Clinical Pharmacology Review

"Filing Review for Divigel® (estradiol gel 0.1%)"

NDA:	22-038
Related IND (s):	51, 246
Sponsor:	Upsher-Smith Laboratories (USL)
Type:	NDA filing meeting package
Drug:	estradiol gel 0.1%
Dose (s) proposed:	0.25 mg, 0.5 mg, 1 mg/day
Indication:	Vasomotor Symptoms (VMS) (b) (4)
Submission date:	May 1, 2006
Draft review:	June 1, 2006
Reviewer:	Sandra Suarez-Sharp, Ph.D.

Executive Summary

At menopause, estrogen levels are decreased and VMS result from estrogen withdrawal. Estrogen for hormone replacement therapy is well established for treatment of postmenopausal symptoms and prevention of osteoporosis, VMS and VVA.

Oral therapies with estradiol for treatment of VMS and VVA include Estrace®, Activella®, and Ortho-Prefest®. These products generally recommend starting treatment at 1 mg estradiol/day with an option to increase to 2 mg/day if needed. Numerous transdermal delivery systems are available and include Combipatch®, Climara®, Alora®, Vivelle®, and Esclim®. The more recently approved products provide delivery rates ranging from 0.025 mg to 0.1 mg/day. These products recommend starting at a delivery rate of 0.025 mg/day and increasing the dose if needed to control symptoms while using the lowest effective dose.

Divigel® (Estradiol Gel, 0.1 %), is an alcohol-based estrogen gel for topical (skin) administration developed by Orion Pharma. This product is intended for once daily administration to postmenopausal women for the treatment of moderate to severe VMS (b) (4) Divigel® is being proposed in three doses of 0.25, 0.5, and 1.0 g for topical application (corresponding to 0.25, 0.5, and 1.0 mg estradiol, respectively). The active ingredient, estradiol, is a naturally occurring hormone (derived from a plant source). The clinical formulation, USL-221, which was used in the submitted Phase 1 and pivotal Phase 3 studies conducted in the U.S., and is the formulation intended for marketing is a smooth and opalescent gel with the active ingredient in dissolved form.

This submission contains six Clinical Pharmacology Studies as follows: **Study P04-003** was conducted to assess the linearity of the pharmacokinetic (PK) profile as the applied topical dose was increased from 0.25 to 1.0 mg estradiol; **Study P04-002** evaluated the potential for transferability from the patient to a non-dosed individual; **Study P04-005** evaluated the effects of washing the site of application at selected times post-administration. In addition to these Phase 1 studies, limited PK samples were collected in the Phase 3 study (**Study P04-001**) to determine serum estradiol and its metabolites concentrations and attempted a population PK analysis. The potential effects of demographic and baseline characteristics and concomitant medications on estradiol and its metabolites PK were investigated using this population PK analysis. A bioequivalence study conducted by Orion Pharma (**Study FR00.037.2**) compared a new formulation (EFI08; now referred to as USL-221) to the original formulation. A pilot study conducted by USL (**Study P04-015**) was also carried out to collect swab samples for analytical method development to determine residual levels of estradiol remaining on the skin before and after washing the application site.

According to the sponsor, the above studies showed that washing the Divigel® application site resulted in a decrease in total exposure of mean baseline-corrected estradiol by approximately 27% and that only up to 1 % of the applied dose was detectable at the application

site by swab analysis at 1 and 8 hours post-dose. Washing the application site for 3 minutes removed all detectable amounts of estradiol from the application site. No significant increases in average concentrations of estradiol, and its metabolites compared with baseline values were found in non-dosed subjects (transferability study). There was no difference in the transfer potential of Divigel® from dosed subjects to non-dosed subjects when contact was made 1 hour after dosing compared with contact made 8 hours after dosing. The sponsor also stated that Estradiol Gel, 0.1 % formulation EFI08 was bioequivalent to the original formulation of Estradiol Gel, 0.1 % based on AUC, but not for Cmax. Based on the population PK analysis median serum estradiol concentrations were stable over time, indicating little or no accumulation of estradiol. The serum estradiol concentrations increased in a dose proportional manner as reflected by median serum estradiol concentrations of 16.2, 30.8, and 61.9 pg/mL during Week 12 for the 0.25, 0.5, and 1.0 g doses, respectively. It was stated that none of the demographic characteristics, renal function, hepatic function, and concomitant medications had a significant effect on the PK of E2, El, or ES. The sponsor is relying on current knowledge base to address the distribution, excretion, metabolism and drug-drug interaction potential of estradiol.

