for VMS and VVA |
| Estradiol plus Progestin Oral Products: | | Nominal daily delivery rate/indication(s): |
| **Activella®** (estradiol/norethindrone acetate tablet) | = | 1 mg plus 0.5 mg daily for VMS, VVA, and prevention of postmenopausal osteoporosis |
| **Prefest™** (estradiol/norgestimate tablet) | = | 1 mg plus 0.09 mg daily for VMS, VVA, and prevention of postmenopausal osteoporosis |
| Estradiol plus Progestin Topical Products: | | Nominal daily delivery rate/indication(s): |
| **ClimaraPro®** (estradiol/levonorgestrel transdermal system) | = | 0.045 mg plus 0.015 mg once weekly for VMS and prevention of postmenopausal osteoporosis |
| **Combipatch™** (estradiol/norethindrone acetate transdermal system) | = | 0.05 mg plus 0.14 mg or 0.25 mg[ ] [ ] for VMS, VVA[ ] [ ] |

## 2.4 Important Issues With Pharmacologically Related Products

Conjugated estrogens tablets, USP (Premarin®) for oral administration contains a mixture of conjugated estrogens obtained exclusively from natural sources, occurring as the sodium salts of water-soluble estrogen sulfates blended to represent the average composition of material derived from pregnant mares' urine. Premarin® is a mixture of sodium estrone sulfate and sodium equilin sulfate. Premarin® contains as concomitant components, as sodium sulfate conjugates, 17α-dihydroequilin, 17α-estradiol, and 17β-dihydroequilin. Premarin® tablets for oral administration are available in 0.3 mg, 0.45 mg, 0.625 mg, 0.9 mg, and 1.25 mg dosage strengths of conjugated estrogens.

After an average follow-up of 5.6 years, the conjugated estrogens (0.625 mg Premarin®) plus medroxyprogesterone acetate (2.5 mg MPA) clinical trial of the Women's Health Initiative (WHI) study was stopped early because of an increased risk of breast cancer (hazard ration [HR] of 1.24 with a 95% CI of 1.01-1.54), increased risk of stroke (HR of 1.31 with a 95% CI of 1.02-1.68), increased risk of coronary heart disease (HR of 1.24 with a 95% CI of 1.00-1.54), increased risk of probable dementia (HR of 2.05 with a 95% CI of 1.21-3.48), and a decreased risk of hip fracture (HR of 0.67 with a 95% CI of 0.47-0.96).

The risk and benefit information available in the WHI study in year 2002 prompted changes in labeling for estrogen class drug products including, but not limited to, the addition of a boxed warning to all estrogen plus progestin product labels and the expansion of the existing boxed warning in all estrogen alone product labels to include the increased risk of myocardial infarction, stroke, invasive breast cancer, pulmonary emboli, and deep vein thrombosis reported in the estrogen plus progestin WHI study. In addition, boxed warning information states that "--- in the absence of comparable data, these risks should be assumed to be similar" for "other doses of conjugated estrogens and medroxyprogesterone acetate, and other combinations and dosage forms of estrogens and progestin", and that "---estrogens with or without progestins should be prescribed at the lowest effective doses and for the shortest duration consistent with treatment goals and risks for the individual women."

After an average follow-up of 6.8 years, the conjugated estrogens alone clinical trial of the WHI study was stopped because the use of conjugated estrogens alone (0.625 mg Premarin®) increased the risk of stroke (estimated hazard ratio [HR] of 1.39 with a 95% confidence interval [CI] for conjugated estrogens versus placebo of 1.10-1.77). Other findings in the conjugated estrogens alone clinical trial included a decreased risk of hip fracture (HR of 0.61 with a 95% CI of 0.41-0.91), no effect on coronary heart disease (HR of 0.91 with a 95% CI of 0.75-1.12), a decreased risk of breast cancer (HR of 0.77 with a 95% CI of 0.59-1.01), an increased risk for probable dementia (HR of 1.49 with a 95% CI of 0.83-2.66), and no decrease in mild cognitive impairment (HR of 1.34 with a 95% CI of 0.95-1.89).

The risk and benefit information available in the estrogen alone WHI study in year 2004 prompted changes in labeling for estrogen class drug products including, but not limited to, the expansion of the boxed warning to include the reported increased risk of stroke in the estrogen alone WHI study.

Risk information available in the Women's Health Initiative Memory Study (WHIMS) in years 2003 and 2004 prompted additional changes in labeling for estrogen class drug products to include the reported increased risk of developing probable dementia in postmenopausal women 65 years of age or older. WHIMS findings for both the estrogen alone substudy and the estrogen plus progestin substudy were added to the boxed warning, and the clinical studies, warnings, and precautions sections of estrogen class labeling.

## 2.5 Presubmission Regulatory Activity

During a pre-IND teleconference on August 8, 2001, the Division of Reproductive and Urologic Products (DRUP) recommended that the Applicant consider conducting a Phase 2 dose-ranging, placebo-controlled clinical trial of four weeks duration to determine the lowest effective dose of estradiol gel for the treatment of moderate to severe vasomotor symptoms associated with the menopause.

A pre-IND meeting was held on August 15, 2001 to discuss BioSante's proposed development plan for estradiol gel. The proposed Phase 2 short-term efficacy and dose-finding Study EST004 was discussed. Agreement was reached that one robust, well-controlled, double-blind, placebo-controlled clinical trial of at least 12-weeks duration which include a dose range could be sufficient to demonstrate efficacy and safety of estradiol gel for the relief of moderate to severe vasomotor symptoms associated with menopause (Study EST005).

On April 24, 2003 an end of Phase 2 meeting was held with the Applicant. The proposed use of the 2.6 gram per day estradiol gel dose in Phase 3 Study EST005 was questioned as the lowest effective dose identified in Study EST004 (the actual dose in Study EST004 was a 2.5 gram per day dose). The Agency recommended that one or more lower doses be included in Study EST005 in order to clearly determine the lowest effective dose of estradiol gel.

In a correspondence to the Applicant dated November 12, 2003, the Agency recommended inclusion criteria based on vulvar and vaginal atrophy symptoms for entry into Study EST005, namely:

"We recommend that the following inclusion criteria be added to Study EST005:

1) The subject self-identifies at least one moderate to severe symptom of vulvar and vaginal atrophy on the Vaginal Atrophy Questionnaire that is most bothersome to her.
2) The subject has a baseline vaginal pH that is greater than 5.0.
3) The subject has $\leq$ 5% superficial cells at baseline on the vaginal cytology smear (maturation index)."

Per the Applicant, such inclusion criteria were not implemented. From information provided by the Applicant, screening for Study EST005 began on September 9, 2003 (first subject screened for inclusion). Per the Applicant, upon receipt of the Agency's November 12, 2003 letter, a decision was reached to not amend the protocol inclusion criteria "because of enrollment reasons and it was felt that at least 80% of subjects would meet the recommended inclusion criteria."

## 2.6 Other Relevant Background Information

BioSante's proposed estradiol gel is not manufactured and distributed in any country.

# 3 SIGNIFICANT FINDINGS FROM OTHER REVIEW DISCIPLINES

## 3.1 CMC (and Product Microbiology, if Applicable)

Estradiol gel is a homogeneous, transparent and non-staining hydroalcoholic gel, containing 17β-estradiol, 0.06%, in a formulation composed of an acrylic polymer (carbomer). It is supplied in a metered-dose pump for application once a day by postmenopausal women.

The formulation of estradiol gel used in primary Phase 3 Study EST005 and secondary Study EST004 and in the biopharmaceutical Studies EST007 and EST003, was the formulation of estradiol gel intended for commercialization.

*Medical Officer's Comments:*

*See the Chemistry, Manufacturing, and Controls Review of NDA 21-813/S-000 for a full discussion of CMC issues.*

## 3.2 Animal Pharmacology/Toxicology

The pharmacology, PK, and toxicology of estradiol treatment in general (including oral and topical administration) have been thoroughly characterized over many years of experience. It is recognized that long-term continuous administration of estrogens in certain animal species increases the frequency of carcinomas of the breast, uterus, cervix, vagina, testes, and liver.

Per the submission, only one *in vivo* animal study was conducted using the [ ] formulation (Study [ ]). As reported, the single-dose topical application was non-irritating in a rabbit skin irritation test. In addition, two *in vitro* skin permeation studies was conducted with this same formulation (Study [ ] on human cadavers and Study [ ] on guinea pigs). Per the Pharmacology/Toxicology Review dated August 9, 2006, it was demonstrated that "there were no significant differences between Bio-E-Gel and Oestrogel (Rontagel, marketed in Europe) regarding cumulative amounts of permeated estradiol at any time point throughout the 24 hours of study drug application using human cadaver skin. In the dose-proportionality study using guinea pig skin, the in-vitro flux rate of estradiol was shown to be dose-related." "Bio-E-Gel had no apparent toxicity in the primary skin irritation study in rabbits." "The pharmacology, pharmacokinetics, and toxicology of transdermally and orally administered estradiol have been extensively investigated previously under various submissions. Also extensive published literature is available to establish the safe use of estradiol. The safety of the excipient, [ ], is provided under [ ] DMF [ ] and has been approved under NDA 21-794 for ACZONE (5% Dapsone gel [ ]) for the topical treatment of acne vulgaris."

Per the submission, no additional animal studies were conducted or require by the FDA, and no additional studies or repetitions of existing studies involving data pertinent to human safety are planned.

*Medical Officer's Comments:*

*Per the Pharmacology/Toxicology Review of NDA 21-813/S-000, "Pharmacology recommends approval of NDA 21-813 for Bio-E-Gel for the treatment of moderate-to-severe vasomotor symptoms associated with menopause." See the Pharmacology/Toxicology Review of NDA 21-813/S-000 dated August 9, 2006 for a full discussion of in vivo animal and in vitro skin permeation findings.*

# 4 DATA SOURCES, REVIEW STRATEGY, AND DATA INTEGRITY

## 4.1 Sources of Clinical Data

Study EST005 was the primary, Phase 3, randomized, double-blind, placebo-controlled, multi-center, parallel-group study conducted to support the safety and effectiveness of estradiol gel in the treatment of moderate to severe vasomotor symptoms [ ] [ ]

Subjects were randomized in an unbalanced manner to one of the following treatment arms:

| Treatment (Grams) | Formulation (Hydroalcololic Gel) | Dose of Estradiol[a] | Nominal Daily Estradiol Dose[b] |
|---|---|---|---|
| Estradiol Gel 0.87 | 0.06% estradiol | 0.52 mg | [ ] mg/day |
| Estradiol Gel 1.7 | 0.06% estradiol | 1.02 mg | 0.0375 mg/day |
| Estradiol Gel 2.6 | 0.06% estradiol | 1.56 mg | 0.0770 mg/day[c] |
| Placebo | Matching gel | 0 mg | 0 mg/day |

a. 0.06% of estradiol gel dose.
b. Based on data from Study EST003 and Study EST007 and calculated by: CL (L/day) x $C_{ave}$ (pg/mL) x [(1000 mL/L)/$10^9$ pg/mg], where CL was clearance rate (1280 L/day), and $C_{ave}$ was baseline adjusted/corrected serum levels for estradiol gel from EST003 and EST007 (0.87 g = 9.2 pg/mL; 1.7 g = 31.9 pg/mL; 2.5 g = 49.8 pg/mL).
c. Based on nominal *in vivo* delivery rate calculated for the 2.5 gram/day dose using data from Study EST008 and rounded to increment most consistent with other marketed estradiol products.

*Medical Officer's Comments:*

*On August 11, 2006, BioSante Pharmaceuticals, Inc. was advised, "We agree that in study EST008, the appropriate baseline estradiol correction is the Subject's own baseline measurement. However, we do not agree with combining the results from study EST008 and EST003, where Bio-E-Gel was applied to the thigh area. The current data is not sufficient to*

*confirm bioequivalence between applications to the upper arm and thigh areas. Additionally, the current data suggest that bioavailability may be different when applied to the 2 sites. We recommend that only data from study EST008 be used to calculate the nominal delivery rate of Bio-E-Gel 2.6 g/day dose, which resulted in a rate of 0.077 mg/24 hours. No further response is needed on this matter."*

## 4.2 Tables of Clinical Studies

Table 1 includes the clinical trials conducted in the estradiol gel development program.

**Table 1: Estradiol Gel Clinical Trials**

| Study Phase and Number | Investigator (Country) | Study Design Duration Efficacy Endpoints | Treatment Groups | Dose of Estradiol Applied (mg)[a] | Nominal Daily Estradiol Delivery (mg)[b] | Number of Subjects[c] |
|---|---|---|---|---|---|---|
| **Placebo-Controlled Clinical Study** Adequate and Well-Controlled Study | | | | | | |
| Phase 3 EST005 | Multicenter (28 US, 2 Canada) | Randomized, double-blind, placebo-controlled, parallel-group 12-week double-blind treatment period Moderate to severe vasomotor symptoms and vulvovaginal atrophy symptoms | Placebo<br>Estradiol gel 0.87 gram<br>Estradiol gel 1.7 gram<br>Estradiol gel 2.6 gram<br>Once daily in the morning | 0<br>0.52<br>1.02<br>1.56 | 0<br>0.0125<br>0.0375<br>0.077[d] | 137 (137)<br>136 (136)<br>142 (142)<br>69 (69)<br>Total: 484 Ages: 28-75 years |
| **Placebo-Controlled Clinical Study** Controlled Supportive Study | | | | | | |
| Phase 2 EST004 | Multicenter (15 US, 2 Canada) | Randomized, double-blind, placebo-controlled, parallel-group 4-week double-blind treatment period Moderate to severe vasomotor symptoms | Placebo<br>Estradiol gel 0.625 gram<br>Estradiol gel 1.25 gram<br>Estradiol gel 2.5 gram<br>Once daily in the morning | 0<br>[redacted]<br>0.75<br>1.5 | 0<br>NA<br>0.025<br>0.060 | 42 (42)<br>42 (41)<br>41 (40)<br>42 (38)<br>Total: 167 |

| Study Phase and Number | Investigator (Country) | Study Design Duration Efficacy Endpoints | Treatment Groups | Dose of Estradiol Applied (mg)[a] | Nominal Daily Estradiol Delivery (mg)[b] | Number of Subjects[c] |
|---|---|---|---|---|---|---|
| | | | | | | Ages: 39-65 years |
| **Clinical Pharmacology Studies (Final Non-Lauryl Alcohol Formulation)** | | | | | | |
| Phase 1<br>EST 003 | Single center (Germany) | Randomized, open-label, multiple-dose, parallel-group 14-days treatment period Postmenopausal women | Estradiol gel 1.25 gram<br><br>Estradiol gel 2.5 gram | 0.75<br><br>1.5 | 0.025<br><br>0.077 | 6 (6)<br><br>6 (6)<br><br>Total: 12 (12)<br>Ages: 45-65 years |
| Phase 1<br>EST006 | Multicenter (2 US) | Open-label, single-dose, parallel-group to examine transfer potential<br>1 dose (1 day)<br>12 women dosed and 12 male partners exposed via skin-to-skin contact | Estradiol gel 2.6 gram (one application to a single arm)<br><br>Estradiol gel 2.6 gram (two applications to each upper are) | 1.56<br><br>3.12 | -<br><br>- | F: 12 (12)<br>M: 12 (12)<br><br>F: 12 (12)<br>M: 12 (12)<br><br>Total : 24 F, 24M<br>Ages: 25-67 years |
| Phase 1<br>EST007 | Single center (US) | Randomized, open-label, single and multiple dose<br>14 day treatment period<br>Postmenopausal women | Estradiol gel 0.87 gram<br><br>Estradiol gel 1.7 gram | 0.52<br><br>1.02 | 0.0125<br><br>0.0375 | 12 (12)<br><br>12 (12)<br><br>Total: 24 (24)<br>Ages: 51-70 years |
| Phase 1<br>EST008 | Single center (US) | Randomized, open-label, 2-period crossover, multiple-dose study of Estradiol gel before and after application of sunscreen<br>44-day treatment period | Estradiol gel 2.6 gram 10 minutes before sunscreen<br><br>Estradiol gel 2.6 gram 25 minutes after sunscreen | 1.56<br><br>1.56 | -<br><br>- | 6 (6)<br><br>6 (6)<br><br>Total: 12 (12) |

| Study Phase and Number | Investigator (Country) | Study Design Duration Efficacy Endpoints | Treatment Groups | Dose of Estradiol Applied (mg)[a] | Nominal Daily Estradiol Delivery (mg)[b] | Number of Subjects[c] |
|---|---|---|---|---|---|---|
| | | Postmenopausal women | | | | Ages: 49-63 years |
| | | | | | | |

Source: NDA 21-813/S-000, Section 8, Integrated Summary of Efficacy, Volume 61, page 29 of 165 and Integrated Summary of Safety, Volume 62, Table 8.8:1-2, pages 21-23.

a. 0.06% of estradiol gel dose.
b. Based on nominal in vivo delivery rate calculated using data from Study EST008 and rounded to increment most consistent with other marketed estradiol products.
c. Number of randomized subjects (number of ITT subjects). Randomization occurred at the beginning of the single-blind placebo lead-in period in Study EST004 and at the beginning of the double-blind treatment period in Study EST005.
d. In the submission, the Applicant reported the rate at 0.060 mg based on the nominal in vivo delivery rate calculated for the 2.5 gram per day dose using data from EST003. The Agency advised the Applicant on August 11, 2006 that only data from Study EST008 be used to calculates the nominal delivery rate of the 2.6 gram per day estradiol gel dose because Study EST003 applied estradiol gel to the thigh area and not to the upper arm areas as used in Study EST008 and the primary efficacy and safety study EST005. The nominal in vivo delivery rate calculated for the 2.6 gram per day estradiol gel dose using data from Study EST008 is 0.077 mg per 24 hours.

## 4.3 Review Strategy

The primary source of efficacy data submitted in support of a treatment of moderate to severe vasomotor symptoms (VMS) indication [ ] is 12-week, Phase 3 Study EST005. Study EST004, a Phase 2 dose-ranging study was conducted prior to the primary Phase 3 Study EST005. Study EST004 was only 4 weeks duration and is considered supportive of the proposed indications.

The primary source of safety data is Phase 3 Study EST005 and Phase 2 Study EST004. A total of 645 subjects are represented in these two studies (484 subjects in Study EST005 and 161 Subjects in Study EST004). Adverse event data was pooled across Studies EST005 and EST004 for the 2.5 gram per day estradiol gel dose in Study EST004 and the 2.6 gram per day estradiol gel dose in Study EST005 and for the placebo treatment groups in these two studies. Adverse event data was presented across all estradiol gel doses in both studies (0.625 gram/day and 1.25 gram/day in Study EST004 and 0.87 gram/day and 1.7 gram/day in Study EST005). Laboratory data collected at baseline and at the end of the study were not pooled due to the difference in double-blind treatment duration between Studies EST004 and EST005 (4 weeks and 12 weeks, respectively).

[ ] Phase 1 investigations were conducted in postmenopausal women in order to evaluate the PK characteristics of estradiol gel. [ ] of these [ ] PK studies used the [ ] formulation and not the [ ] formulation planned for marketing (Studies [ ] [ ] The remaining four PK studies used the [ ] formulation (Studies EST003, EST006, EST007, and EST008).