Study P04-001 is being considered as the primary efficacy study in this submission. Study P04-001, is a 12-week, randomized, placebo-controlled, double-blind, Phase 3 multi-center study that included three dose levels of topical Estradiol Gel, 0.1 % (0.25, 0.5 and 1 mg/day).

The sponsor has submitted a reviewable Clinical Pharmacology package for this NDA and therefore, there are no filing issues. The following comments should be conveyed to the sponsor:

1. Information on the effect of sunscreen products, topically applied skin creams or lotions on the systemic exposure to Divigel® was not included in the present submission. Provide available information on this issue. Otherwise, this lack of information and the potential impact on safety (based on available information from other related products) will be specified in the final Package Insert for Divigel®.
2. Submit the following datasets to support the population analysis:
    - All datasets used for model development and validation should be submitted as a SAS transport files (*.xpt). A description of each data item should be provided in a Define.pdf file. Any concentrations and/or subjects that have been **excluded from the analysis** should be flagged and maintained in the datasets.
    - Model codes or control streams and output listings should be provided for all major model building steps, e.g., base structural model, covariates models, final model, and validation model. These files should be submitted as ASCII text files with *.txt extension (e.g.: myfile_ctl.txt, myfile_out.txt).
    - A model development decision tree and/or table which gives an overview of modeling steps.

RECOMMENDATION

The Office of Clinical Pharmacology, the Division of Clinical Pharmacology III (OCP/DCP-III) has reviewed the NDA 22-038 package for filing. The NDA is filable from an OCP standpoint.

Sandra Suarez-Sharp, Ph.D.
Pharmacokinetics Reviewer, DPEIII, OCP

Concurrence:

Ameeta Parekh Ph. D.
Team Leader, DCPIII, OCP

cc:
HFD-580 Div., Patsner, Lyght, Slaughter
OCP/DCPIII Hunt, Parekh, Bashaw, Gobburu, Suarez-Sharp

INTRODUCTION

Formulation

Estradiol Gel, 0.1% is a smooth, clear to opalescent gel in which the active ingredient, estradiol, is dissolved. Estradiol is absorbed following application of the gel to the skin. The 0.1 % bulk gel is packaged into three different weight single-dose foil-laminate packets (0.25, 0.5 and 1 g, corresponding to 0.25, 0.5 and 1.0 mg of estradiol, respectively). The 0.1 % bulk gel is manufactured, packaged, tested and released at Orion Pharma in Turku, Finland and is distributed by the NDA holder, Upsher-Smith Laboratories, Inc. in Minneapolis, MN. The formulation is provided in Table 1.

Table 1. Formulation Summary- Theoretical Delivered Dose

INGREDIENT	COMPLIES WITH USP/NF SPECIFICATIONS	FUNCTION	FORMULATION USL-221/ EF108 0.1% GEL (MG/G)	AMOUNT PER 0.25 G DOSE	AMOUNT PER 0.5 G DOSE	AMOUNT PER 1.0 G DOSE
Estradiol	Estradiol, USP	Active ingredient	1.0*	0.25 mg	0.5mg	1.0mg
Carbomer (b) (4)						
Triethanolamine						
Propylene Glycol						
Ethanol (b) (4)						
Purified Water						
			Total Fill	0.325 g	0.575 g	1.075 g

*Quantity adjusted according to assay and water content

Formulation Development

The history of the proposed U.S. commercial formulation consists of two main formulations. The original formulation was used in clinical studies sponsored by Orion and was available commercially outside of the U.S. from 1994 until 2002/2003. (b) (4)

Estradiol Gel, 0.1 % has been registered and marketed in over 30 countries. According to the sponsor, bioequivalence according to the protocol definition between the current Estradiol Gel, 0.1 % (USL-2211EFI08) and the original Estradiol Gel, 0.1 % was demonstrated in bioavailability study FR 00.037.2.