Data from the two placebo-controlled clinical studies and [ ] of the [ ] PK studies are included in the Integrated Summary of Safety (Study [ ] [ ] The Integrated Summary of Safety (ISS) summarizes data on a total of 756 subjects (645 subjects in Studies EST004 and EST005 and 111 subjects in Studies [ ] EST003, EST006, EST007, and EST008).

## 4.4 Data Quality and Integrity

Per the submission, Good Clinical Practice (GCP) audits of five sites were conducted by [ ] on behalf of BioSante Pharmaceuticals, Inc. between November 2004 and February 2005:

1. Site 02, Dr. Celine Bouchard, Clinique de Recherche en Sante des Femmes, Inc., Quebec City, Quebec, Canada.
2. Site 07, Dr, William Koltun, Medical Center for Clinical Research, San Diego, CA.
3. Site 18, Dr. Stephan Sharp, Clinical Research Associates, Inc., Nashville, TN.
4. Site 24, Dr. Douglas Young, Northern California Research Corporation, Carmichael, CA.
5. Site 28, Dr. Ronald Ackerman, Comprehensive Clinical Trials, LLC, West Palm Beach, FL.

None of these sites were discontinued as a result of these GCP audits.

The applicant was requested to provide the following information (or indicated where such information can be located in the paper submission) to assist in determining the need for a DSI audit:

- Number of subjects randomized per center.
- Number of subjects treated per center.
- Number of subjects discontinued per center.
- Number of protocol violations per center.
- Number of major protocol violations per center.

From the information received from the Applicant on April 10, 2006, the following three centers were recommended by this reviewer for DSI audits:

1. Center 24, Dr. Douglas Young
   Northern California Research Corp.
   3720 Mission Ave., Suite 18
   Carmichael, CA 95608

[38 enrolled subjects, 38 treated subjects, 3 discontinuations (8.0%), 7 major protocol violations (18.4%), and 26 protocol deviations (68.4%)]:
- Majority of protocol deviations are coded 3 "Other protocol deviations" (blood sample collected outside window, clinic visit outside protocol, inconsistent gel application, etc.)
- Seven subjects listed as protocol deviations had no code provided (Subjects 573, 677, 678, 681, 686, 687, and 689).
- Four protocol deviations are coded as 1 "Inclusion/exclusion criteria deviations" (Subjects 102, 371, 662, and 663).
- Six subjects were excluded from the evaluable subject data set (Subjects 101, 272, 274, 353, 371, and 689).

2. Center 23, Dr. Arthur Waldbaum
Downtown Women's Health Center
1860 Larimer Street, Suite 280
Denver, CO 80202

[42 enrolled subjects, 40 treated subjects, 3 discontinuations (7.5%), 2 major protocol violations (5.0%), and 33 protocol violations (82.5%)]:
- Majority of protocol deviations are coded 3 "Other protocol deviations" (blood sample collected outside window, clinic visit outside protocol, inconsistent gel application, etc.).
- Eight subjects listed as protocol deviations had no code provided (Subjects 193. 266, 376, 581, 612, 618, 664, and 665).
- Two protocol deviations are coded as 1 "Inclusion/exclusion criteria deviations" (Subjects 264 and 375).
- One protocol deviation was coded as 2 "Excluded concomitant medication deviations" (Subject 268).
- Two subjects were excluded from the evaluable subject data set (Subjects 106 and 267.

3. Center 10, Dr. Michele Moreau
Montreal Clinical Study Center, Inc.
5554 St. Zotique Street East
Montreal, Quebec
Canada H1T 1P6

[41 enrolled subjects, 40 treated subjects, 1 discontinuation (2.5%), 3 major protocol violations (7.5%), and 39 protocol violations (97.5%)]:
- Majority of protocol deviations are coded 3 "Other protocol deviations" (blood sample collected outside window, clinic visit outside protocol, inconsistent gel application, etc.)
- 2 protocol deviations coded as 1 "Inclusion/exclusion criteria deviations" (Subjects 160 and 324)
- 2 protocol deviations coded as 2 "Excluded concomitant medication deviations" (Subject 733 and 803)
- 3 subjects excluded from evaluable subject data set (Subjects 160, 163, and 803)

The Good Clinical Practice Branch I of the Division of Scientific Investigation (DSI) conducted an investigation of Center 24 (Dr. Douglas Young, Carmichael, CA) July 11-17, 2006; Center 21 (Dr. Stephen Swanson, Lincoln, NE) August 8-10, 2006, and Center 10 (Dr. Michele Moreau, Montreal, Quebec) October 23-27, 2006.

The DSI Clinical Inspection Summary, submitted to DRUP on November 6, 2006, indicates:

Center 24: There were no violations observed of FDA regulations and there was no under reporting of adverse events observed at this site. The data from this site can be used in support of NDA 21-813.

Center 21: The "Vaginal Maturation Index" data of all subjects in the study were not kept at the site after the study was completed and un-blinded. As a result, the field investigator could not verify the data of this efficacy parameter. Apart from one of the efficacy parameters (Vaginal Maturation Index) which could not be verified, and the un-certainty regarding use of "Premarin Cream" by subject # 869, the remaining data can be used in support of the NDA.

Center 10: The DSI Clinical Inspection Summary indicates, per a telephone conversation with the field inspector, that her inspection "revealed no violations" and that the recommended classification is NAI (No deviation from regulations. Data acceptable.). The field investigator's written report is pending.

*Medical Officer's Comments:*

*An addendum to this review will be prepared should any violations be reported in the written inspection report for Center 10.*

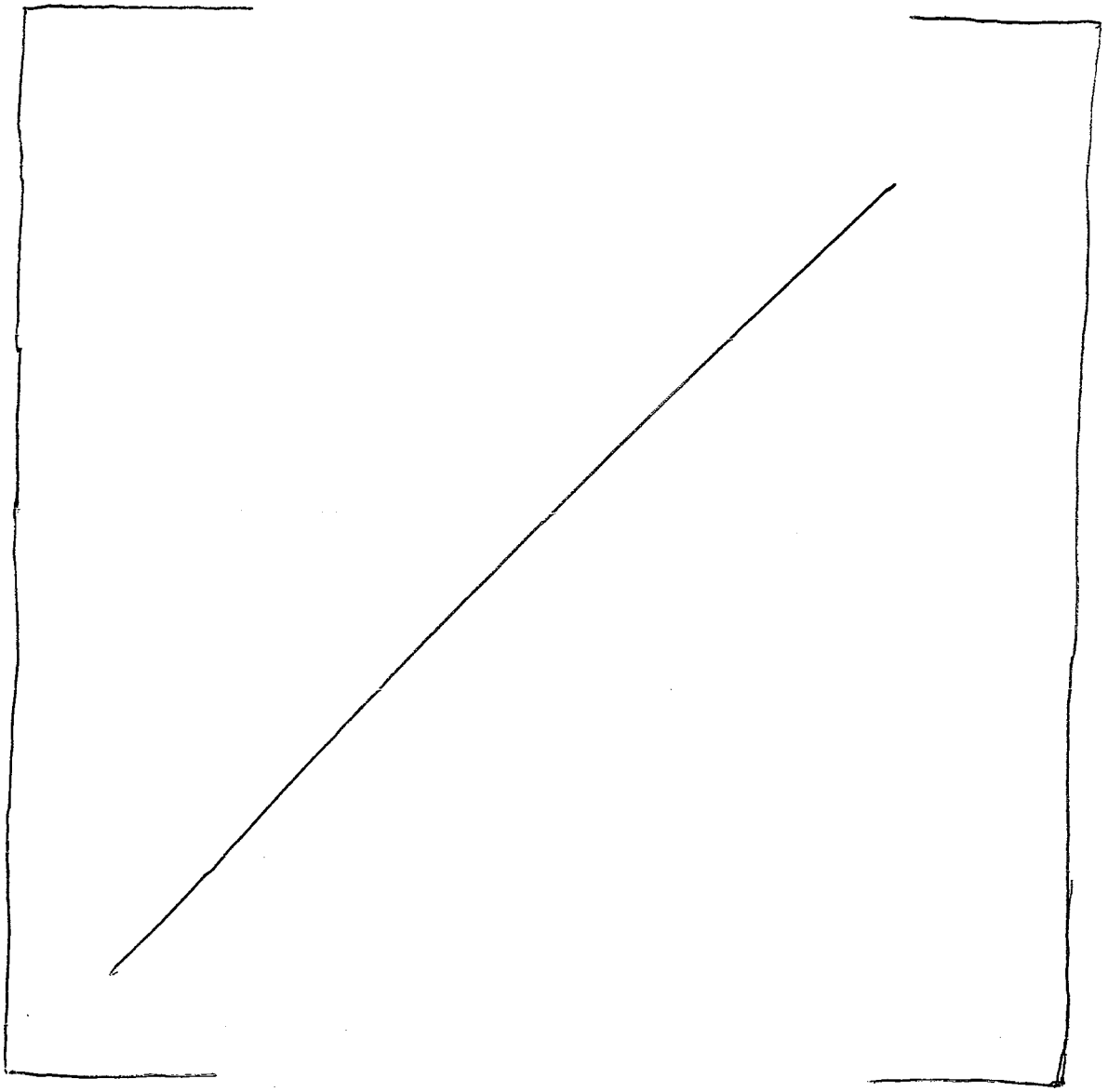
## 4.5 Compliance with Good Clinical Practices

The primary, Phase 3, efficacy and safety Study EST005 appears to have been conducted in accordance with regulations pertaining to Good Clinical Practice (GCP) (International Conference on Harmonization: Good Clinical Practice Consolidation Guidelines, Notice of Availability, *Federal Register* 25692, May 6, 1997 and the Declaration of Helsinki (revised Hong Kong, 1989).

An adequate informed consent form was signed and dated by the subject, her witness, and the investigator during screening as specified in the study protocol. The original signed informed consent form was retained in the subject's study file.

## 4.6 Financial Disclosures

[ ] investigators enrolled and completed subjects in primary, Phase 3 Study EST005:



One additional site was initiated [ ] but did not enroll any study subjects and did not receive any study medication.

Form FDA 3454 (2/03), dated January 5, 2006 and signed by Stephen M. Simes, Vice Chairman, President, and Chief Executive Officer, BioSante Pharmaceuticals, Inc. was included in the submission. [ ]

# 5 CLINICAL PHARMACOLOGY

## 5.1 Pharmacokinetics

An estradiol transdermal formulation applied once daily directly to the skin has potential advantages over oral estradiol formulations since estradiol would not be subject to the first-pass metabolism occurring after oral administration, thus a greater proportion of circulating estrogen would be available as estradiol than as its less potent metabolites. With transdermal application, the liver is not exposed to high concentrations of estrogens via the portal circulation as occurs after administration of oral estrogens, thereby potentially reducing the induction of a number of proteins in the coagulation pathway and their associated thrombotic risks. In addition, oral estrogen may increase SHBG and other binding globulins thereby reducing the availability of estrogens systemically.

The formulation of estradiol gel used in primary, Phase 3 Study EST005 and secondary Study EST004 and in the biopharmaceutical Studies EST003, EST006, EST007, and EST008 was the formulation of estradiol gel intended for commercialization.

Results from Study EST007 showed that following topical application of estradiol gel, serum levels of estradiol increased within 1-2 hours with steady state being reached after approximately 3 days of daily application. Average serum estradiol concentrations (i.e., $C_{ave}$) of 9.2 pg/mL and 31.9 pg/mL were observed after application of 0.87 gram per day estradiol gel and 1.7 gram per day estradiol gel, respectively, for two weeks. The nominal estradiol delivery rate, based on a reported clearance value of 1280 L/day and $C_{ave}$ was calculated to be 0.0125 mg per 24 hours and 0.375 mg per 24 hours, respectively.

[ ] pharmacokinetic studies were conducted with estradiol gel:

- Study EST003 was a Phase 2, open-label, multiple-dose study of skin tolerability and the pharmacokinetic (PK) profile of estradiol and its metabolites during application of the 1.25 gram daily dose of estradiol gel containing 0.75 mg of estradiol and the 2.5 gram daily dose of estradiol gel containing 1.5 mg of estradiol to the thigh area for 14 days. The 1.25 gram estradiol gel dose was applied daily to 375 $cm^2$ area of the front and inner thigh. The 2.5 gram estradiol gel dose was applied daily to 750 $cm^2$ area of the front and inner thigh.

  Six subjects at each dose (total of 12 subjects) were exposed.

  Adverse events, vital signs, and skin tolerability were monitored and recorded. No subject had observable skin irritation at the application site. No subject discontinued medication due to an adverse event (AE). No serious adverse events (SAEs) were reported.

  Study EST003 reported that mean trough estradiol serum concentrations (steady state) was reached by day 4 and day 5, respectively for the estradiol gel 1.25 gram and 2.5 gram doses.

Mean unadjusted $AUC_{0-24}$ on day 14 was 568 pg.h/mL and 1282 pg.h/mL, respectively. The 2.5 gram dose unadjusted $AUC_{0-24}$ was 2.3 times the 1.25 gram dose unadjusted $AUC_{0-24}$.

- Study EST007 was a Phase 1, open-label, single and multiple-dose PK study of the 0.87 gram dose of estradiol gel containing 0.52 mg of estradiol and the 1.7 gram dose of estradiol gel containing 1.02 mg of estradiol applied once daily to the upper arm for 14 days. Each dose was applied to a 320 $cm^2$ area of the upper arm. The same arm was used each day for the study gel application and the arm was washed each day with soap and water 15 minutes prior to application.

  Eleven (11) subjects were in the 0.87 gram per day group and 10 subjects were in 1.7 gram per day group.

  Blood samples for PK analyses ($AUC_{0-24}$, $C_{max}$, $C_{ave}$, $C_{min}$, $AUC_{inf}$, $T_{max}$, $K_{el}$, $t_{1/2}$, and AI) were drawn on days one and 14 prior to dosing (0 hour) and at 1, 2, 4, 6, 8, 12, 16, 20, and 24 hours post dose. Trough serum estradiol, estrone and estrone sulfate serum levels were measured on days 2-5, 7, 9, 11, 13 and 14 prior to gel application.

  AEs and SAEs were collected, monitored, and reported throughout the treatment period and for six days following treatment. Per the submission, no subject experienced a SAE during the study. Fourteen (14) of the 24 subjects treated experienced an adverse event. No subject had observable skin irritation at the application site. No subject discontinued medication due to an AE.

  Pharmacokinetic results of Study EST007 are shown in Table 2.

**Table 2: Estradiol Pharmacokinetic Parameters for Unadjusted Serum Concentrations (Single Dose, Day 1, and Multiple Doses, Day 14)**

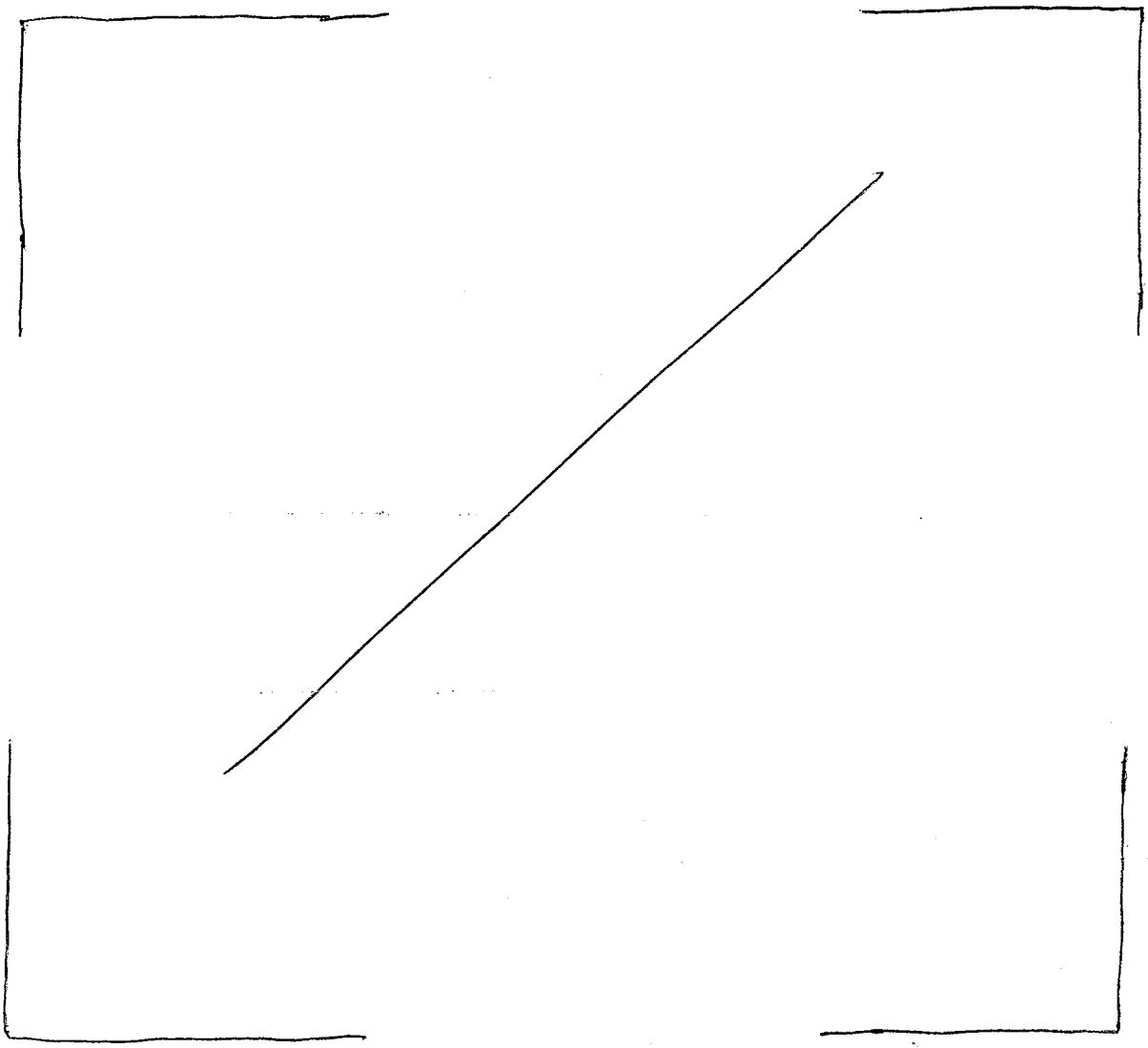
| Pharmacokinetic Parameters | Estradiol Gel 1.7 gram/day (1.02 mg/day E2) Mean ± SD[a] | Estradiol Gel 0.87 gram/day (0.52 mg/day E2) Mean ± SD[a] | Comparison of Between Group Difference P-value[b] |
|---|---|---|---|
| **Day 1 (Single Dose)** | | | |
| **$AUC_{0-24}$ (pg.h/mL)** | 421.9 ± 296.3 | 179.0 ± 113.0 | 0.54 |
| **$C_{max}$ (pg/mL)** | 31.4 ± 22.9 | 13.0 ± 6.4 | 0.47 |
| **$C_{ave}$ (pg/mL)** | 17.6 ± 12.3 | 8.2 ± 4.2 | 0.85 |
| **$T_{max}$** | 20.0 | 18.0 | -- |
| **Day 14 (Steady-state)** | | | |
| **$AUC_{0-24}$ (pg.h/mL)** | 940.0 ± 623.8 | 335.2 ± 166.0 | 0.20 |
| **$C_{max}$ (pg/mL)** | 66.7 ± 38.3 | 21.6 ± 13.7 | 0.11 |
| **$C_{ave}$ (pg/mL)** | 39.2 ± 26.0 | 15.4 ± 5.4 | 0.32 |
| **$T_{max}$** | 4.0 | 18.0 | -- |
| **Fluctuation Index[c]** | 1.16 | 0.80 | -- |
| **E2:E1 ratio[d]** | 0.98 | 0.53 | -- |
| **Accumulation Index** | 2.91 ± 1.96 | 2.06 ± 1.45 | -- |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 25, Table 1, page 53 of 87.
a. Median data for $T_{max}$ are presented; all other parameters are mean data.

b. p-values from a 2-sided t-test for between group differences. Dose-normalized values were used in statistical comparisons for dose-dependent parameters (e.g., AUC, $C_{max}$, $C_{ave}$, ss).
c. Fluctuation index was calculated as follows $C_{max}$,-$C_{min}$/$C_{ave}$, ss.
d. E2:E1 ratio was calculated based on the $C_{ave}$ values at steady state.

*Medical Officer's Comments:*

*Study EST007 reported that mean trough estradiol serum concentrations was reached by day 3 for both dose levels. Mean unadjusted $AUC_{0-24}$ on day 14 was 335 pg.h/mL and 940 pg.h/mL, respectively. Unadjusted $AUC_{0-24}$, $C_{ave}$ and $C_{max}$ were approximately 2 to 3 fold the respective values with the 0.87 gram per day dose, indicating the dose and serum estradiol concentrations to be linearly related but not dose-proportional (i.e., doubling the dose led to a 2.5 to 3.0 increase in PK parameters).*



- Study EST006 was a Phase 1, open-label, study of skin-to-skin transfer between estradiol gel treated subject and an untreated male partner. Two parallel groups were randomized. Group 1 couples engaged in five minutes of skin-to-skin contact two hours after the 2.6 gram per day estradiol gel dose was applied to the upper arm of the female subject. Group 2 couples engaged in contact eight hours after the application of estradiol gel to the upper arm. Twelve couples were assigned to group 1 and 12 couples were assigned to group 2.

  To establish baseline estradiol levels, males in both groups underwent serum estradiol sampling on day 1 prior to skin contact at 1, 2, 4, 8, and 24 hours relative to the projected skin contact.

  To determine residual estradiol on the skin, group 1 females applied an additional 2.6 gram dose of estradiol gel to the opposite arm and separate 20 $cm^2$ areas were swabbed two hours and eight hours after application. The arm was immediately washed and swabbed again and assayed for estradiol content. Cotton swabs were extracted into a methanol: water (50:50) solution.

  PK parameters for $AUC_{0-24}$, $C_{max}$, $C_{ave}$, $C_{min}$, $T_{max}$, $AUC_{inf}$, $K_{el}$, and $t_{1/2}$ were analyzed for serum estradiol concentrations in the male subjects.

  Study EST006 reported that PK parameters in the untreated males were not significantly different compared to before skin contact at 2 or 8 hours. The adjusted $AUC_{0-24}$ and $C_{ave}$ after skin contact were negative (that is a decreased exposure to estradiol compared to baseline). The mean percent of estradiol recovered from the skin at 2 and 8 hours post-application was 4.6 ± 4.0% and 7.8 ± 5.8% of the applied dose of estradiol, respectively.

  Five percent of the applied dose of estradiol was recovered from the female subject at two hours and 8% at eight hours post application. [redacted] 1% of the applied dose was recovered after washing the site.

*Medical Officer's Comments:*

*No detectable absorption of estradiol in male partners after 5 minutes of skin-to-skin contact with estradiol gel indicates that the potential for estradiol transfer is negligible. A low amount of residual estradiol (< 10% of the applied dose) was demonstrated at two and eight hours after estradiol gel application. Washing the application site with soap and water resulted in* [redacted]

*1% of the dose remaining at the application site and suggest that washing of the application site area substantially decreased the potential for transfer of estradiol gel.*

- Study EST008 was a Phase 1, randomized, open-label, 2-period crossover, multiple-dose study to determine concentrations of estradiol when estradiol gel was applied either before or after the application of sunscreen. Twelve (12) postmenopausal women applied 2.6 grams of estradiol gel daily to 320 $cm^2$ of the upper arm for 15 days. Blood draws were performed pre-dose on days 13-14 to determine trough steady-state serum hormone levels ($C_{min, ss}$ or $trough_{ss}$). Serial blood draws were taken throughout a 24 hour period on day 15 for hormone analyses ($AUC_{0-24, ss}$).

  Sunscreen ([ ] SPF 30, UVA, UVB) was applied 10 minutes before each application of estradiol gel to the same upper arm site of days 16-22 (group one, sequence one, six subjects). Serial blood draws were taken pre-dose and throughout a 24-hour period on day 22.

  Following another 15 days of once daily dose application of estradiol gel (days 23-37), serial blood draws were taken again throughout a 24 hour period on day 37. Subsequently, these subjects applied sunscreen 25 minutes after the application of estradiol gel for the final seven days (days 38-44). On the day of final application of estradiol gel and sunscreen (day 44), serial blood draws were taken pre-dose and throughout a 24 hour period.

  The second group of six subjects (sequence two) received the therapies, but received the sunscreen application in the opposite sequence: sunscreen application 25 minutes after the estradiol gel application on days 16-22 and sunscreen application 10 minutes before estradiol gel application for days 38-44.

  The PK parameters of $AUC_{0-24}$, $C_{max}$, and $C_{ave}$ were calculated for steady-state, serum estradiol, estrone and estrone sulfate concentrations during applications of estradiol gel alone and in combination with sunscreen.

  Adverse events were assessed prior to each drug application and for six days following the last application.

  Study EST008 reported that when sunscreen was applied 10 minutes before applying estradiol gel for seven days $C_{ave}$ and $AUC_{0-24}$ for estradiol, estrone, and estrone sulfate increased by 55%, 34%, and 36%, respectively compared when applied alone.

**Table 3: Estradiol PK Parameters and Mean Ratios (%) for All Subjects After Estradiol Gel (A) was Administered Daily for 15 Days, Followed by Sunscreen Administered 10 Minutes Prior to Estradiol Gel (B) Daily for 7 Days**

| PK parameter | Descriptive Statistics | A | B | Individual Subject B/A Ratios[a] (%) |
|---|---|---|---|---|
| $C_{ave}$ (pg/mL) | N | 11 | 11 | 11 |
| | Mean | 100.8 | 159.8 | 154.6 |
| | SD | 61.4 | 108.8 | 34.5 |
| | CV% | 60.9 | 68.1 | 22.3 |
| | GeoMean | 89.0 | 134.0 | 150.5 |
| $AUC_{0-24}$ (pg.mL) | N | 11 | 11 | 11 |
| | Mean | 2419.9 | 3835.3 | 154.6 |
| | SD | 1474.4 | 2611.4 | 34.5 |
| | CV% | 60.9 | 68.1 | 22.3 |
| | GeoMean | 2137.1 | 3216.7 | 150.5 |
| $C_{max}$ (pg/mL) | N | 11 | 11 | 11 |
| | Mean | 224.8 | 332.7 | 156.7 |
| | SD | 163.7 | 219.7 | 41.4 |
| | CV% | 72.8 | 66.0 | 26.4 |
| | GeoMean | 181.0 | 275.2 | 152.0 |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 25, Table 1, page 76 of 87.
Period A = Steady-state period when estradiol gel was administered alone.
Period B = Steady-state period when sunscreen was applied 10 minutes prior to estradiol gel.
a. Ratio (B/A) was calculated for each subject as the parameter value under B divided by the parameter value under A. The descriptive statistics summarize these ratios across all subjects.

When the sunscreen was applied 25 minutes after estradiol gel application for seven days, no significant change in $C_{ave}$ and $AUC_{0-24}$ for estradiol or its metabolites were observed.

**Table 4: Estradiol PK Parameters and Mean Ratios (%) for All Subjects After Estradiol Gel (C) was Administered Daily for 15 Days, Followed by Sunscreen Administered 25 Minutes After Estradiol Gel (D) Daily for 7 Days**

| PK parameter | Descriptive Statistics | C | D | Individual Subject D/C Ratios[a] (%) |
|---|---|---|---|---|
| $C_{ave}$ (pg/mL) | N | 11 | 11 | 11 |
| | Mean | 126.8 | 98.4 | 102.9 |
| | SD | 110.3 | 37.8 | 43.5 |
| | CV% | 87.0 | 38.4 | 42.3 |
| | GeoMean | 95.5 | 88.8 | 93.0 |
| $AUC_{0-24}$ (pg.mL) | N | 11 | 11 | 11 |
| | Mean | 3043.1 | 2361.2 | 102.9 |
| | SD | 2646.5 | 906.3 | 43.5 |
| | CV% | 87.0 | 38.4 | 42.3 |
| | GeoMean | 2291.1 | 2130.5 | 93.0 |
| $C_{max}$ (pg/mL) | N | 11 | 11 | 11 |
| | Mean | 325.9 | 228.0 | 108.3 |
| | SD | 323.8 | 111.5 | 61.0 |
| | CV% | 99.4 | 48.9 | 56.4 |
| | GeoMean | 212.1 | 187.6 | 88.4 |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 25, Table 1, page 79 of 87.
Period C = Steady-state period when estradiol gel was administered alone.
Period D = Steady-state period when sunscreen was applied 25 minutes after estradiol gel.

a. Ratio (D/C) was calculated for each subject as the parameter value under D divided by the parameter value under C. The descriptive statistics summarize these ratios across all subjects.

In the Agency's 74-day letter to the Applicant dated April 27, 2006, Clinical Pharmacology requested responses to the following questions:

1. "Regarding Study EST008, we are concerned with the increased estradiol exposure in the group where Bio-E-Gel was applied after sunscreen (mean increase of 55% with individual increase as high as /% relative to Bio-E-Gel alone) and the increased estradiol exposure in all groups in the second crossover period (mean increase of [ ] fold relative to the first period).

   - [ ]

   - Provide rationale for the higher exposure to estradiol in the second crossover period (i.e., days 37 and 44) as compared to the first period (i.e., days 15 and 22) in study EST008. Specifically, address whether this was related to the application of sunscreen on days 16-22 or other factors that may be responsible for this observation (e.g., change in SHBG and estradiol binding)."

2. "The nominal delivery rate estimate for the 2.6 gram dose appears to be low. For calculations of the nominal delivery rate for the 2.6 gram dose, you used data from Study EST003, where 2.5 grams of gel was applied to the front and inner thigh area, to estimate a nominal delivery rate of 0.064 mg/day. The mean unadjusted average estradiol concentration ($C_{avg}$) in this study was 52.4 pg/ml. We noted that in Study EST008, where 2.6 grams was applied to the upper arm (i.e., same dose and application site as in the proposed labeling), the mean unadjusted $C_{avg}$ for estradiol on day 15 were 74 and 75 pg/ml for group 1 and 2, respectively. Considering baseline mean estradiol levels of 4-8.1 pg/ml in your studies EST007 and EST003 and applying the same equation that you used, the estimate nominal delivery rate would be approximately in the range of 0.084 to 0.091 mg/day. [ ]
   [ ]

In a response dated June 13, 2006, the Applicant noted that the effect of sunscreen on estradiol gel was similar to that of a currently approved and marketed topical product for the treatment of vasomotor symptoms. [ ]
[ ]

*Medical Officer's Comments:*

*Sunscreen [ ] application ten minutes before the application of estradiol gel was found to increase estradiol absorption by 55%. Per the reported results of Study EST008, no significant changes in estradiol were observed when sunscreen was applied 25 minutes after estradiol gel.*

*This significant increase in absorption when sunscreen is applied ten minutes before the application of estradiol gel is concerning for climates and lifestyles that foster the frequent application of sunscreen. The Applicant proposes to address these reported results in labeling by recommending that estradiol gel be applied at least 25 minutes after the application of sunscreen. This recommendation is acceptable to this reviewer.*

Regarding the higher exposure to estradiol in the second crossover period in Study EST008, the Applicant noted several possible explanations:

1. "Comparison over short periods such as days 15 and 22 or days 37 and 45 are much more reasonable to make than is a comparison of days 15 and 37 in which small, unnoticeable, incremental changes may go unrecognized that may impact baseline assessments.
2. In Study EST008, attempts were made to control dosing variability. Study drug was first dispensed onto a scale with a 3-4 inch square of aluminum foil or some similar product. The study drug was then transferred to the coordinators gloved hand, then applied to the subject's skin and rubbed in with the gloved fingers of the coordinator.
3. There are two AUC calculations for individual subjects that appear to drive much of the apparent increases in AUC between the first half and second half of Study EST008. On day 37 Subject 103 and on day 44 Subject 206 had much higher AUCs than other subjects, both > 10,000 pg.hr/mL.
4. SHBG was not measured in Study EST008. However, in Study EST003, the mean SHBG increased from baseline of approximately 73.3 to 82.8 nmol/L at day 15. In Study EST005, SHBG increased in the 2.6 gram/day group at baseline to approximately 105 nmol/L at day 28 and to 110 nmol/L at day 56. It could be inferred from SHBG measurement in Studies EST003 and EST005 that one might expect the SHBG to increase approximately 15% between day 15 and day 37 in Study EST008. This change in SHBG might account for a percentage of the apparent increase in estradiol exposure at day 37 over that of day 15 in Study EST008, both at baseline and for the AUC.
5. If there was a depot effect of enhancer or estradiol in the arm of subjects using estradiol gel, as suggested, the Phase 3 clinical trial Study EST005 would show a gradual rise in serum estradiol, estrone or estrone sulfate over time. Per the Applicant, for all three doses of Bio-E-Gel in Study EST005 the mean serum estradiol, estrone and estrone sulfate trough measures are similar at the respective 4, 8, and 12 week measures, suggesting a steady state delivery of estradiol over time."

BioSante Pharmaceuticals believes "that the primary reason for the apparent discordance in the two halves of the EST008 study in baseline values and AUCs is technical and is confounded by

period effects and by small increases in SHBG that may have occurred in subjects between the times of these assessments."

*Medical Officer's Comments:*

*The Agency's Clinical Pharmacology Reviewer agrees with the Applicant that sex hormone binding globulin (SHBG) can account for ≤ 15% of the observed 110% increase in estradiol $AUC_{0-24}$ on day 37. However, it remains unclear why there is a [ ]-fold increase in estradiol $AUC_{0-24}$ on day 37 relative to day 15 in Study EST008. These findings will be reflected in labeling.*

*In response to question number 2, the Applicant agreed that the mean unadjusted $C_{ave}$ is somewhat higher in the EST008 study than the EST003 study. However, the Applicant believed that the appropriate baseline measurement for Study EST008 would be the subject's own baseline measurement. The baseline values for Study EST008 prior to starting study medication ranged from <10 pg/mL to 18 pg/mL.*

*The Applicant recalculated the adjusted $C_{ave}$ in Study EST008, and then performed the additional operation of dose-normalizing the 2.5 gram per day dose to the 2.6 gram per day dose for the Study EST003 data and recalculated the mean adjusted $C_{ave}$ for each subject. The Applicant combined the dose-normalized $C_{ave}$ data from both studies and determined nominal delivery rates that ranged between 66 and 77 mcg per day with an average nominal delivery of approximately 72.5 mcg per day.*

*On August 11, 2006, BioSante Pharmaceuticals, Inc. was advised in a letter from the Agency, "We agree that in study EST008, the appropriate baseline estradiol correction is the Subject's own baseline measurement. However, we do not agree with combining the results from study EST008 and EST003, where Bio-E-Gel was applied to the thigh area. The current data is not sufficient to confirm bioequivalence between applications to the upper arm and thigh areas. Additionally, the current data suggest that bioavailability may be different when applied to the 2 sites. We recommend that only data from study EST008 be used to calculate the nominal delivery rate of Bio-E-Gel 2.6 g/day dose, which resulted in a rate of 0.077 mg/24 hours. No further response is needed on this matter."*

*See the Clinical Pharmacology and Biopharmaceutics Review of NDA 21-813/S-[ ] for a full discussion of PK issues.*

## 5.2 Pharmacodynamics

No pharmacodynamic studies related to efficacy were conducted with estradiol gel.

### 5.3 Exposure-Response Relationships

An increased incidence of shifts from baseline TVU measurements ≤ 4 mm to > 4 mm at the end-of-study visit in Study EST005, and normal to abnormal endometrial biopsies was observed with the 1.7 gram per day and the 2.6 gram per day estradiol gel treatment groups in 12-week Study EST005. These findings are further discussed in Section 7.1.3 Dropouts and Other Significant Adverse Events of this review.

## 6 INTEGRATED REVIEW OF EFFICACY

### 6.1 Indication

NDA 21-813/S-000 is seeking approval of [ ]estradiol gel dosage strengths (0.87 gram per day, 1.7 gram per day[ ]) for[ ] indications:

1. Treatment of moderate to severe vasomotor symptoms associated with the menopause.

[ ]

#### 6.1.1 Methods

The clinical program to evaluate the efficacy and safety of estradiol gel included a single randomized, double-blind, placebo-controlled, Phase 3 Study EST005 and one supportive, randomized, double-blind, placebo-controlled Phase 2 dose-ranging Study EST004.

Primary, Phase 3 Study EST005 will be discussed further in the review. Secondary, Phase 2 Supportive Study EST004 was a four-week, randomized, double-blind, placebo-controlled study initiated to provide dose ranging information. In Study EST004, 167 subjects were screened and 161 subjects were subsequently enrolled to receive study drug. The 161 treated subjects in Study EST004 are included in the Integrated Summary of Safety (ISS) but are not included in any efficacy analyses due to the limited duration of treatment (four weeks) and the difference in dosage strengths (0.625 gram per day, 1.25 gram per day and 2.5 gram per day).

#### 6.1.2 General Discussion of Endpoints

Variability in estrogen levels at menopause leads to an array of potentially bothersome symptoms. These symptoms are related to the vascular system (vasomotor symptoms, such as hot flushes and night sweats), the genitourinary tract (symptoms discussed below), and other body symptoms (systemic symptoms, such as fatigue).

Vasomotor symptoms in postmenopausal women are commonly known as hot flushes or hot flashes. The cause of the hot flush is unknown but believed to occur due to induced lability in the thermoregulatory center of the hypothalamus with declining levels of estrogen and progesterone resulting in peripheral vasodilation. "Hot flush" is descriptive of a sudden onset of reddening of the skin over the head, neck, and chest, accompanied by a feeling of intense body heat and concluded by sometimes profuse perspiration.

The severity of vasomotor symptoms is defined clinically as follows:

| | |
|---|---|
| Mild: | Sensation of heat without sweating. |
| Moderate: | Sensation of heat with sweating, able to continue activity. |
| Severe: | Sensation of heat with sweating, causing cessation of activity. |

Per the Agency's 2003 draft clinical evaluation guidance document, the Agency recommends that one or more 12-week, randomized, double-blind, placebo-controlled clinical trials be conducted that:

1) have appropriate inclusion and exclusion criteria;
2) conduct appropriate study analyses; and
3) evaluate the following four co-primary endpoints:

- Mean change in frequency of moderate to severe vasomotor symptoms from baseline to week 4.
- Mean change in frequency of moderate to severe vasomotor symptoms from baseline to week 12.