(b) (4)

Thirty-four of the clinical studies conducted by Orion, (b) (4) USL are included in this NDA submission. The majority of the studies included in this submission (conducted by Orion (b) (4)) utilized the original formulation of Estradiol Gel, 0.1 %,

although both sponsors also conducted a few clinical studies with the EF108 formulation. In contrast, USL's clinical development program (four Phase 1 studies and one Phase 3 study) used only the EFI08 formulation of Estradiol Gel, 0.1 % (USL-221).

Currently Marketed Estradiol Formulations

Numerous estradiol transdermal delivery systems are also available in the United States. Although few studies provide a direct comparison of oral versus transdermal estradiol, available data suggest that 1 mg daily oral dose of estradiol is approximately equivalent to transdermal delivery of approximately 0.5 mg/day. Some of the commonly prescribed transdermal systems include Estraderm®, Alora®, Climara®, Vivelle-Dot® and Esclim™. The more recently approved products (Climara®, Vivelle-Dot® and Esclim™) provide systemic delivery rates ranging from 0.025 to 0.1 mg/day. The Cmax levels from these products at the approved doses range from 32 pg/mL to 145 pg/mL[1]. Generally, the recommended starting systemic delivery rate for these products is 0.025 mg/day, increasing the dose if needed to control symptoms while recommending to ultimately determine the lowest effective dose for the patient. Older products such as Estraderm® and Alora® lack the 0.025 mg/day systemic delivery rate; a 0.05 mg/day systemic delivery is the lowest available dose for these two products. In clinical studies, systemic delivery rates of 0.025 and 0.0375 mg/day transdermally have been shown to provide significant reductions in frequency and severity of VS in many women.

Topical therapies that provide systemic delivery of estradiol are available including EstroGel® and Estrasorb™. Although both products recommend use of the lowest effective dose to treat menopausal symptoms, only one dose is currently approved for each product. The estradiol Cmin following Estrasorb 2.5 mg administration was 63 pg/mL at steady state.

Clinical Pharmacology

Study P04-003 was considered a key study with the primary objective of evaluating the single-dose and multiple-dose PK profiles of estradiol, estrone and estrone sulfate following topical administration of USL-221 at three dose levels of estradiol (0.25,0.5 and 1.0 mg) when dosed for 14 consecutive days to postmenopausal women (N=21). This was a randomized, open-label, multiple-dose study utilizing a three-way crossover design with blood sampling on Days 1 and 14 for PK analysis. Each treatment arm consisted of an application of USL-221 to a 200 cm^2 area of the thigh.

According to the sponsor, this study demonstrated linear and dose-proportional estradiol PK at steady state for both AUC_{0-24} and C_{max} following once daily dosing (Table 2).

Table 2. Mean (%CV) AUC_{0-24} and C_{max} for Estradiol on Day 14 Following Multiple Daily Doses of Divigel

Parameter (units)	DIVIGEL 0.25 g	DIVIGEL 0.5 g	DIVIGEL 1.0 g
AUC_{0-24} (pg•h/mL)	236 (94)	504 (149)	732 (81)
C_{max} (pg/mL)	14.7 (84)	28.4 (139)	51.5 (86)

[1] Data taken from PDR online.

The effects of washing at various times after application of USL-221 were evaluated in **Study P04-005**. This was a randomized, open-label, single-dose, three-way, incomplete block, crossover study with four treatments, each consisting of an initial single application of 1.0 g of USL-221 (containing 1.0 mg estradiol) to a 200 cm^2 area of the thigh. All subjects (N=16) received the first two treatments consisting of the application of USL-221 followed by: (1) washing of the application site 60 minutes later and (2) no washing of the application site. Following the completion of the first two treatments, the subjects were randomly assigned to one of two treatments for determination of residual estradiol remaining on the skin at the application site. A 10 cm^2 area of the application site was swabbed prior to dosing and at either 60 minutes or 8 hours after application of USL-221. The application site was washed and a third swab was obtained 15 minutes after the start of washing. All washing was conducted with mild hypo allergenic soap and a washcloth for 30 seconds followed by rinsing with warm water for 2.5 minutes. There was a 14-day washout period between treatments.