  For study inclusion, study participants should have a minimum of 7 to 8 moderate to severe hot flushes per day at baseline, or 50 to 60 moderate to severe hot flushes per week at baseline.

  The primary efficacy analysis should show a statistically significant reduction in frequency, within 4 weeks of initiation of treatment and maintained throughout 12 weeks of treatment in the treated group compared to the placebo group.

  The primary efficacy analysis should also show a clinically significant reduction in frequency identified as a reduction of at least two moderate to severe hot flushes above placebo at week 4 and week 12.

- Mean change in severity of moderate to severe vasomotor symptoms from baseline to week 4.

- Mean change in severity of moderate to severe vasomotor symptoms from baseline to week 12.

For study inclusion, study participants should have a minimum of 7 to 8 moderate to severe hot flushes per day at baseline, or 50 to 60 moderate to severe hot flushes per week at baseline.

The primary efficacy analysis should show a statistically significant reduction in hot flush severity, within 4 weeks of initiation of treatment and maintained throughout 12 weeks of treatment in the treated group compared to the placebo group.

The Agency recommends that hot flush severity be "scored" accordingly: mild x 1, moderate x 2, and severe x 3.

The Agency's 2003 draft clinical evaluation guidance document recommends three co-primary endpoints for the treatment of moderate to severe symptoms of vulvar and vaginal atrophy to address the resulting estrogen deprived changes in the genitourinary tract. In the vulvar area and vagina, the vaginal epithelium becomes dry and atrophic, which causes inflammation, discomfort, itching, and dyspareunia. A lateral wall vaginal cytology smear (allowing the cytological examination of vaginal mucosa epithelial cells) demonstrates an increased proportion of parabasal vaginal epithelial cells and a decreased proportion of superficial vaginal epithelial cells. Vaginal pH increases from the normal 3.5 to 4.0 (a pH which favors lactobacilli) to 6.0 to 8.0 (a pH which favors pathogenic organisms).

Per the Agency's 2003 draft clinical evaluation guidance document, the Agency recommends that one or more 12-week, randomized, double-blind, placebo-controlled clinical trials be conducted that:

1) have appropriate inclusion and exclusion criteria;
2) conduct appropriate study analyses; and
3) evaluate the following three co-primary endpoints:

- The mean change from baseline to week 12 in the vaginal maturation index (superficial and parabasal cells). For study inclusion, study participants would have no greater than 5 percent superficial cells on a vaginal smear at baseline. The primary efficacy analysis should show a statistically significant increase in superficial cells and a statistically significant decrease in parabasal cells.

- The mean change from baseline to week 12 in vaginal pH. For study inclusion, study participants should have a vaginal pH > 5.0 at baseline. The primary efficacy analysis should show a statistically significant lowering of vaginal pH.

- The mean change from baseline to week 12 in the moderate to severe self-assessed symptom identified by the subject as being the most bothersome to her. For study inclusion, study participants would have self-identified at least one moderate to severe vulvar and vaginal atrophy symptom. The primary efficacy analysis should show statistically significant improvement in the moderate to severe symptom identified by the

subject as most bothersome. The recommended subject self-assessed symptoms of vulvar and vaginal atrophy include:

1. Vaginal dryness (categorized as none, mild, moderate or severe).
2. Vaginal and/or vulvar irritation/itching (categorized as none, mild, moderate or severe).
3. Dysuria (categorized as none, mild, moderate or severe).
4. Vaginal pain associated with sexual activity (categorized as none, mild, moderate or severe).
5. Vaginal bleeding associated with sexual activity (categorized as none, mild, moderate or severe).

### 6.1.3 Study Design

Postmenopausal women who had experienced ≥ 60 moderate to severe hot flushes per week at baseline participated in Study EST005. Per the submission, study subjects were not required to meet minimum criteria for vulvar and vaginal atrophy symptoms for entry, but the efficacy analyses for each of the three variables (most bothersome symptom, vaginal pH, and vaginal maturation index) took into account subjects who met the minimum criterion for the respective variable at baseline (i.e., self-identified at least one moderate to severe symptom most bothersome to her, ≤ 5% superficial cells on a vaginal smear, and vaginal pH > 5.0).

In Study EST005, 503 subjects underwent screening procedures and 484 were randomized. The sample size was calculated to detect differences between treatment groups of ≥ 2 hot flushes in the mean change from baseline to weeks 4 and 12. For three study groups in Study EST005 (0.87 grams per day estradiol gel corresponding to an application of 0.52 mg of estradiol per day, 1.7 grams per day estradiol gel corresponding to an application of 1.02 mg of estradiol per day, and placebo), the planned sample size for inclusion in the primary efficacy analysis was 127 subjects per treatment group, based on an estimated standard deviation of change from baseline of 5.0 hot flushes per day and an 80% power to detect differences at the 0.5 level of statistical significance.

For the fourth study group in Study EST005 (2.6 grams per day estradiol gel corresponding to an application of 1.56 mg of estradiol per day), it was expected that the difference between the treatment group and placebo in mean change in daily hot flush rate from baseline to week 12 would be at least 3.0 hot flushes per day. Accordingly, a sample size of 50 subjects in this treatment group was determined to give approximately 80% power to detect a difference from placebo (Amendment 7 for Study EST005).

The original protocol for Study EST005 included only two doses of estradiol gel (1.7 gram per day dose and 2.6 grams per day dose). BioSante amended the protocol (Amendment 7; per the Applicant, in response to the published WHI findings) to include a lower dose of estradiol gel (0.87 gram per day dose) and to reduce the number of subjects randomized to receive the 2.6 gram per day dose to 50 subjects.

Study EST005 was conducted in 32 sites. A total of 30 (28 U.S. and two Canadian) sites randomized subjects into the double-blind treatment period and two U.S. sites entered subjects only into the single-blind placebo lead-in period.

BioSante Pharmaceuticals, Inc., 111 Barclay Boulevard, Suite 280, Lincolnshire, IL 60069 is the applicant for NDA 21-813/S-000.

Drug supply manufacturer was DPT Laboratories, San Antonio, TX, and the drug supply distributor was [ ]

The clinical laboratory evaluations and hormone assays were performed by [ ] [ ] Vaginal maturation indexes, endometrial biopsy samples and Pap smear evaluations were performed by the [ ]

Per the submission, the primary objectives of this study were to evaluate the safety and efficacy of 2.6 gram per day estradiol gel containing 1.56 mg of estradiol, 1.7 gram per day estradiol gel containing 1.02 mg of estradiol, and 0.87 gram per day estradiol gel containing 0.52 mg of estradiol administered as a daily regimen, as compared to that of placebo gel, in the treatment of moderate to severe vasomotor symptoms and moderate to severe symptoms of vulvar and vaginal atrophy associated with the menopause.

In Study EST005, subjects were randomized in an unbalanced manner to one of the following treatment groups:

- Estradiol gel 0.87 gram (0.52 mg of estradiol) providing a nominal daily estradiol delivery rate of 0.015 mg per day.
- Estradiol gel 1.7 gram (1.02 mg of estradiol) providing a nominal daily estradiol deliver rate of 0.0375 mg per day.
- Estradiol gel 2.6 gram (1.56 mg of estradiol) providing a nominal daily estradiol delivery rate of 0.077 mg per day.
- Placebo

The formulation used in Study EST005 was 0.06% estradiol in a hydroalcoholic gel. The dose of estradiol applied was determined from data obtained in Studies EST003 and EST007 and was calculated by the following:

- CL (L/day) x $C_{ave}$ (pg/mL) x [(1000 mL/L)/$10^9$ pg/mg], where CL was clearance rate (1280 L/day), and $C_{ave}$ was baseline adjusted/corrected serum levels for estradiol gel from EST003 and EST007 (0.87 g = 9.2 pg/mL; 1.7 g = 31.9 pg/mL; 2.5 g = 49.8 pg/mL).

Inclusion Criteria:

Any subject who met the following criteria, as determined from assessments completed during screening and a placebo run-in period, was eligible for study participation:

1. Female and at least 18 years of age.
2. In good health as determined by the investigator on the basis of medical history, physical and gynecologic examinations, screening mammography, and clinical laboratory tests.
3. Undergone natural menopause, defined as at least 12 months amenorrhea prior to the first screening visit or surgical menopause (bilateral oophorectomy with or without hysterectomy) occurring at least six months prior to the first screening visit. Subjects who had undergone a hysterectomy without bilateral oophorectomy were to be $\geq$ 52 years old.
4. Estradiol serum concentration $\leq$ 2.0 ng/dL and follicle stimulating hormone (FSH) serum concentration > 40 mIU/mL.
5. Experienced $\geq$ 60 moderate to severe hot flushes each week during the first two weeks (14 days) of the screening period.
6. No pathological findings on the screening Pap smear (required on all subjects with a cervix) performed within nine months prior to or at screening visit one (day -21). Acceptable findings were: normal results, atypical squamous cells of undetermined significance (ASCUS), or atypia without dysplasia.
7. No evidence of endometrial hyperplasia or carcinoma, as evidenced by the screening endometrial biopsy. If the screening biopsy specimen result indicated insufficient endometrial tissue for diagnosis, a transvaginal ultrasound (TVUS) was to be performed prior to or at visit two (day -7), and the results must have indicated (prior to placebo administration) an endometrial double-wall thickness of $\leq$ 4 mm.
8. Mammography performed within nine months prior to or at screening indicated no questionable findings, including pre-cancerous or cancerous findings.
9. Clinical breast exam at visit one had no suspicion of breast malignancy.
10. Thyroid stimulating hormone (TSH) levels during screening were within the normal reference range for the central laboratory.
11. Free of any physical signs and symptoms of vaginal and/or urinary tract infection at visit one.
12. BMI was $\geq$ 18 and $\leq$ 35 kg/m$^2$ at the first screening visit.
13. Agreement not to use any medications on the "excluded medication" list including herbal or soy products and vitamins on that list, during the course of the study. If the subject regularly used a selective serotonin reuptake inhibitor (SSRI), she was to be on stable dose and frequency for at least six months prior to the first screening visit, and the SSRI must not have been prescribed for the treatment of hot flushes.
14. Cooperative, anticipated availability for the entire study, willing to complete a daily diary and apply study gel on a daily basis, for the duration of the study.
15. Signed both the abbreviated and full versions of the written informed consent agreement forms.

Exclusion Criteria:

1. History of any estrogen-dependent neoplasia (e.g., breast cancer, endometrial cancer); malignant melanoma; or other malignancies unless treated with no evidence of recurrence within the last five years. Treated basal cell carcinoma was not exclusionary.
2. Undiagnosed vaginal/uterine bleeding within 12 months prior to the first screening visit.
3. History of allergic reaction to estradiol therapy.

4. History of reactions to transdermally administered medications.
5. Any systemic skin disease or local skin abnormalities in the area of study drug application.
6. Elevated sitting blood pressure (systolic > 144 mm Hg and/or diastolic > 94 mm Hg) on two readings taken at least five minutes apart at visit one.
7. Serious cardiac disease.
8. Active hepatic or gallbladder disease (unless surgically corrected) with six months prior to study drug administration.
9. Serious renal disease (serum creatinine > 1.5 times the upper limit of normal).
10. Serious hepatic disease (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] > two times the upper limit of normal.
11. History of atherosclerotic vascular disease, thrombotic disorders, or angina.
12. The estradiol serum concentration of blood drawn at visit two (day -7) was > 2.0 ng/dL.
13. History of alcohol or substance abuse within six months prior to the first screening visit.
14. Vitamins, herbal or soy therapies on the "excluded medication" list taken within two weeks prior to the first screening visit.
15. Vaginal non-steroidal products used within two weeks prior to visit one.
16. Use of any of the excluded prescribed medications.
17. Vaginal (excluding Femring) or transdermal steroid hormone therapy (estrogen, progestin, androgens, gonadotropins, gonadotropin releasing factors, or corticosteroids) used within four weeks prior to the first screening visit.
18. Oral or intrauterine steroid hormone therapy (estrogen, progestin, androgens, gonadotropins, gonadotropin releasing factors, or corticosteroids), or Femring® received within eight weeks prior to the first screening visit.
19. Injectable or implanted hormones (estrogen, progestin, androgens, gonadotropins, gonadotropin releasing factors, or corticosteroids) received within six months prior to the first screening visit.
20. Endocrine disease (including diabetes), with the exception of medication-controlled thyroid disease as evidenced by a normal TSH at screening.
21. Any investigational medication received within 30 days prior to the first screening visit, or any investigational drug other than Estradiol gel scheduled to be received during the course of the study.
22. Previous participation in a clinical trial for the treatment of postmenopausal vasomotor symptoms.
23. According to subject history, the subject would experience no improvement in frequency or severity of hot flushes with estrogen therapy.
24. Unable to provide informed consent, not available for close follow-up, or is unwilling to maintain a record of hot flushes throughout the study.
25. Any condition that the investigator thought would make the subject unsuitable for participation in the trial, including dementia or evidence of mental incapacity that precluded compliance with the protocol.

A subject was withdrawn from the study if she developed symptoms that required medical intervention or drug discontinuation, developed an intercurrent illness that would require a medication prohibited or would interfere with the subject's continued participation. Each subject had the right to withdraw from the study at any time without prejudice.

If, for any reason, a subject who received treatment was withdrawn before completing the study, all end-of-study procedures were performed at the time of withdrawal.

Following the screening period, all eligible subjects entered a one-week, single-blind, placebo gel lead-in period (days -7 to -1).

Study drug was provided in metered-dose pump bottles that delivered 0.87 gram of estradiol gel or matching placebo per pump actuation. In order to maintain the study blind, study drug was provided in two pump bottles: Bottle A and Bottle B.

Using the randomization schedule, subjects received one of the following three treatments according to a 1:1:1 randomization scheme until approximately 50 subjects per treatment group were enrolled:

- Estradiol gel 1.7 gram per day (1.02 mg of estradiol)
- Estradiol gel 2.6 gram per day (1.56 mg of estradiol)
- Placebo gel

Once approximately 50 subjects were enrolled in the 2.6 gram per day estradiol gel treatment group, eligible subjects then received one of the following three treatments according to a 4:2:2 randomization scheme until approximately 127 subjects per treatment group were enrolled:

- Estradiol gel 0.87 gram per day (0.52 mg of estradiol)
- Estradiol gel 1.7 gram per day (1.02 mg of estradiol)
- Placebo gel

The daily dose of randomized study drug was applied topically by the subject at the same time each morning during the study to facilitate trough level blood draws.

The gel from Bottle A was applied to the left upper arm/shoulder area. The gel from Bottle B was applied to the right upper arm/shoulder area. Daily bathing was to be done with soap and water prior to gel application. Subjects were instructed not to wash the application sites for at least six hours (and preferably not until the next morning). Subjects were instructed to allow the gel to dry three to five minutes before covering the application sites with clothing or before coming into contact with another person. Subjects were also cautioned against applying lotions, ointments, gels, sunscreen or other skin care products to the skin areas used for gel application during the study and to wash their hands after gel application. Subjects were instructed not to apply the gel to the breast or intravaginally.

Study drug dosing was as follows:

| **Treatment** | **Bottle A** | **Bottle B** |
|---|---|---|
| Before adding the 0.87 gram per day estradiol gel treatment group: | | |

| | | | |
|---|---|---|---|
| Placebo | 3 pumps | 2 pumps | Both bottles placebo |
| 1.7 gram per day | 3 pumps | 2 pumps | Bottle B active drug |
| 2.6 gram per day | 3 pumps | 2 pumps | Bottle A active drug |

After adding 0.87 gram per day estradiol gel treatment group:

| | | | |
|---|---|---|---|
| Placebo | 1 pump | 2 pumps | Both bottles placebo |
| 1.7 gram per day | 1 pump | 2 pumps | Bottle B active drug |
| 0.87 gram per day | 1 pump | 2 pumps | Bottle A active drug |

BioSante Pharmaceuticals, Inc. supplied study drug in quantities sufficient to satisfy the protocol requirements:

- Estradiol gel (0.06% estradiol in a hydroalcoholic gel formation), Batch TFE.
- Placebo gel, Batch TEFC.

Each bottle of study drug was primed and weighed before being dispensed. Bottles were re-weighed at the next clinic visit and the results were recorded on the study drug CRF.

### 6.1.4 Efficacy Findings

For the treatment of moderate to severe vasomotor symptoms associated with the menopause, the Agency's January 2003 draft Guidance for Industry entitled, "Estrogen and Estrogen/Progestin Drug Products to Treat Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms – Recommendations for Clinical Evaluation" recommends that one or more 12-week, randomized, double-blind, placebo-controlled clinical trial be conducted that evaluate the following four co-primary endpoints:

- Mean change in frequency of moderate to severe vasomotor symptoms from baseline to week 4. The primary analysis should show a statistically and clinically significant reduction in the frequency of moderate to severe hot flushes. A clinically significant reduction is defined as at least two more than placebo per day or at least 14 more than placebo per week.
- Mean change in frequency of moderate to severe vasomotor symptoms from baseline to week 12. The primary analysis should show a statistically and clinically significant reduction in the frequency of moderate to severe hot flushes. A clinically significant reduction is defined as at least two more than placebo per day or at least 14 more than placebo per week.
- Mean change in severity of moderate to severe vasomotor symptoms from baseline to week 4.
- Mean change in severity of moderate to severe vasomotor symptoms from baseline to week 12.

Per the Agency's draft guidance document, the severity of vasomotor symptoms is defined clinically as follows:

- Mild: Sensation of heat without sweating.
- Moderate: Sensation of heat with sweating, able to continue activity.
- Severe: Sensation of heat with sweating, causing the cessation of activity.

In primary Phase 3 Study EST005, subjects graded the severity of their hot flushes according to the following classifications:

- Mild: Severity score of 1; sensation of heat without perspiration.
- Moderate: Severity score of 2; sensation of heat with perspiration, able to continue activity.
- Severe: Severity score of 3; sensation of heat with perspiration, causing the subject to stop an activity until the event passed. If the sensation occurred when the subject was asleep, the subject was awakened and resumed sleep with difficulty.

For the treatment of moderate to severe symptoms of vulvar and vaginal atrophy associated with the menopause, the Agency's January 2003 draft Guidance for Industry recommends that one or more 12-week, randomized, double-blind, placebo-controlled clinical trial be conducted that evaluate the following three co-primary endpoints:

- The mean change from baseline to week 12 in the vaginal maturation index (proportions of superficial and parabasal cells). The primary analysis should show a statistically significant increase in superficial cells and a statistically significant decrease in parabasal cells.
- The mean change from baseline to week 12 in vaginal pH. The primary analysis should show a statistically significant lowering of vaginal pH.
- The mean change from baseline to week 12 in the moderate to severe self-assessed symptom identified by the subject as being the most bothersome to her. The primary analysis should show statistically significant improvement in the moderate to severe symptom identified by the subject as most bothersome to her.

Phase 3 Study EST005:

In primary Phase 3 Study EST005, a specimen was obtained from the vaginal wall during visit one (day -21) and visit 6 (day 85) or last visit for subjects who discontinued prematurely. Vaginal wall specimens were sent to the [ ] to determine the percentage of parabasal, intermediate, and superficial cells. A vaginal maturation index (VMI) score was calculated by weighing the percentage of intermediate cells (multiplied by 0.5) and the percentage of superficial cells (multiplied by 1) and summing the weighted percentages, thus:

$$VMI = 0.5 \times [100 \times I/(P+I+S)] + [100 \times S/(P+I+S)]$$

where I is the number of intermediate cells, P is the number of parabasal cells, and S is the number of superficial cells in the sample. Per the submission, higher VMI values are indicative of decreased parabasal cells and increased superficial cells, consistent with more mature vaginal mucosa.