According to the sponsor, washing the application site with soap and water 1 hour after application removed all detectable amounts of estradiol from the surface of the skin, and resulted in a 27% decrease in the mean total 24-hour exposure to estradiol.

Study P04-002 was designed to assess the potential transfer of USL-221 from the skin of a dosed individual to a non-dosed individual following direct contact. This was a randomized, open-label, single-dose, three-way crossover study with a 14-day washout period in healthy adult male and postmenopausal female volunteers. Since statistical comparisons were made on change from Baseline in each treatment group, data from all non-dosed subjects who participated in the study and completed one treatment according to the protocol were used in the PK analysis. Dosed subjects each received treatments consisting of a single application of 1.0 g of USL-221 (containing 1.0 mg estradiol) to a 200 cm^2 area of the thigh. One treatment period consisted of contact with an unclothed application site at 60 minutes after dosing; a second treatment period consisted of contact with a clothed application site at 60 minutes after dosing and a third treatment period consisted of contact with an unclothed application site at 8 hours after dosing. For each treatment period, the non-dosed subject rubbed the anterior portion of his /her forearm over the dosed subject's application site for 5 minutes (10-15 rubs per minute) and then maintained contact with the same forearm at the application site for another 10 minutes without the rubbing motion. Blood sampling of non-dosed subjects was performed for 72 hours after contact for pharmacokinetic analysis.

According to the sponsor, no increase in mean serum concentration of estradiol was observed in non-dosed subjects after 15 minutes of direct contact with the application site at 1 or 8 hours post-dose, indicating no evidence of transfer.

The primary objective of **Study FR00.037.2** was to assess the bioequivalence of two benzene-free Estradiol Gel, 0.1 % test formulations to the original formulation that was marketed in Europe. This was a randomized, three-way crossover study in 27 healthy, postmenopausal female patients who received the original formulation, test formulation EFI07 and test formulation EFI08. Each treatment period consisted of application of 1 g of gel on a skin area of 400 cm^2 (thigh) once daily for 14 days. There was no washout between the treatment periods.

Study P04-015 was a study conducted to collect swab samples to aid in analytical method development to determine the residual amount of estradiol remaining on the skin before and after washing the application site, in postmenopausal women dosed with USL-221 versus placebo gel. The study was designed as an open-label, single-dose, pilot study consisting of 3 subjects dosed once in a single study period.

The objective of the **population** PK analysis was to develop population PK models for estradiol (E2), estrone (El), and estrone sulfate (ES), following administration of Estradiol Gel, 0.1 %. These models were then applied to estimate the population and individual PK parameters and steady-state concentrations of E2, El, and ES in postmenopausal patients following once daily application of USL-221 at three estradiol dose amounts (0.25, 0.5, and 1 mg) (Study P04-001) in postmenopausal female patients. The potential effects of demographic and baseline characteristics and concomitant medications on E2, El, and ES pharmacokinetics following USL-221 application were also investigated using the population PK analysis.

Sparse PK samples were collected at baseline ant then within 1-10 hrs of the morning dose at weeks 4, 8, and 12 from Study P04-001 for measurements of serum concentrations of E2 and its two metabolites (El and ES). Serum samples (n=1,291) collected from 327 female patients were included in the population PK analysis. PK data obtained from postmenopausal women in two Phase 1 studies (P04-003 and P04-005) utilizing an intensive sampling schedule were used to develop the structural PK models for E2 and its two metabolites. According to the sponsor, one-compartmental models with linear disposition and sequential zero-order and first-order absorption incorporating lag time best described the serum profiles of E2 and its metabolites. The following covariates were included in the population PK analysis: uterus status, estradiol, sex hormone binding globulin (SHBG), and PSH levels at screening, estradiol dose, race, age, body weight, BMI, renal and hepatic functions and concomitant medications.