The pH of the vaginal vault was measured in the clinic during visit one (day -21) and visit 6 (day 85) or last visit for subjects who discontinued prematurely. An indicator strip was used to assess the relative alkalinity or acidity value.

Vulvar and vaginal atrophy symptoms were evaluated based on a subject vaginal health self-assessment. Subjects completed a Vaginal Health Self-Assessment Questionnaire by checking the selected response using a four-point scale ("None", "A little (mild)", "Quite a bit (moderate)", "Extremely (severe)", or "No sexual activity" to the following questions:

1. "Do you experience vaginal dryness (decreased vaginal lubrication, secretions, fluid, or mucus)?"
2. "Do you experience vaginal (or vaginal area) irritation or itching?"
3. "Do you experience pain or difficulty passing urine?"
4. "Do you experience vaginal pain with sexual activity?"
5. "Do you experience vaginal bleeding with sexual activity?"

The subject determined which symptom rated moderate or severe was the most bothersome to her. The Vaginal Health Self-Assessment Questionnaire was completed at visit one (day -21), at visit two (day -7) and weekly (based on her experience the previous week) for the remainder of the 12-week study.

In addition, a physician assessment of vaginal atrophy was performed in Study EST005 during visit one and visit six (or last day for subjects who discontinued prematurely). The physician rated the severity of observed vaginal atrophy based on a four-point scale (none, mild, moderate, or severe) of each of the following: vaginal atrophy, vaginal pallor, vaginal dryness, vaginal tissue integrity/friability, and vaginal tissue petechiae.

Per the submission, other assessments of efficacy included subject global efficacy questions, subject quality of life questionnaire, a subject opinion survey, and trough serum concentrations of estradiol, estrone, estrone sulfate, and SHBG.

The secondary efficacy variables for evaluation of vasomotor symptoms in Study EST005 were:

- Mean change from baseline to all non-primary post-baseline time points during double-blind treatment in daily moderate to severe hot flush rate.
- Mean change from baseline to all non-primary post-baseline time points during the double-blind treatment in daily hot flush mean severity.
- Mean percent change from baseline to all post-baseline time points during double-blind treatment in daily moderate to severe hot flush rate.
- Mean percent change from baseline to all post-baseline time points during double-blind treatment in daily hot flush mean severity.
- Time to first 100% reduction from baseline in daily moderate to severe hot flush rate.
- Percent of subjects who achieved a 50%, 80%, 90%, 95%, and 100% reduction from baseline to all post-baseline time points during double-blind treatment in daily moderate to severe hot flush rate.

- Percent of subjects who achieved a 50%, 80%, 90%, 95%, and 100% reduction from baseline to all post-baseline time points during double-blind treatment in daily hot flush mean severity.

The secondary efficacy variables for evaluation of vulvar and vaginal atrophy symptoms were:

- Change from baseline to all post-baseline time points during double-blind treatment in subject vaginal health self-assessment: vaginal dryness, vaginal irritation, pain or difficulty passing urine, pain with sexual activity, and vaginal bleeding during sexual activity.
- Change from baseline to day 85 in physical assessment of vaginal atrophy: vaginal atrophy, vaginal pallor, vaginal dryness, vaginal tissue integrity/friability, and vaginal tissue petechiae.

Statistical significance was declared if the 2-sided p-value was $\leq 0.05$. For all efficacy variables an adjustment for multiple testing was performed using Dunnett's test. Before the implementation of parametric methods of analysis, the distribution of primary analysis variables were examined to determine if model assumptions were satisfied and transformations or non-parametric methods of analysis were used if satisfied.

Three study populations were used for analyses in Study EST005:

- Safety: all subjects who were randomized and received at least one application of double-blind study medication.
- Intent-to-Treat (ITT): all randomized subjects who received double-blind study drug and provided a diary response for at least one full day during the double-blind treatment period.
- Evaluable: all randomized subjects who completed $\geq$ 28 days of double-blind treatment, satisfied compliance criteria with respect to diary completion and study medication dosing, and who did not have documented major protocol deviations, which were determined prior to unblinding.

The duration of double-blind treatment was calculated as the number of days from the date of first dose of double-blind study drug to the date of last dose. Compliance was estimated as the percent of study medication actually used compared to the theoretical amount of drug that could have been used during each subject's duration of double-blind treatment. Per the submission, no adjustment was made for missed doses or interruption of study medication.

All efficacy variables were analyzed using both the ITT and evaluable populations. Primary conclusions were drawn from the ITT population, using last observation carried forward (LOCF) to estimate missing data for those subjects who withdrew early from the study.

Investigative sites with fewer than four subjects in any treatment group in the ITT population were pooled. In total, 24 centers were pooled into six pooled centers.

To demonstrate clinical effectiveness of estradiol gel, an analysis of covariance (ANCOVA) model was used that included factors of covariate, center, treatment, covariate-by-treatment

interaction, where the covariate was the baseline value of the variable being analyzed. The analyses were repeated without the interaction factor(s) if they were not statistically significant ($p>0.10$). Each of the estradiol gel doses were compared with placebo using Dunnett's test, with comparisons based on the least squares (LS) means derived from the ANCOVA, with statistical significance declared if the 2-sided p-value was $\leq 0.05$. In addition, a clinically meaningful difference between placebo and estradiol gel was declared for a time point if there was a difference of at least 2 moderate to severe hot flushes per day in the mean change from baseline.

Age group analyses (<50, 50 to 59, and >59 years of age) were performed to demonstrate mean change from baseline in the daily moderate to severe hot flush rate and hot flush severity at weeks four and 12 of treatment. The estimate of the baseline hot flush rate was determined from the first 14 qualifying days of diary completion during the screening period.

Per the submission, the daily moderate to severe hot flush rate for a given day during double-blind treatment in Study EST005 was calculated as the total number of moderate to severe hot flushes recorded in the diary during the seven days immediately preceding and including that study day, divided by the number of those seven days with diary entries completed. For example, if a subject had 63 moderate to severe hot flushes recorded during days 57 to 63, and no diary record completed for day 61, the calculated daily hot flush rate at day 63 was 10.5 hot flushes per day (63 divided by six days). The baseline hot flush rate was based on the first 14 qualifying days of diary completion during the screening period.

Likewise, the daily hot flush mean severity for a given day during double-blind treatment was calculated as the sum of the average daily hot flush severity rating (mild=1, moderate=2, severe=3) recorded in the diary during the seven days immediately preceding and including that study day, divided by the number of those seven days with diary entries completed. Any day with a completed diary entry indicating no hot flushes was assigned an average daily hot flush severity of zero (0). For example, if a subject had two moderate and two severe hot flushes recorded each day during days 15 and 16, no hot flushes recorded on day 17, did not complete a diary record for day 18, and had two mild and two moderate hot flushes recorded each day during days 19 to 21, the calculated daily hot flush mean severity at day 21 was 1.58 (2.5 + 2.5 + 0 + 1.5 +1.5 +1.5 divided by six days). The baseline for flush mean severity was based on the first 14 qualifying days of diary completion during the screening period. If the severity of a hot flush was unknown, it was assigned a severity of unknown in the database and was not used in the analysis.

All subjects who self-identified at least one moderate to severe symptom of vulvar and vaginal atrophy that was most bothersome at baseline; had a vaginal pH > 5.0 at screening; and had $\leq 5\%$ vaginal superficial epithelial cells in the vaginal smear at baseline were included in the primary VVA analysis (modified ITT [mITT] cohort).

All protocol deviations occurring on an individual subject basis were reviewed before the study blind was broken. Per the submission, no subjects were excluded from the ITT data set.

In Study EST005, 76 subjects were considered to have one or more important deviations significant enough to exclude them from evaluable subjects data set (15 of these 76 subjects were excluded for more than one reason). The following table shows the number of excluded subjects by study treatment group and the reason for exclusion.

**Table 5: Protocol Deviations for Which a Subject Was Excluded From the Evaluable Subject Data Set (All Randomized Subjects)**

| | | Treatment group | | | |
|---|---|---|---|---|---|
| Procedure | | **Estradiol Gel 0.87 g/day** | **Estradiol Gel 1.7 g/day** | **Estradiol Gel 2.6 g/day** | **Placebo** |
| Admission | Did not meet natural or surgical menopause criteria | 2 | 1 | 2 | 1 |
| | Estradiol ≥ 2.0 ng/dL | 1 | 1 | 0 | 4 |
| | FSH < 40 mIU/mL | 1 | 1 | 0 | 0 |
| | < 120 moderate to severe hot flushes at baseline | 0 | 1 | 0 | 0 |
| | TSH levels outside normal range | 3 | 7 | 1 | 3 |
| | Use of hormone therapy within 8 weeks | 1 | 1 | 1 | 2 |
| | Washout period for other meds not met | 1 | 1 | 0 | 0 |
| | Discontinuation or dose change of baseline medication | 1 | 1 | 0 | 0 |
| | SSRI for < 6 months | 1 | 0 | 0 | 0 |
| | Started excluded medication after randomization | 1 | 2 | 2 | 4 |
| Study Drug Administration | Compliance < 80% (as measured by bottle weight) | 4 | 10 | 6 | 8 |
| | < 28 days on study medication | 3 | 1 | 1 | 4 |
| | Misdosed | 0 | 1 | 2 | 1 |
| Study Conduct | Investigator questioned validity of primary efficacy data | 0 | 0 | 0 | 1 |
| | > 20% of hot flush dairy incomplete | 0 | 0 | 0 | 1 |

Source: Adapted from NDA 21813/S-000, Table 10.2-1, Section 8, Volume 26, page 73 (page 93 of 369).
a. Fifteen subjects were excluded for more than one reason.

*Medical Officer's Comments:*

*Protocol deviations reported in Study EST005 were fairly evenly distributed among estradiol gel and placebo treatment groups. The majority of protocol deviations were related to admission procedures related to relevant inclusion/exclusion criteria.*

*Fewer subjects receiving the 0.87 grams per day estradiol gel dose were excluded from the evaluable subject data set because of < 80% compliance.*

The following table shows the demographic characteristics for the ITT population in Study EST005.

**Table 6: Demographic Characteristics (Study EST005) (All Randomized Subjects/ITT Population)**

| Characteristic | Estradiol Gel 0.87 gram/day (N = 136) | Estradiol Gel 1.7 gram/day (N = 142) | Estradiol Gel 2.6 gram/day (N = 69) | Placebo (N = 137) |
|---|---|---|---|---|
| **Age (years)** | | | | |
| Mean ± SD | 54.4 ± 6.3 | 53.9 ± 6.2 | 55.3 ± 8.5 | 54.4 ± 5.8 |
| Range | 31 – 73 | 30 – 69 | 28 – 74 | 40 - 71 |
| **Race, n (%)** | | | | |
| White | 120 (88.2) | 119 (83.8) | 57 (82.6) | 113 (82.5) |
| Black | 10 (7.4) | 11 (7.7) | 9 (13.0) | 17 (12.4) |
| Hispanic | 5 (3.7) | 10 (7.0) | 3 (4.3) | 7 (5.1) |
| American Indian | 1 (0.7) | 1 (0.7) | 0 (0.0) | 0 (0.0) |
| Other | 0 (0.0) | 1 (0.7) | 0 (0.0) | 0 (0.0) |
| **BMI ($kg/m^2$)** | | | | |
| Mean ± SD | 26.4 ± 4.0 | 26.2 ± 3.8 | 26.6 ± 3.6 | 25.8 ± 3.8 |
| Range | 18 – 35 | 17 – 35 | 20 – 35 | 19 - 35 |
| **Height (in)** | | | | |
| Mean ± SD | 64.0 ± 3.0 | 64.0 ± 2.6 | 64.6 ± 2.7 | 64.3 ± 2.9 |
| Range | 53 – 72 | 58 – 71 | 59 – 73 | 59 - 72 |
| **Weight (lb)** | | | | |
| Mean ± SD | 154.1 ± 25.7 | 152.2 ± 26.0 | 157.9 ± 25.8 | 151.9 ± 27.6 |
| Range | 107 – 240 | 88 – 224 | 111 – 207 | 101 - 250 |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 61, Table 8.7.3-2, page 54 of 165.

*Medical Officer's Comments:*

*There were no statistically significant differences among treatment groups for any demographic characteristics.*

## Primary Analyses of Efficacy:

## Vasomotor Symptoms:

At the week four primary endpoint, the mean change from baseline in the daily frequency of moderate to severe vasomotor symptoms was statistically significantly greater for subjects receiving 1.7 gram per day estradiol gel and 2.6 gram per day estradiol gel than for the placebo treatment group (-8.2 and -9.5, respectively versus -5.4 for the placebo group; $p<0.0001$ for both comparisons). The reduction in these two estradiol gel treatment groups was also clinically significant (> 2 hot flush reduction over placebo per day).

The change from baseline in daily moderate to severe hot flush rate for subjects receiving the 0.87 gram per day estradiol gel dose was not statistically significantly different from placebo at week 4 (-6.6 versus -5.4, respectively, $p=0.0965$) and was not clinically significantly different from placebo (< 2 [mean of 1.2] hot flush reduction over placebo per day). Clinically and statistically significant difference from placebo were observed beginning at week 5 in Study EST005 (-7.7 versus -5.5, respectively; $p=0.0001$; > 2 hot flush reduction over placebo per day) and maintained through week 12.

At the week 12 primary endpoint, the change from baseline in the daily moderate to severe hot flush rate was statistically and clinically greater for subjects receiving 0.87 gram per day estradiol gel, 1.7 gram per day estradiol gel, and 2.6 gram per day estradiol gel than for placebo (-9.1, -10.7, -11.3, and -6.1, respectively; $p<0.0001$ for all comparisons; 3.0, 4.6, and 5.2 reduction in hot flushes over placebo, respectively).

The mean change in frequency of hot flushes is illustrated in Table 7.

**Table 7: Mean Change ± Standard Deviation (SD) From Baseline in Daily Moderate to Severe Hot Flush Rate (ITT-LOCF)**

| Evaluation | Mean Change From Baseline[a] | | | |
|---|---|---|---|---|
| | **Estradiol Gel 0.87 gram/day N = 137** | **Estradiol Gel 1.7 gram/day N = 142** | **Estradiol Gel 2.6 gram/day N = 69** | **Placebo N = 137** |
| **Baseline (Mean ± SD)[b]** | **13.3 ± 4.6** | **13.1 ± 6.5** | **12.9 ± 4.5** | **13.5 ± 4.5** |
| **Week 4** | **-6.6 ± 5.0** | **-8.2 ± 5.8** | **-9.5 ± 6.5** | **-5.4 ± 5.8** |
| **P-value vs. Placebo[c]** | **0.0965** | **<0.0001** | **<0.0001** | - |
| **Week 5** | **-7.7 ± 4.8** | -9.0 ± 5.9 | -10.0 ± 6.1 | -5.5 ± 6.0 |
| **P-value vs. Placebo[c]** | **0.0002** | <0.0001 | <0.0001 | - |
| Week 6 | -7.9 ± 4.8 | -9.5 ± 5.9 | -10.4 ± 5.6 | -5.7 ± 5.9 |
| P-value vs. Placebo[c] | 0.0002 | <0.0001 | <0.0001 | - |
| Week 7 | -8.5 ± 4.8 | -9.9 ± 6.0 | -10.9 ± 5.4 | -6.0 ± 5.9 |
| P-value vs. Placebo[c] | <0.0001 | <0.0001 | <0.0001 | - |
| Week 8 | -8.6 ± 4.7 | -10.1 ± 6.1 | -11.0 ± 5.6 | -6.0 ± 6.0 |
| P-value vs. Placebo[c] | <0.0001 | <0.0001 | <0.0001 | - |
| Week 9 | -8.7 ± 4.8 | -10.3 ± 6.3 | -11.4 ± 5.8 | -6.0 ± 6.1 |
| P-value vs. Placebo[c] | <0.0001 | <0.0001 | <0.0001 | - |
| Week 10 | -9.0 ± 4.6 | -10.5 ± 6.4 | -11.3 ± 6.0 | -6.0 ± 6.0 |
| P-value vs. Placebo[c] | <0.0001 | <0.0001 | <0.0001 | - |
| Week 11 | -9.0 ± 4.6 | -10.5 ± 6.5 | -11.3 5.8 | -6.1 6.2 |
| P-value vs. Placebo[c] | <0.0001 | <0.0001 | <0.0001 | - |
| **Week 12** | **-9.1 ± 4.6** | **-10.7 ± 6.6** | **-11.3 ± 5.9** | **-6.1 ± 6.2** |
| **P-value vs. Placebo[c]** | **<0.0001** | **<0.0001** | **<0.0001** | - |

Source: adapted from NDA 21-813/S-000, Table 11.4-1, Section 8, Volume 26, page 83 (page 103 of 369).

a. Differences from baseline to each week based on LS means derived from the ANCOVA model with factors for baseline, treatment, site, and treatment-by-baseline interaction.

b. Unadjusted means and standard deviation. Baseline based on the first 14 days of the screening period.

c. Treatment comparison with placebo (Dunnett's test).

The reduction from baseline in daily hot flush severity (score) was statistically significantly greater for subjects receiving the 1.7 gram per day dose and the 2.6 gram per day dose than for placebo (-0.7 and -1.0, respectively, versus -0.3 for the placebo treatment group; $p<0.0001$ for both comparisons). A clinically significant difference from placebo was observed at week 5 for the 0.87 gram per day dose (-0.6 versus -0.3, P=0.0083) and maintained through week 12.

At the week 12 primary endpoint in Study EST005, the change from baseline in daily hot flush severity was statistically significantly greater for subjects receiving all three estradiol gel dosage

strengths than for placebo (-0.9, -1.3, and -1.6, respectively, versus -0.4; p<0.0001 for all comparisons.

The mean change in severity of hot flushes is illustrated in Table 8.