According to the sponsor, median serum estradiol concentrations were stable over time, indicating little or no accumulation of estradiol. The serum estradiol concentrations also increased in a dose proportional manner as reflected by median serum estradiol concentrations of 16.2, 30.8, and 61.9 pg/mL during Week 12 for the 0.25, 0.5, and 1.0 g doses, respectively (Table 3). None of the demographic characteristics (age, uterus status, race, and body weight), renal function, hepatic function, and concomitant medications taken by the patients in the Phase 3 study had a significant effect on the PK of E2, El, or ES following daily administration of USL-221 across the 0.25 to 1.0 mg estradiol dose levels.

Table 3: Median Serum Estradiol and Estrone Concentrations and E2/E1 Ratios during Week 12 with Daily Dosing of DIVIGEL

	DIVIGEL 0.25 g (N=122)	**DIVIGEL 0.5 g (N=123)**	**DIVIGEL 1.0 g (N=125)**	**Placebo (N=125)**
Estradiol (E2) (pg/mL)	16.2	30.8	61.9	2.99
Estrone (E1) (pg/mL)	33.3	44.9	65.2	19.0
E2/E1 Ratio*	0.49	0.69	0.95	0.16

*Normal ratio observed in the early follicular phase of premenopausal women is 0.5 to 1.0

Bioanalytical Methods

The measurement of serum concentrations of estradiol and estrone in one method and for estrone sulfate in a separate assay was accomplished by LC/MS/MS. Both methods were developed and validated at PPD Development, Richmond VA. According to the sponsor, the lower limits of quantification (LLOQ) were 2.5, 5.0 and 50.0 pg/mL for estradiol, estrone and estrone sulfate, respectively. An assay was also developed and validated at PPD to assess the residual concentrations of estradiol remaining on skin by swabbing a proscribed skin surface area at various times after dose application. An assay range of 50 to 1000 ng/swab was established for use in the analytical method for swab analysis and the method was determined by the sponsor to be acceptable for quantifying estradiol samples via HPLC with MS/MS detection for use in subsequent studies using USL-221. The analytical method used for the measurement of estradiol in human serum samples from the Orion-conducted bioequivalence study (Study FR00.037.2) was a radioimmunoassay (RIA) that was developed and validated at Medix Diacor Laboratory Services, Espoo Finland.

***In vitro* Release Testing**

A method for evaluation of in vitro release, using an automated Franz Cell apparatus with a polysulfone synthetic membrane, was developed and validated by K.A.B.S. Laboratories. The amount of drug released per unit area ($\mu g/cm^2$) is plotted against the square root of time and the release rate is determined by calculating the slope of the line. An average of six determinations (slopes) is used for each sample.

In vitro release tests were carried out for different Estradiol Gel, 0.1 % batches and formulations. According to the sponsor, studies with different formulations of Estradiol Gel, 0.1 % showed a slight difference in estradiol release rate between the Original Formulation and the USL-221/EF108 formulation, whereas no difference was seen between the non-equivalent formulations of EFI07 and EFI08. SUPAC-SS comparisons showed no statistical differences between the three formulations tested. The sponsor stated that freshly manufactured Estradiol Gel, 0.1 % batches showed minimal variation on the release rate of estradiol as a function of viscosity. Aging of the product had no effect on the release rate since the release rates of the long-term stability samples were similar to the results for the freshly manufactured batches.

According to the sponsor, the proposed specification limits are intended to encompass the variability between both the batches of product and between different runs of the in vitro test. Using data from the study and three times standard deviation -around the minimum and maximum observed values, the proposed acceptance criteria for the average slope is between 4.40 and 8.15 $\mu g/cm^2/min^{0.5}$. The average slope will be based on a minimum of five out of six individual determinations.

Clinical Studies

Study P04-00 1 is being considered as the primary efficacy study in this submission. Study P04-001 was a randomized, parallel, placebo-controlled, double-blind, multicenter study in postmenopausal women with MSVS. Patients (495 enrolled patients; aprox. 120 /group) received treatment with USL-221 (Estradiol Gel, 0.1%) or placebo for 12 weeks. Additionally, the study evaluated postmenopausal women with complaints of VVA. This study consisted of a screening period, four study visits (Visits 2-5) for

patients without an intact uterus, and five study visits (Visit 2-6) for patients with a uterus.