**Table 8: Mean Change ± Standard Deviation (SD) From Baseline in Daily Moderate to Severe Hot Flush Severity (ITT-LOCF)**

| Evaluation | Mean Change From Baseline[a,b] | | | |
|---|---|---|---|---|
| | Estradiol Gel 0.87 gram/day N = 137 | Estradiol Gel 1.7 gram/day N = 142 | Estradiol Gel 2.6 gram/day N = 69 | Placebo N = 137 |
| **Baseline (Mean ± SD)[c]** | **2.4 ± 0.3** | **2.4 ± 0.3** | **2.4 ± 0.3** | **2.4 ± 0.3** |
| **Week 4** | **-0.5 ± 0.7** | **-0.7 ± 0.8** | **-1.0 ± 0.9** | **-0.3 + 0.6** |
| **P-value vs. Placebo[d]** | **0.0714** | **<0.0001** | **<0.0001** | - |
| **Week 5** | **-0.6 ± 0.8** | -0.8 ± 0.8 | -1.1 ± 1.0 | -0.3 ± 0.6 |
| **P-value vs. Placebo[d]** | **0.0083** | <0.0001 | <0.0001 | - |
| Week 6 | -0.6 ± 0.8 | -0.9 ± 0.9 | -1.2 ± 0.9 | -0.3 ± 0.6 |
| P-value vs. Placebo[d] | 0.0057 | <0.0001 | <0.0001 | - |
| Week 7 | -0.7 ± 0.8 | -1.0 ± 0.9 | -1.3 ± 0.9 | -0.3 ± 0.6 |
| P-value vs. Placebo[d] | 0.0014 | <0.0001 | <0.0001 | - |
| Week 8 | -0.7 ± 0.9 | -1.1 ± 1.0 | -1.4 ± 1.0 | -0.3 ± 0.7 |
| P-value vs. Placebo[d] | 0.0003 | <0.0001 | <0.0001 | - |
| Week 9 | -0.8 ± 0.9 | -1.1 ± 1.0 | -1.5 1.0 | -0.3 ± 0.7 |
| P-value vs. Placebo[d] | 0.0003 | <0.0001 | <0.0001 | - |
| Week 10 | -0.8 ± 0.9 | -1.2 ± 1.0 | -1.6 ± 1.0 | -0.3 ± 0.6 |
| P-value vs. Placebo[d] | <0.0001 | <0.0001 | <0.0001 | - |
| Week 11 | -0.9 ± 1.0 | -1.2 ± 1.0 | -1.5 ± 1.0 | -0.3 ± 0.7 |
| P-value vs. Placebo[d] | <0.0001 | <0.0001 | <0.0001 | - |
| **Week 12** | **-0.9 ± 1.0** | **-1.3 ± 1.0** | **-1.6 ± 1.0** | **-0.4 0.7** |
| **P-value vs. Placebo[d]** | **<0.0001** | **<0.0001** | **<0.0001** | - |

Source: Adapted from NDA 21-813/S-000, Table 11.4-2, Section 8, Volume 26, page 86 (page 106 of 369).
a. Differences from baseline to each week based on LS means derived from the ANCOVA model with factors for baseline, treatment, and site.
b. Severity score: 0=none, 1=mild, 2=moderate, 3=severe.
c. Unadjusted means and standard deviation. Baseline based on the first 14 days of the screening period.
d. Treatment comparison with placebo (Dunnett's test).

*Medical Officer's Comments:*

*As noted previously in this review, the Agency's January 2003 draft clinical evaluation guidance for industry recommends that the primary analysis should show a statistically and clinically significant mean change in the frequency and severity of moderate to severe hot flushes from baseline to week four that is maintained through week 12 for a VMS indication.*

*As shown in Table 7, the mean change from baseline in the daily frequency of moderate to severe vasomotor symptoms was statistically significantly greater for subjects receiving the 1.7 gram per day estradiol gel dose and the 2.6 gram per day estradiol gel dose than for the placebo treatment group at week 4 and this statistically significant reduction was maintained through week 12. The hot flush reduction in these two estradiol gel treatment groups was also clinically*

*significant (> 2 hot flush reduction over placebo per day) at weeks 4 and 12. Similarly, the reduction from baseline in daily hot flush severity (score) was statistically significantly greater for subjects receiving the 1.7 gram per day and 2.6 gram per day doses than for placebo at week 4, and this statistically significant reduction was maintained through week 12.*

*However, for subjects receiving the 0.87 gram per day estradiol gel dose, the mean changes in the daily frequency and severity of moderate to severe hot flushes were not statistically significant at week 4 but were delayed until week 5 as shown in Tables 7 and 8, but once achieved were maintained through week 12. Likewise, the reduction in the 0.87 gram per day treatment group was not clinically significant (i.e., > 2 hot flush reduction over placebo per day) until week 5, but once achieved, was maintained through week 12.*

In Study EST005, several secondary efficacy variables for evaluation of the relief of vasomotor symptoms were proposed (see page 53 of this review). Four of the secondary endpoints have previously been discussed in this review, namely, the results of the week 5 time point for the 0.87 gram per day estradiol gel dose for hot flush frequency and severity. Two additional secondary endpoints are of interest, namely, the percent change from baseline in daily moderate to severe hot flush frequency and severity rates over time. These two secondary endpoints are discussed below in support of the four primary endpoints.

In Study EST005, the percent of subjects who achieved a 50%, 80%, 90%, 95%, and 100% reduction from baseline to all post-baseline time points during double-blind treatment in daily moderate to severe hot flush frequency and severity was evaluated. The number and proportion of subjects with a ≥ 50% to a 100% reduction in daily moderate to severe hot flush frequency and severity are shown in Table 9 and Table 10, respectively for weeks 4 and 12. Data for other time points (weeks 1-3 and weeks 5-11) may be found in the NDA submission.

**Table 9: Number and Proportion of Subjects With a ≥ 50% to a 100% Reduction in Daily Moderate to Severe Hot Flush Rates at Week 4 and Week 12 (Study EST005) (ITT-LOCF Data Set)**

| Evaluation | Number (%) of Subjects | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Estradiol Gel 0.87 g/day (N=136) | | Estradiol Gel 1.7 g/day (N=142) | | Estradiol Gel 2.6 g/day (N=69) | | Placebo (N=137) | |
| | Week 4 | Week 12 | Week 4 | Week 12 | Week 4 | Week 12 | Week 4 | Week 12 |
| ≥ 50% Reduction | 75 (56) | 109 (81) | 100 (70) | 122 (86) | 53 (77) | 61 (88) | 54 (40) | 62 (46) |
| P-value vs. Placebo[a] | <0.01 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | - | - |
| ≥ 80% Reduction | 34 (25) | 71 (53) | 60 (42) | 98 (69) | 41 (59) | 55 (80) | 25 (19) | 30 (22) |
| P-value vs. Placebo[a] | >0.05 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | - | - |
| ≥ 90% Reduction | 25 (19) | 54 (40) | 44 (31) | 83 (59) | 33 (48) | 49 (71) | 14 (10) | 21 (16) |
| P-value vs. Placebo[a] | <0.05 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| ≥ 95% Reduction | 19 (14) | 45 (33) | 34 (24) | 72 (512) | 27 (39) | 46 (67) | 10 (7) | 15 (11) |
| P-value vs. Placebo[a] | >0.05 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | - | - |
| ≥ 100% Reduction | 8 (6) | 34 (25) | 16 (11) | 52 (37) | 16 (23) | 36 (52) | 5 (4) | 12 (9) |
| P-value vs. Placebo[a] | >0.05 | <0.001 | <0.05 | <0.0001 | <0.0001 | <0.0001 | - | - |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 61, Table 8.7.3-9, page 71 of 165.
a = Treatment comparison with placebo based on the Cochran-Mantel-Haenszel general association statistic with center as the stratification factor.

*Medical Officer's Comments:*

*Overall, Table 9 shows that more estradiol gel-treated subjects experienced at least a 50% reduction in daily moderate to severe hot flush frequency compared to placebo-treated subjects at weeks four and 12 (56% to 77% across the three estradiol gel treatment groups versus 40% for the placebo treatment group at week four; 81% to 88% versus 46% at week 12). The difference from placebo in the percent of subjects with at least 50% reduction in hot flush frequency at week four was statistically significant for the 0.87 gram per day treatment group (p=0.0095) and for the 1.7 gram per day and 2.6 gram per day doses (p=0.0017 and p<0.0001, respectively). Between 19% (0.87 gram per day group, 25 of 136 subjects) and 48% (2.6 gram per day group, 33 of 69 subjects) of subjects experienced at least 90% reduction in moderate to severe hot flush frequency compared with the 10% reported for the placebo treatment group (14 of 137 subjects) at week 4.*

**Table 10: Number and Proportion of Subjects With a ≥ 50% to a 100% Reduction in Daily Moderate to Severe Hot Flush Severity at Week 4 and Week 12 (Study EST005) (ITT-LOCF Data Set)**

| Evaluation | Number (%) of Subjects | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Estradiol Gel 0.87 g/day (N=136) | | Estradiol Gel 1.7 g/day (N=142) | | Estradiol Gel 2.6 g/day (N=69) | | Placebo (N=137) | |
| | Week 4 | Week 12 | Week 4 | Week 12 | Week 4 | Week 12 | Week 4 | Week 12 |
| ≥ 50% Reduction | 20 (15) | 43 (32) | 35 (25) | 77 (54) | 25 (36) | 46 (67) | 9 (7) | 15 (11) |
| P-value vs. Placebo[a] | <0.05 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | - | - |
| ≥ 80% Reduction | 11 (8) | 31 (23) | 15 (11) | 48 (34) | 15 (22) | 36 (52) | 3 (2) | 8 (6) |
| P-value vs. Placebo[a] | <0.05 | <0.0001 | <0.01 | <0.0001 | <0.0001 | <0.0001 | - | - |
| ≥ 90% Reduction | 7 (5) | 29 (22) | 9 (6) | 41 (29) | 10 (15) | 32 (46) | 3 (2) | 6 (4) |
| P-value vs. Placebo[a] | >0.05 | <0.0001 | >0.05 | <0.0001 | <0.01 | <0.0001 | - | - |
| ≥ 95% Reduction | 6 (4) | 27 (20) | 9 (6) | 35 (25) | 6 (9) | 31 (45) | 2 (2) | 6 (4) |
| P-value vs. Placebo[a] | >0.05 | <0.0001 | >0.05 | <0.0001 | >0.05 | <0.0001 | - | - |
| ≥ 100% Reduction | 6 (4) | 27 (20) | 9 (6) | 35 (25) | 5 (7) | 31 (45) | 2 (2) | 6 (4) |
| P-value vs. Placebo[a] | >0.05 | <0.0001 | >0.05 | <0.0001 | >0.05 | <0.0001 | - | - |

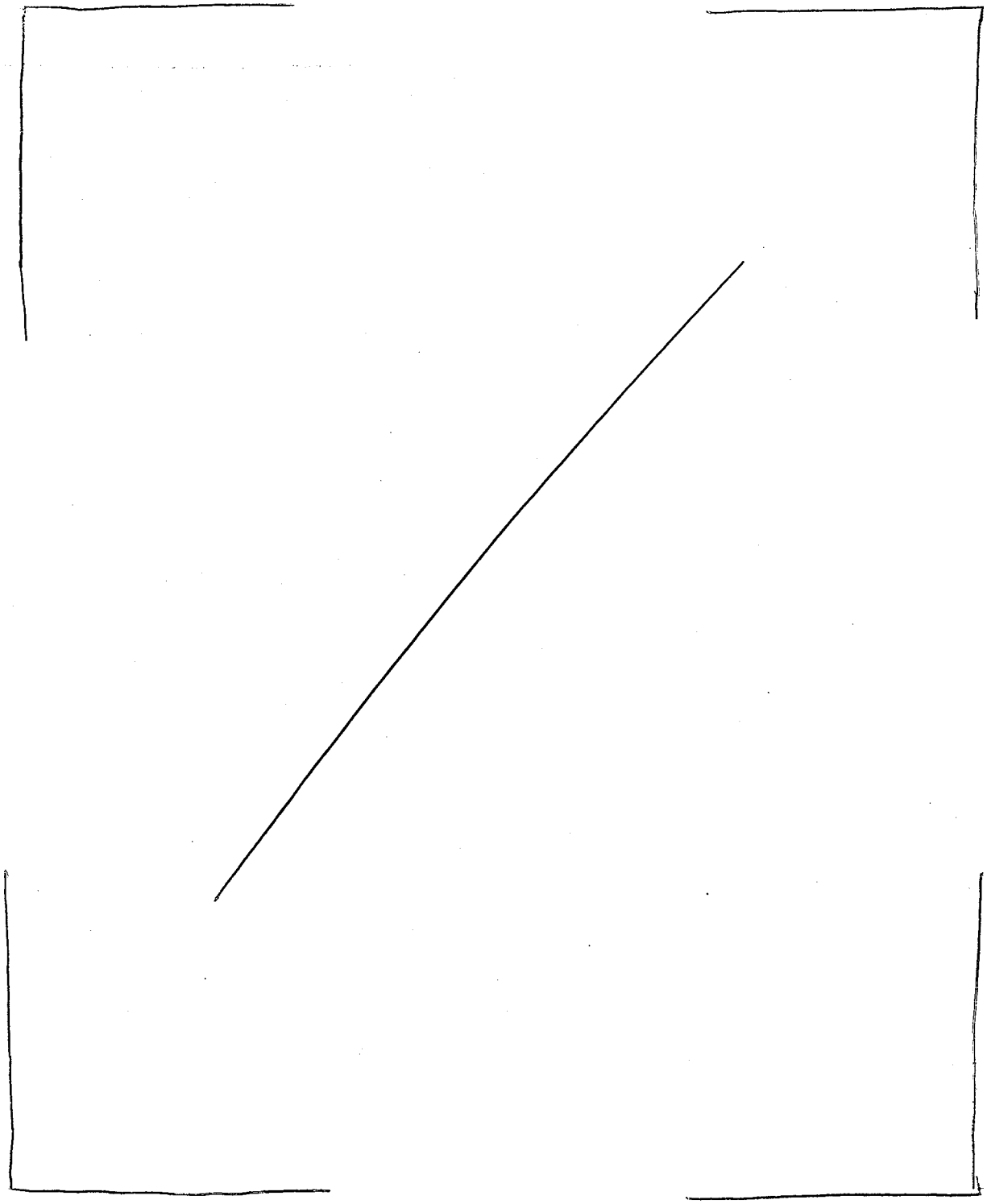
Source: Adapted from NDA 21-813/S-000, Section 8, Volume 61, Table 8.7.3-10, page 72 of 165.
a = Treatment comparison with placebo based on the Cochran-Mantel-Haenszel general association statistic with center as the stratification factor.

*Medical Officer's Comments:*

*As shown in Table 10, a statistically significantly greater proportion of estradiol gel-treated subjects (15% to 36% across the estradiol gel treatment groups) had achieved at least a 50% reduction in daily hot flush severity compared to 7% for placebo-treated subjects at week 4. The difference from placebo in the percent of subjects with at least 50% reduction in hot flush severity at week 4 was statistically significant for all three estradiol gel dosage strengths (p=0.0262, p<0.0001, and p<0.0001, respectively). A similar finding was reported for a ≥ 80% reduction in hot flush severity at week 4.*

*This reviewer believes that an important proportion of women improve on treatment at week 4 with the 0.87 gram per day estradiol gel dosage strength and that this proportion increases through week 12. These secondary analyses support the recommended approval of the 0.87 gram per day estradiol gel dose even though the primary endpoints were not met at week 4. A*

*significant proportion of symptomatic women could benefit from this low daily dose of estradiol gel.*



Redacted 11 page(s)

of trade secret and/or

confidential commercial

information from

Medical Review (pg 63-73)
(12/14/06)

In Study EST005, subjects were asked to respond to two Global Efficacy Questions at week 12:

1. "In your opinion, how do you feel the study gel affected your hot flashes?" Categories for responses included:

   - No improvement
   - Slight improvement
   - Moderate improvement
   - Great improvement

2. "In your opinion, how do you feel the study gel affected your vaginal atrophy (eg, dryness, itching)?" Categories for responses included:

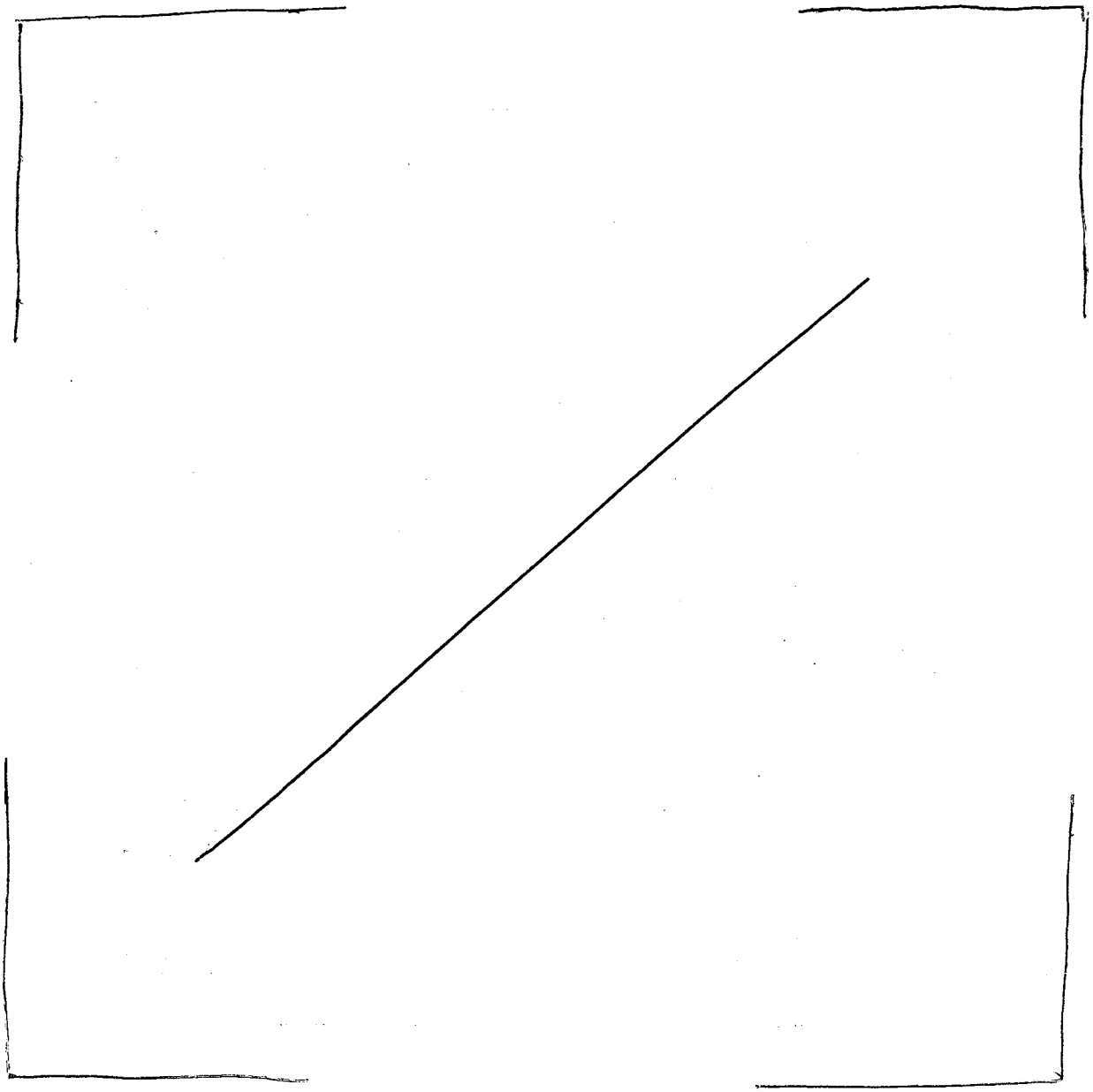
   - No improvement
   - Slight improvement
   - Moderate improvement
   - Great improvement
   - Not applicable

Per the reported results of the subject Global Efficacy Questions, compared to placebo, a greater proportion of subjects receiving estradiol gel treatment felt that study gel produced moderate to great improvement in their hot flushes at week 12: 79.9% (107 of 136 subjects), 87.7% (122 of 142 subjects), and 89.8% (62 of 69 subjects) versus 50.7% (67 of 137 subjects) for the 0.87 gram per day group, 1.7 gram per day group, and the 2.6 gram per day group versus the placebo group, respectively. A considerably fewer estradiol gel-treated subjects felt that treatment had produced no to slight improvement: 20.0% (27 of 136 subjects), 12.3% (17 of 142 subjects), and 10.1% (7 of 69 subjects) versus 49.3% (65 of 137 subjects), respectively.

*Medical Officer's Comments:*

*Overall, each dose of estradiol gel was significantly different from placebo treatment ($p<0.0001$) with respect to the distribution of subjects who reported no improvement or slight, moderate, or great improvement in their hot flushes [redacted] The percentages reported for moderate to great improvement for [redacted] VMS [redacted] are dose-dependent.*

In addition, the Utian Quality of Life Scale (UQOL), and the Mean Change from Baseline in Menopause-Specific Quality of Life Questionnaire (MENQOL) were completed at baseline and week 12 (or last visit) in Study EST005. The findings of these assessments are not discussed in this review.



Redacted 2 page(s)

of trade secret and/or

confidential commercial

information from

Medical Review (pgs. 76-77)
(12/14/06)

### 6.1.5 Clinical Microbiology

DRUP submitted a request for consultation to HFD-805 on February 16, 2006 to confirm that microbial limits are in compliance with established standards.

Per the Microbiology Review dated October 12, 2006, NDA 21-813/S-000 is "recommended for approval from the standpoint of product quality microbiology." "The drug product is a non-sterile topical gel containing ⁄% ethanol. The results of preservative effectiveness testing and microbial limits specifications were provided in the application." "No deficiencies were identified based upon the information provided."

### 6.1.6 Efficacy Conclusions

**Moderate to Severe Vasomotor Symptoms:**

The results from 12-week, primary, Phase 3 Study EST005 demonstrate the delayed effect of the 0.87 gram per day estradiol gel dose (containing 0.52 mg of estradiol providing a nominal delivery rate of 0.0125 mg of estradiol per day), and the effectiveness of the 1.7 gram per day estradiol gel dose (containing 1.02 mg of estradiol providing a nominal delivery rate of 0.0375 mg of estradiol per day) in producing a statistically significant reduction compared with placebo in the frequency and severity of hot flushes. A third 2.6 gram per day estradiol gel dose in Study EST005 also demonstrated efficacy compared to placebo for the frequency and severity of hot flushes. [ ] [ ] [ ]

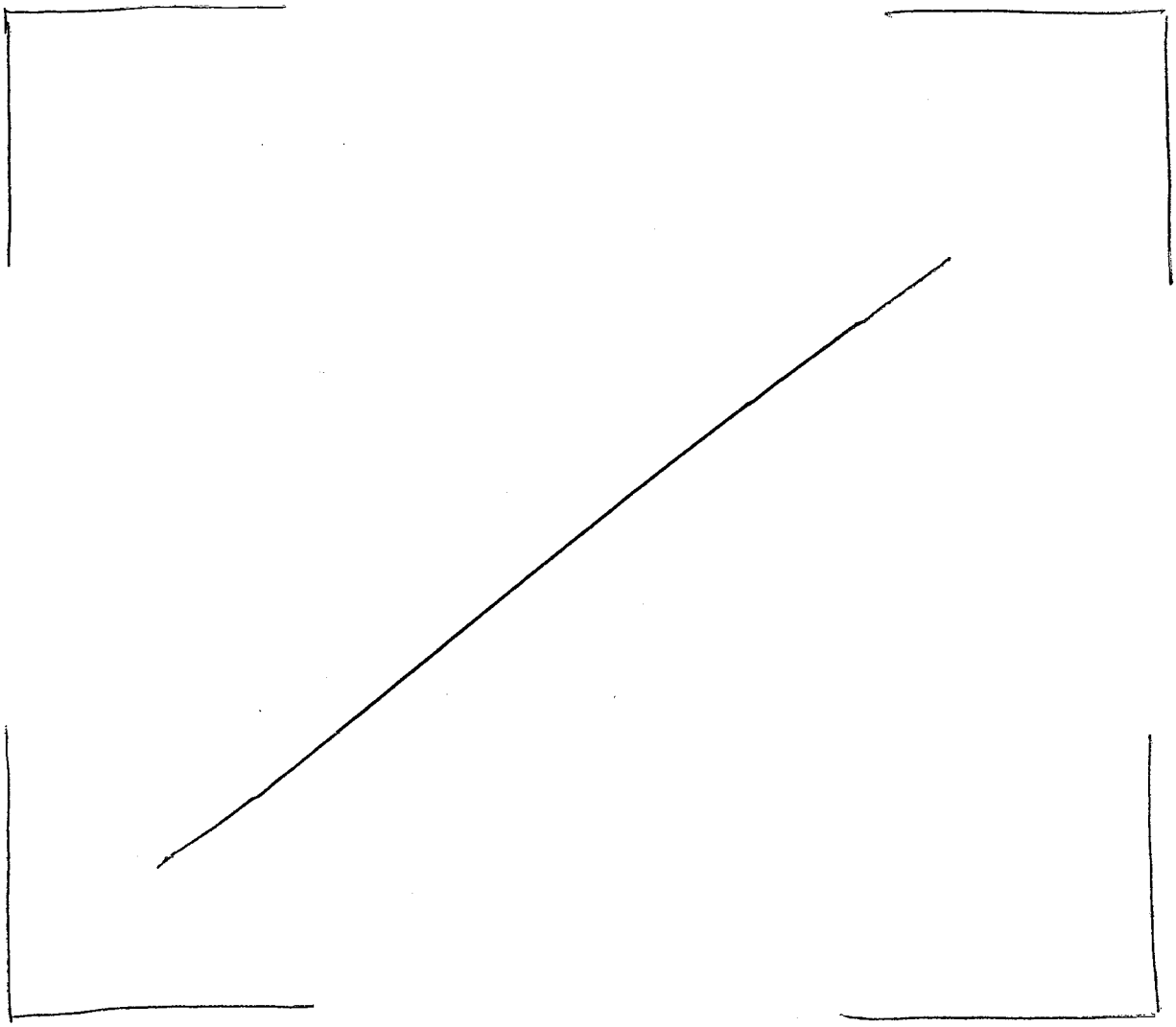
A statistically significant reduction in daily moderate to severe hot flush frequency compared to placebo was observed at week 5 for the 0.87 gram per day estradiol gel treatment group ($p<0.001$) and at week 4 for the 1.7 gram per day estradiol gel treatment group ($p<0.0001$). The 0.87 gram per day estradiol gel treatment group did not demonstrate statistical significance compared to placebo at week four in Study EST005 ($p=0.0965$). Statistically significant reductions in daily moderate to severe hot flush frequency compared to placebo were demonstrated at week 12 for the 0.87 gram per day and 1.7 gram per day estradiol gel treatment groups ($p<0.0001$ for both dosage strengths).

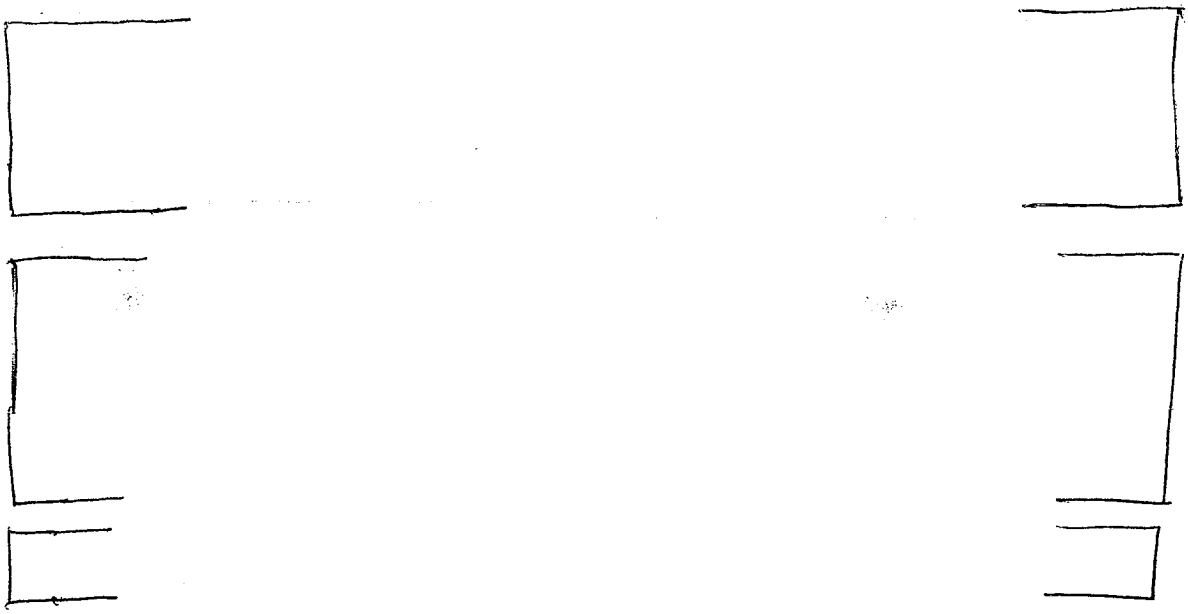
A clinically meaningful reduction in daily hot flush frequency compared with placebo was observed for the 0.87 gram per day estradiol gel treatment group at week 5 (> 2 difference in the number of moderate to severe hot flushes per day over placebo) and at week 4 for the 1.7 gram per day estradiol gel treatment group (> 2 difference in the number of moderate to severe hot flushes per day over placebo). The reduction in the number of hot flushes over placebo was not clinically meaningful for the 0.87 gram per day estradiol gel treatment group at week 4 (1.2 difference in the number of moderate to severe hot flushes per day compared with placebo at week 4).

Reduction in hot flush severity was statistically significantly different from placebo treatment by week 5 for the 0.87 gram per day estradiol gel treatment group ($p<0.01$ at week five, $p=0.714$ at week four), and by week 4 for the 1.7 gram per day estradiol gel treatment group ($p<0.0001$). Statistically significant reductions in daily moderate to severe hot flush severity compared to placebo were demonstrated at week 12 for both estradiol gel doses ($p<0.0001$ for the 0.87 gram per day and 1.7 gram per day dosage strengths).

Based on these efficacy analyses, this reviewer recommends approval of the 0.87 gram per day and 1.7 gram per day estradiol gel dosage strengths for the treatment of moderate to severe vasomotor symptoms associated with the menopause.

Product labeling must clearly delineate that VMS effectiveness for the1.7 gram per day estradiol gel dose was achieved at week 4 in Study EST005, and that the VMS effectiveness of the 0.87 gram per day estradiol gel dosage strength was delayed until week 5.





# 7 INTEGRATED REVIEW OF SAFETY

## 7.1 Methods and Findings

Per the submission, the clinical development program for estradiol gel was "designed to address the minimum effective dose issue" and included two placebo-controlled efficacy and safety studies (Study EST004 and Study EST005).

Study EST004 was a Phase 2, four-week, dose-finding study conducted to investigated the safety and efficacy of the 0.625 gram per day, 1.25 gram per day , and 2.5 gram per day estradiol gel dosage strengths in the treatment of moderate to severe vasomotor symptoms (VMS). Study EST005, conducted after Study EST004, was a Phase 3, 12-week, well-controlled study designed to investigate the safety and efficacy of the 0.87 gram per day, 1.7 gram per day, and 2.6 gram per day estradiol gel dosage strengths in the treatment of moderate to severe vasomotor symptoms (VMS) and moderate to severe symptoms of vulvar and vaginal atrophy associated with the menopause (VVA).

In addition, [ ]Phase 1 investigations were conducted in postmenopausal women in order to evaluate the pharmacokinetic (PK) characteristics of estradiol gel. [ ] of the[ ]Phase 1 studies utilized a[ ] formulation of estradiol gel not planned for marketing (Study [ ] and Study [ ]

Data from the two placebo-controlled clinical trials and[ ] of the[ ]clinical pharmacology studies are included in the Integrated Summary of Safety (ISS) in the submission. Study [ ]
[ ]

The ISS summarizes data on a total of 759 subjects (645 subjects in the two placebo-controlled clinical trials and 111 subjects in the PK studies [including 24 male subjects]) in clinical trials conducted between 1999 and 2005. A total of 577 subjects received six different doses of estradiol gel (466 in clinical studies and 111 in the PK studies including 15 female subjects exposed to the [ ] formulation and 24 male subjects exposed in transfer Study EST006). A total of 179 subjects in the clinical trials were exposed to placebo treatment.

Only limited pooling of data was completed for analysis of safety data across Study EST004 and EST005 due to the differences in doses and duration of treatment in these two studies. However, adverse events were pooled for the 2.5 gram per day estradiol gel dose utilized in Study EST004 and the 2.6 gram per day estradiol gel dose utilized in Study EST005, and for the placebo treatment groups in these two studies. Data were also pooled for subject disposition, demography, and extent of exposure. No laboratory data were pooled due to the difference in double-blind duration of treatment in Studies EST004 and EST005.

Safety measurements in Phase 3 Study EST005 included a complete physical examination and vital signs performed at visit one (day -21 and visit six (day 85) or last visit for subjects who discontinued prematurely. All physical examination findings, vital signs, height, weight, and calculated BMI were recorded on the appropriate case report form (CRF).

Gynecological examination including a Pap smear (for women with a cervix), mammogram (if not performed within the previous nine months with a normal written report available), endometrial biopsy and transvaginal ultrasound (TVUS) for women with a uterus was performed. Pap smear specimens and endometrial biopsy specimens were sent to the [ ] [ ] Mammograms and TVUS assessments were read locally.

Safety measurements in Phase 2 Study EST004 included a complete physical examination including a pelvic examination with Pap smear (if not performed within 12 months prior to screening), vital signs, height and weight, mammogram (if not performed within nine months prior to screening), blood samples for clinical chemistry, lipids, hematology, coagulation after a minimum of 8-hour fasting, and urine for urine dipstick.

Subjects with systemic skin diseases or local skin abnormalities in the area of gel application (upper arm) were not eligible for Studies EST004 and EST005. The study drug application site was assessed for skin irritation at visit 2 (day -7) and at all subsequent study visits, with severity of irritation rated according to the following scores:

0 = No erythema
1 = Minimal erythema
2 = Moderate erythema with sharply defined borders
3 = Intense erythema with or without edema
4 = Intense erythema with edema and erosion/blistering

Results of the application area inspection were recorded on the CRF; scored ≥ 1 were recorded as adverse events.

Clinical laboratory tests:

Blood and urine samples were obtained in the two studies for the following clinical laboratory tests at screening and end-of-study):

| | |
|---|---|
| Hematology | hematocrit, hemoglobin, platelet count, red blood cells, white blood cells with differential, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and reticulocytes in Study EST005 only. |
| Chemistry: | sodium, potassium, chloride, glucose, calcium, blood urea nitrogen (BUN), creatinine, total bilirubin, total protein, albumin, alkaline phosphatase, phosphorus, lactate dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (AST/SGOT), and serum glutamic-pyruvic transaminase (ALT/SGPT). |
| Lipid Profile: | triglycerides, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). |
| Urinalysis: | appearance, color, pH, specific gravity, leukocytes, nitrites, protein, glucose, ketones, and blood. |
| Coagulation (EST004): | (activated) partial thromboplastin time (aPTT), and prothrombin time (PT). |
| Coagulation profile: (EST005) | protein-C activity, protein-S antigen (protein S), antithrombin III activity, and Factor V Leiden (activated protein-C resistance). |
| Miscellaneous: (EST005) | C - reactive protein |

Clinical laboratory testing was performed by [redacted]

Per the submission, clinical laboratory data, vital signs, and weight were evaluated based on mean change from baseline to week 12 (or final visit). For the final laboratory sample, only data collected within seven days of study drug completion were included in the analysis.

Other evaluations:

- Mammogram (unless performed within nine months prior to screening with a written report indicating no questionable findings, including pre-cancerous or cancerous findings).
- Cervical Pap smear (unless performed within 12 months prior to screening with a written normal report in Study EST004 and 9 months in Study EST005).
- Transvaginal ultrasound (TVUS) in Studies EST004 and EST005 (summarized as ≤ 4 mm [normal] versus > 4 mm [abnormal]) and an endometrial biopsy at screening and week 12 (day 84) or at premature discontinuation in women with a uterus in Study EST005.

Per the submission, endometrial biopsy specimens were evaluated by one pathologist (Dr. [redacted], Director of Pathology at [redacted]). Change in endometrial biopsy results by treatment group were classified into one of 11 categories:

Normal:
1. Strips of benign surface and glandular lining epithelium.
2. Inactive/atrophic endometrium.
3. Proliferative endometrium.
4. Progestational secretory endometrium.
5. Menstrual type endometrium.
6. Polyp.

Abnormal:
7. Polyp.
8. Simple hyperplasia without atypia.
9. Complex hyperplasia without atypia.
10. Atypical hyperplasia.
11. Carcinoma.

*Medical Officer's Comments:*

*Per the Agency's 2003 draft clinical evaluation guidance document, the use of three independent expert pathologists, blinded to treatment group and to each other's readings, is recommended, particularly for estrogen plus progestin drug products. The concurrence of two of the three pathologists is accepted as the final diagnosis. In the case of a single pathologist conducting endometrial safety reads, as utilized in Study EST005, the single diagnosis is maintained as the final diagnosis.*

*The histologic classifications utilized by the single pathologist were similar but not as descriptive as the histologic classification recommended in the Agency's 2003 draft clinical evaluation guidance document.*

### 7.1.1 Deaths

No deaths occurred during the conduct of primary, Phase 3 Study EST005, Phase 2 Study EST004, or any of the six PK studies conducted under the estradiol gel clinical development program.

### 7.1.2 Other Serious Adverse Events

Serious adverse events (SAEs) were defined as adverse events that were fatal, life-threatening, disabling, or required hospitalization or prolongation of hospitalization. In addition, based on appropriate medical judgment, any important medical event that jeopardized the subject or

required medical or surgical intervention could be considered a SAE. SAEs were reported to BioSante Pharmaceuticals, Inc. (or designee, per the submission) immediately upon discovery of the event. SAEs occurring during the conduct of the study were to be followed with appropriate medical management until resolved.

Per the submission, three subjects in Study EST005 had serious adverse events, one during the single-blind placebo lead-in period and two during the double-blind treatment period:

- Subject 913 experienced chest pain which required hospitalization during the single-blind placebo lead-in period. Placebo medication was discontinued. The event was not considered related to study drug.
- Subject 106 (1.7 gram per day estradiol gel, 50 years of age) experienced a severe staphylococcal infection in her left thumb at a site where she had a previous surgery with pin insertion which required hospitalization (study day 76). Medication was discontinued. The event was not considered related to study drug.
- Subject 261 (2.6 gram per day estradiol gel, 54 year of age) experienced a worsening of a cervical cyst noted at study entry and an increase in endometrial thickness at end-of-study (4 mm at baseline, 6 mm at end-of-study). She required hospitalization approximately three months after the last dose of study medication and underwent a transabdominal hysterectomy and bilateral salpingoophorectomy. The event was considered possibly related to study drug.

### 7.1.3 Dropouts and Other Significant Adverse Events

#### 7.1.3.1 Overall profile of dropouts

Of the 503 subjects who entered the single-blind placebo lead-in period of Study EST005, 19 subjects discontinued before randomization (3.8%, 19 of 503 subjects). Two of the 19 discontinuations during this period were due to adverse events (Subject 908 for "moodiness" and Subject 913 for "chest pain resulting in hospitalization"). Nine of the 19 discontinuations during this period were due to estradiol levels being > 20 pg/mL. Other reasons for discontinuation included withdrawn consent (two subjects), use of corticosteroids or exclusionary medications (Subject 906 and Subject 903), Applicant request (Subject 905) or Applicant closed site (Subject 901), elevated screening glucose (Subject 907), and laboratory error for serum estradiol concentration (Subject 915).