The primary objective was to compare the change from baseline in mean daily frequency and severity of moderate to severe vasomotor symptoms (MSVS) at weeks 4 and 12 between USL-221 and placebo. The secondary objective was to assess the effect of USL-221 versus placebo on vulvar and vaginal atrophy (VVA): specifically – the change in the moderate to severe symptom identified as most bothersome by the patient, between baseline and week 12; the change in vaginal pH between baseline and week 12; and the change in vaginal maturation index (VMI) between baseline and week 12.

This reviewer's Comments

The table below summarizes the overall content of the Clinical Pharmacology information provided by the sponsor to support the request for the approval of this NDA. The sponsor has submitted a reviewable package for this NDA and therefore, there are no filing issues.

Study Title/Description	Tabular listing/PK summary	Analytical method	PK parameters	Statistical analysis
Study P04-002: Randomized, Open-Label, Single-Dose, 3-Way Cross-over Study of the Transferability of USL-221 During Skin-to-Skin Contact With and Without clothing.	√	√	√	√
Study P04-003: Randomized, Open-Label, Multiple-Dose, 3-Way Cross-over Pharmacokinetic Study Evaluating Three Dose Levels of USL-221.	√	√	√	√
Study P04-005: Randomized, Open-Label, Single-Dose, 3-Way Crossover Study of the Washability of USL-221.	√	√	√	√
Study P04-001: Placebo-Controlled, Randomized, Double-Blind, Multicenter Study, to Demonstrate the Efficacy of 12 Weeks of Treatment With USL-221 on Moderate to Severe Vasomotor Symptoms and Vulvar/Vaginal Atrophy in Postmenopausal Patients.	√	√	√	√
Study P04-015: Single dose, open label study for analytical method development to determine residual levels of estrogen remaining in the skin before and after washing the application site in postmenopausal women (3).	√	√	√	√
Study FR00.037.2: To asses the BE of two newly developed estradiol gel, 0.1% formulations (EF107 and EF108) versus the original formulation.	√	√	√	√

The study report for the population PK analysis (Study P04-001) did not include the necessary information for review as electronic submission. Therefore, the sponsor is requested to submit the following information:

1. Submit the following datasets to support the population analysis:
   - All datasets used for model development and validation should be submitted as a SAS transport files (*.xpt). A description of each data item should be provided in a Define.pdf file. Any concentrations and/or subjects that have been **excluded from the analysis** should be flagged and maintained in the datasets.
   - Model codes or control streams and output listings should be provided for all major model building steps, e.g., base structural model, covariates models,

final model, and validation model. These files should be submitted as ASCII text files with *.txt extension (e.g.: myfile_ctl.txt, myfile_out.txt).

- A model development decision tree and/or table which gives an overview of modeling steps.

It has been shown that application of sunscreen prior to or after the application of estradiol topical emulsion increased the exposure to estradiol by approximately 35% and 15%, respectively.[2] The sponsor did not submit information on this for Divigel®; therefore the following comment is being conveyed to the sponsor:

2. Information on the effect of sunscreen products, topically applied skin creams or lotions on the systemic exposure to Divigel® was not included in the present submission. Provide available information on this issue. Otherwise, this lack of information and the potential impact on safety (based on available information from other related products) will be specified in the final Package Insert for Divigel®.

[2] Taken from PDR online for Estrasorb®.

Office of Clinical Pharmacology and Biopharmaceutics
New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	22-038	**Brand Name**	Divigel® (estradiol gel 0.1%)
OCPB Division (I, II, III)	II	**Generic Name**	estradiol
Medical Division	DPADP	**Drug Class**	Estrogen (hormone)
OCPB Reviewer	Sandra Suarez-Sharp	**Indication(s)**	Treatment of Vasomotor Symptoms (b) (4)
OCPB Team Leader	Ameeta Parekh	**Dosage Form**	Topical gel
PM Reviewer		**Dosing Regimen**	0.25, 0.5, or 1 mg/day
Date of Submission	May 1, 2006	**Route of Administration**	Topical (skin)
Estimated Due Date of OCPB Review	December 2006	**Sponsor**	Upsher-Smith Laboratories
PDUFA Due Date	March 4, 2007	**Priority Classification**	Standard
Division Due Date	January, 2007		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	**Number of studies submitted**	**Number of studies reviewed**	**Critical Comments If any**
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	**X**			
Tabular Listing of All Human Studies	**X**			
HPK Summary	**X**			
Labeling	**X**			
Reference Bioanalytical and Analytical Methods	**X**			Analytical method reports are in electronic submission as part of individual study reports.
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	**x**			**Study P04-002:** Transferability of estrogen gel during skin-skin contact to partner (electronic submission).
multiple dose:				
Patients-				
single dose:	**x**	**2**		**Study P04-005:** 3-Way Crossover Study of the Washability of estrogen gel (electronic submission). **Study P04-015:** Single dose, open label study for analytical method development to determine residual levels of estrogen remaining in the skin before and after washing the application site in postmenopausal women (3) (paper submission, vol. 1.22).