Of the 167 subjects who entered the single-blind placebo lead-in period in Study EST004, six subjects (3.6%, 6 of 167 subjects) discontinued before randomization. A total of 3 subjects discontinued due to an adverse event (skin rash at the application site for Subject 180, erythema of the legs and back for Subject 241, and diarrhea for Subject 297). Three subjects withdrew consent.

Four hundred eighty-four (484) subjects subsequently were randomized into Study EST005: 136 subjects to the 0.87 gram per day estradiol gel treatment group, 142 subject to the 1.7 gram per

day estradiol gel treatment group, 69 subjects to the 2.6 gram per day estradiol gel treatment group, and 137 subjects to the placebo treatment group. Per the submission, more than 93% of subjects in each treatment group completed the study: 132 subjects in the 0.87 gram per day treatment group (97.1%, 132 of 136 subjects), 133 subject in the 1.7 gram per day treatment group (93.7%, 133 of 142 subjects), 64 subjects in the 2.6 gram per day treatment group (92.8%, 64 of 69 subjects), and 128 subjects in the placebo treatment group (93.4%, 128 of 137 subjects).

Subject disposition in Study EST005 is shown in the following table.

**Table 21: Subject Disposition (Study EST005) (All Randomized Subjects)**

| | Number (%) of Subjects | | | |
|---|---|---|---|---|
| **Subject Disposition** | **Estradiol Gel 0.87 gram/day (N = 136)** | **Estradiol Gel 1.7 grams/day (N = 142)** | **Estradiol Gel 2.6 grams/day (N = 69)** | **Placebo (N = 137)** |
| **Randomized** | 136 (100%) | 142 (100%) | 69 (100%) | 137 (100%) |
| **Completed Study** | 132 (97.1%) | 133 (93.7%) | 64 (92.8%) | 128 (93.4%) |
| **Prematurely Discontinued** | 4 (2.9%) | 9 (6.3%) | 5 (7.2%) | 9 (6.6%) |
| **Reasons Discontinued** | | | | |
| Adverse Event | 1 (0.7) | 5 (3.5) | 2 (2.9) | 9 (6.6) |
| Non-compliance | 0 (0.0) | 0 (0.0) | 1 (1.4) | 1 (0.7) |
| Lack of Efficacy | 1 (0.7) | 0 (0.0) | 1 (1.4) | 1 (0.7) |
| Estradiol ≥ 2.0 ng/dL | 1 (0.7) | 0 (0.0) | 0 (0.0) | 1 (0.7) |
| Withdrew Consent | 0 (0.0) | 3 (2.1) | 1 (1.4) | 1 (0.7) |
| Lost to Follow-up | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (1.5) |
| Other | 1 (0.7) | 1 (0.7) | 0 (0.0) | 2 (1.5) |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 26; Table 10.1-1; page 71 (page 91 of 369) and Volume 61, Table 8.7.3-1, page 52 of 165.

*Medical Officer's Comments:*

*As shown in Table 21, a total of 27 subjects (5.6%, 27 of 484 subjects) prematurely discontinued: 33.3% due to an adverse event (9 of 27 subjects), 18.5% due to withdrawn consent (5 of 27 subjects), 14.8% due to "other" (4 of 27 subjects), 11.1% due to lack of efficacy (3 of 27 subjects), and 7.4 % due to non-compliance and loss to follow-up (2 of 27 subjects for each).*

*In summary, the rates of discontinuations in Phase 3 Study EST005 were low compared with discontinuations rates from other controlled clinical trials submitted to DRUP and similar between groups. The discontinuation rates observed in Study EST005 do not raise safety concerns.*

Subject disposition in Study EST005 and Study EST004 across all treatment groups is shown in the following table.

**Table 22: Subject Disposition: All Subjects Entering the Double-Blind Treatment Period (Study EST005)**

| | Number (%) of Subjects | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject Disposition** | **Estradiol Gel 0.625 g/day 4 weeks** | **Estradiol Gel 0.87g/day 12 weeks** | **Estradiol Gel 1.25 g/day 4 weeks** | **Estradiol Gel 1.7 g/day 12 weeks** | **Estradiol Gel 2.5/2.6 g/d 4 weeks[a]** | **Estradiol Gel 2.6 g/day 12 weeks** | **Placebo 4 weeks/12 weeks** |
| **Entered DB Period[b]** | 41 (6.4) | 136 (21.1) | 40 (6.2) | 142 (22.0) | 107 (16.6) | 69 (10.7 | 179 (27.8) |
| **ISS Safety Population[c]** | 41 (100.0) | 136 (100.0) | 40 (100.0) | 142 (100.0) | 107 (100.0) | 69 (100.0) | 179 (100.0) |
| **Completed DB Period[c]** | 41 (100.0) | 132 (97.1) | 37 (92.5) | 133 (93.7) | 106 (99.1) | 64 (92.8) | 169 (94.4) |
| **Discontinued DB Period** | 0 | 4 (2.9) | 3 (7.5) | 9 (6.3) | 1 (0.9) | 5 (7.2) | 10 (5.6) |
| **Reasons for Discontinued[c]** | | | | | | | |
| Adverse event[d] | 0 | 1 (0.7) | 3 (7.5) | 5 (3.50 | 1 (0.9) | 2 (2.9) | 1 (0.6) |
| Withdrew consent | 0 | 0 | 0 | 3 (2.1) | 0 | 1 (1.4) | 1 (0.6) |
| Lack of efficacy | 0 | 1 (0.7) | 0 | 0 | 0 | 1 (1.4) | 1 (0.6) |
| Non-compliance | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 1 (0.6) |
| Lost to follow-up | 0 | 0 | 0 | 0 | 0 | 0 | 2 (1.1) |
| Other[e] | 0 | 2 (1.5) | 0 | 1 (0.7) | 0 | 0 | 4 (2.2) |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 62, Table 8.8.3-4, page 46 of 277.
DB = Double-blind.

a. Includes EST004 subjects assigned to 2.5 g/day estradiol gel for four weeks and EST005 subjects assigned to 2.6 g/day estradiol gel for 12 weeks. In this column, EST005 subjects were considered completed if their treatment duration was ≥28 days; those treated estradiol gel All Doses 4-12 weeks" columns (subjects counted once in latter column).
b. Randomization to DB treatment groups occurred at the beginning of the single-blind placebo lead-in period in Study EST004 and at the beginning of the DB treatment period in EST005. Percentages are based on the total number of all subjects <28 days were considered discontinued. Completion/withdrawal status of EST005 subjects for the entire 12-week DB treatment period is presented in the estradiol gel 2.6 g/day 12 weeks and estradiol gel groups who entered the DB treatment period across all treatment groups combined for both studies (i.e., 161 EST004 + 484 EST005 = 645 DB subjects) to show the proportional contribution from each treatment group.
c. Percentages are based on the number of subjects who entered the DB treatment period for each treatment group (or pooled treatment group) shown.
d. Three EST004 1.25 g/day estradiol gel subjects discontinued due to adverse events (AEs) of headache (Subject 255), dizziness (Subject 146), and gastroenteritis (Subject 262). Nine EST005 subjects discontinued due to AEs of pressure sensation in legs (Subject 846), weight gain (Subject 130), vaginal bleeding (Subject 251), migraine (Subject 256), rash (Subject 692), muscle pain (Subject 606), thickened endometrial lining ( Subject 684), staphylococcus infection of thumb (Subject 106), and breast swelling (Subject 880).
e. Other reasons subjects discontinued during the DB period were, for EST004, an abnormal screening mammogram (Subject 211) and, for EST005, subject did not like gel formulation (Subject 178), initiation of excluded medications (Subject 284), abnormal mammogram (Subject 381), did not meet entry criteria (Subject 711), and serum estradiol >2.0 ng/dL (Subjects 640 and 890).

*Medical Officer's Comments:*

*The discontinuation rates during the double-blind treatment in Studies EST004 and EST005, as shown in Table 22, were low across all treatment groups and do not appear to be clearly related to the estradiol gel dose or to the duration of treatment. However, when compared to discontinuations rates in the placebo-treated subjects across Studies EST004 and EST005 (0.6%, 1 of 179 subjects), discontinuations due to adverse event across all estradiol gel treatment groups combined are greater in the estradiol gel-treated subjects (2.4%, 11 of 466 subjects).*

### 7.1.3.2 Adverse events associated with dropouts

Among the different study periods in Study EST005, adverse events leading to discontinuation included:

- Placebo (single-blind) = "moodiness" (Subject 908) and "chest pain resulting in hospitalization" (Subject 913).
- Placebo (double-blind) = "breast swelling" (Subject 880).
- 0.87 gram/day estradiol gel = "muscle pain" (Subject 606).
- 1.7 gram/day estradiol gel = "Staphlococcal infection" (Subject 106), "weight gain" (Subject 130), "thickened endometrial lining" (Subject 684), "rash" (Subject 692), and "pressure sensation in legs" (Subject 846).
- 2.6 gram/day estradiol gel = "vaginal bleeding" (Subject 251) and "migraine" (Subject 256).

Among the different study periods in Study EST004, adverse events leading to discontinuation included:

- 1.25 gram/day estradiol gel = "Headache" (Subject 255), "dizziness" (Subject 146), and gastroenteritis (Subject 262).

*Medical Officer's Comments:*

*The discontinuation rates observed across Studies EST004 and EST005 do not raise safety concerns.*

### 7.1.3.3 Other significant adverse event

An increased incidence of shifts from baseline transvaginal ultrasound (TVUS) measurements ≤ 4 mm to > 4 mm at the end-of-study visit in Study EST005, and normal to abnormal endometrial biopsies was observed with the 1.7 gram per day and the 2.6 gram per day estradiol gel treatment groups in 12-week Study EST005.

Endometrial Hyperplasia:

Endometrial hyperplasia is known to occur with unopposed estrogen use in women with a uterus. Endometrial hyperplasia is frequently used as a surrogate endpoint for the evaluation of endometrial safety. The hard endpoint for endometrial safety is endometrial cancer. However, the observance of endometrial cancer often requires long-term treatment in a large number of women with a uterus. Published literature reports that 1.6% of cases of hyperplasia without atypia will convert to cancer (women followed for an average period of 13.4 years), and 23% of cases of hyperplasia with atypia will convert to cancer.[1]

In four-week Study EST004, subjects with a uterus had a TVUS performed at baseline, followed by saline infusion in the event the TVUS was not effective. In 12-week Study EST005, subjects with a uterus had an endometrial biopsy performed at baseline and end-of-study. A TVUS was performed subsequent to the endometrial biopsy in Study EST005, in the event that the

1 Kurman RJ et al. The Behavior of Endometrial Hyperplasia, A Long-Term Study of "Untreated" Hyperplasia in 170 Patients. Cancer. 1985; 56(2):403-12.

endometrial biopsy did not provide sufficient endometrial tissue for assessment. A total of 304 subjects with a uterus participated in Study EST005 as shown in Table 23.

**Table 23: Number of Randomized Subjects with Endometrial Biopsies and Transvaginal Ultrasounds at Baseline and End-of-Study (Study EST005)**

| | Number of Subjects | | | | |
|---|---|---|---|---|---|
| | **Estradiol Gel 0.87 g/day (N=136)** | **Estradiol Gel 1.7 g/day (N=142)** | **Estradiol gel 2.6 g/day (N=69)** | **Placebo (N=137)** | **Total (N=484)** |
| **Randomized Subjects with a Uterus (n, %)** | 81 (59.5) | 95 (66.9) | 45 (65.2) | 83 (60.6) | 304 (62.8) |
| **Baseline** | | | | | |
| EMB Only | 72 | 80 | 39 | 69 | 260 |
| Both EMB and TVUS | 0 | 3 | 0 | 3 | 6 |
| TVUS Only | 9 | 12 | 6 | 11 | 38 |
| **End of Study** | | | | | |
| EMB Only | 64 | 79 | 37 | 63 | 243 |
| Both EMB and TVUS | 2 | 4 | 2 | 4 | 12 |
| TVUS Only | 13 | 11 | 4 | 8 | 36 |
| Neither EMB or TVUS | 2 | 1 | 2 | 8 | 13 |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 62, Table 8.8.6-4, page 129 of 277.
EMB = Endometrial biopsy.
TVUS = Transvaginal ultrasound.

*Medical Officer's Comments:*

*Sixty-three percent (62.8%) of the 484 treated subjects in 12-week Study EST005 had uteri. The percentage of randomized subjects with a uterus per treatment group in Study EST005 appears similar.*

*Table 23 shows that 87.5% of the 304 subjects with a uterus in Study EST005 underwent an endometrial biopsy at baseline (266 of the 304 subjects with a uterus), while 12.5% (38 of 304 subjects) had only transvaginal ultrasounds performed. Similar percentages are observed for end-of-study endometrial assessments (83.9%, 255 subjects had an endometrial biopsy performed; and 11.8%, 36 subjects had only a TVUS performed). However, at end-of study, 13 subjects (4.3%) had neither procedure performed.*

*Overall, the percentage of subjects who had an endometrial biopsy and/or a TVUS performed at end-of-study is high, 95.7% (291 of 304 subjects with a uterus).*

In 12-week Study EST005, a total of 6 cases of endometrial hyperplasia were reported: one case of complex hyperplasia with atypia in the 1.7 gram per day estradiol gel treatment group (incidence rate of 1.05%, one case per 95 subjects with a uterus) and five cases of simple hyperplasia in the 2.6 gram per day estradiol gel treatment group (incidence rate of 11.1%, five cases per 45 subjects with a uterus). Complex hyperplasia with atypia is felt to be the histologic classification most likely to progress to endometrial cancer.

*Medical Officer's Comments:*

*See Section 7.1 Methods and Findings for a description of the endometrial histologic classifications used in Study EST005 to determine "normal endometrium" from "abnormal endometrium".*

The following information was provided in the ISS regarding abnormal endometrial biopsy results at final visit in Study EST005.

**Table 24: Subjects With Abnormal Endometrial Biopsy at Final Visit (Study EST005) (ISS Safety Population)**

| Study Drug | Subject Number (Study day) | Age (year) Race | Biopsy Description[a] | | Trough Estradiol (pg/mL) | Comments |
|---|---|---|---|---|---|---|
| | | | Baseline | End-of-Study | | |
| Estradiol Gel 1.7 g/day | 313 (day 90) | 54 White | Strips of benign surface | Atypical hyperplasia (10) | Baseline:<10<br>Week 4: 15<br>Week 8: 18<br>Week 12: 13 | Biopsy showed complex hyperplasia with atypia. Fractional dilatation and curettage pathology: focal squamous metaplasia, benign endometrial polyps with focal hyperplasia, simple and complex, without atypia. Follow-up: 3-months progestin treatment followed by dilatation and curettage. Subject discontinued progestin after < 1 month. No further treatment (as of Jan. 2005) |
| Estradiol Gel 2.6 g/day | 129 (day 92) | 62 White | Inactive/ atrophic | Simple hyperplasia without atypia (8) | Baseline:<10<br>Week 4: 21<br>Week 8: 69<br>Week 12:<10 | Subject received Provera. Repeat biopsy approximately 3 months later showed inactive/ atrophic endometrium |
| | 173 (day 86) | 60 White | Strips of benign surface | Simple hyperplasia without atypia (8) | Baseline:<10<br>Week 4: 39<br>Week 8: 47<br>Week 12:<10 | Subject received Provera. Repeat biopsy approximately 3 months later showed inactive/ atrophic endometrium |
| | 271 (day 86) | 69 White | Inactive/ atrophic | Simple hyperplasia without atypia (8) | Baseline:<10<br>Week 4: <10<br>Week 8: 20<br>Week 12: 47 | Subject received progestin and had bleeding. No other follow-up |
| | 302 (day 93) | 56 White | Inactive/ atrophic | Simple hyperplasia | Baseline:<10<br>Week 4: 39 | Subject received Novo-Medtrone (3 days light |

| Study Drug | Subject Number (Study day) | Age (year) Race | Biopsy Description[a] Baseline | Biopsy Description[a] End-of-Study | Trough Estradiol (pg/mL) | Comments |
|---|---|---|---|---|---|---|
| | | | | without atypia (8) | Week 8: 52<br>Week 12: 41 | bleeding). Repeat biopsy showed no evidence of malignancy or hyperplasia. |
| | 375<br>Day 89) | 54<br>White | Inactive/ atrophic | Simple hyperplasia without atypia (8) | Baseline:<10<br>Week 4: 37<br>Week 25: 25<br>Week 12: 17 | Subject received Prometrium (no bleeding). No other follow-up |

Source: Adapted from NDA 21-813/S-000, Section 8, Volume 62, Table 8.8.6-5, page 131 of 277 and Table 2.3 in submission dated June 13, 2006, page 1247, and Appendix 16.2.7.2: Adverse Events, Volume 44, pages 1-114.

a. Endometrial biopsy results were classified into 1 of 11 categories of which 1-6 were normal and 7-11 were abnormal: 1=strips of benign surface and glandular lining epithelium; 2=inactive/atrophic endometrium; 3=proliferative endometrium; 4=progestational secretory endometrium; 5=menstrual type endometrium; 6=polyp; 7=polyp; 8=simple hyperplasia without atypia; 9=complex hyperplasia without atypia; 10=atypical hyperplasia; 11=cancer.

*Medical Officer's Comments:*

*As noted in Table 24, no subjects in the 0.87 gram per day estradiol gel treatment group were diagnosed with endometrial hyperplasia in 12- week Study EST005. Subject 313 in the 1.7 gram per day estradiol gel treatment group was diagnosed with complex hyperplasia with atypia by endometrial biopsy collected on [ ]. Her baseline endometrial biopsy, collected on [ ], showed strips of benign surface and glandular endometrium. The single examining pathologist recommended "follow-up procedures to rule out possibility of an adenocarcinoma remaining in uterine cavity." A fractional dilation and curettage, performed on [ ], showed "endometrial curettings and polyps: benign endometrial polyps with focal hyperplasia, simple and complex, without atypia. Per the submission, Subjects 313 discontinued the recommended three months of progestin therapy after < one month. No further information is available for this subject. This case of complex hyperplasia with atypia in the 1.7 gram per day estradiol gel treatment group, although worrisome for the severity of the hyperplasia, is only a single case of hyperplasia. In DRUP's experience, one or no cases of complex hyperplasia with atypia or endometrial cancer have been previously reported in 12-week clinical trials.*

*The five remaining cases of endometrial hyperplasia, all in the 2.6 gram per day estradiol gel treatment group, were diagnosed as simple hyperplasia without atypia (see Table 24). These five cases of simple hyperplasia because of the incidence rate in the 2.6 gram per day estradiol gel treatment group raise safety concerns for this reviewer.*

BioSante Pharmaceuticals, Inc. was advised by DRUP that the reported finding of one case of endometrial hyperplasia with atypia by scheduled endometrial biopsy at the 1.7 gram per day estradiol gel dose and the five cases of simple hyperplasia without atypia by scheduled endometrial biopsy at the 2.6 gram per day estradiol gel dose in 12-week Study EST005 raised serious safety concerns.