multiple dose:	**x**	**2**		**Study P04-003:** 3-Way Cross-over PK Study Evaluating Three Dose Levels of estrogen gel (electronic submission) and **Study P04-001** which was the pivotal efficacy and safety study. The PK data from this study was analyzed using a population PK method (paper submission, vol. 1.37).
Dose proportionality -	**x**	**1**		**Study P04-003:** 3-Way Cross-over PK Study Evaluating Three Dose Levels of estrogen gel (electronic submission).
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:	**x**	**1**		Population PK analysis of estradiol, estrone and estrone sulfate following once daily administration of estrodiol gel in postmenopausal women (data from Study P01-001)
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:	**x**	**1**		**Study FR00.037.2:** assessed the BE of two newly developed estradiol gel, 0.1% formulations (EF107 and EF108) versus the original formulation.
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
QTC STUDIES (PHASE 1)				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**6**		
Filability and QBR comments				

	"X" if yes	Comments
Application filable ?	X	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm ?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.
QBR questions (key issues to be considered)	**1. Dose-Response for efficacy and safety** **2. Sunscreen and other topical product effect on the systemic exposure of estradiol gel** **3. Transferability to partner** **4. Effect of washing on the systemic exposure of estradiol gel**	
Other comments or information not included above		
Primary reviewer Signature and Date		
Secondary reviewer Signature and Date		

CC: NDA 20-907, HFD-580 (Lyght, Patsner, Slaugther), DCPIII (Parekh, Hunt, Bashaw)

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/s/

Sandra Suarez
6/14/2006 02:10:53 PM
PHARMACOLOGIST

Ameeta Parekh
6/16/2006 03:55:35 PM
BIOPHARMACEUTICS

CLINICAL PHARMACOLOGY REVIEW

NDA:	22-038
Proprietary Drug Name:	**DIVIGEL®**
Generic Name:	estradiol (E2)
Proposed Indications:	Treatment of Moderate to Severe Vasomotor Symptoms (MSVS) [redacted] (b) (4)
Dosage Form:	Topical gel
Proposed Strengths:	0.1%
Route of Administration:	Topical (skin)
Applicant:	Upsher-Smith Laboratories, Inc.
Clinical Division:	DRUP (HFD-580)
Type of Submission:	NDA
Submission Dates:	05/05/06; 08/17/06; 09/11/06; 10/24/06; 04/06/07; 05/22/07
Reviewer:	Sandra Suarez-Sharp, Ph.D.
Pharmacometrics Consultant:	Atul Bhattaram, Ph.D.
Team Leader:	Myong-Jin Kim, Pharm. D.

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

1.1 Recommendation

The Office of Clinical Pharmacology/ Division of Clinical Pharmacology III (OCP / DCP-III) has reviewed NDA 22-038 submitted on May 5, 2006. We found this NDA acceptable from an OCP standpoint provided that the sponsor agrees with the Agency's labeling recommendations.

1.2 Comments to Medical Officer

- Although there was a trend for a dose-response relationship in terms of mean changes from baseline in the daily frequency and daily severity of hot flushes, these changes were not statistically significant from placebo at week 4 (primary endpoints) for the 0.25 mg/day dose. In addition, an analysis of the estradiol average concentration (Cavg) across several estradiol products (gels/emulsions) approved for the treatment of moderate to severe vasomotor symptoms (MSVS) showed that the Cavg for these products ranges from 15 pg/mL to 44 pg/mL following multiple administration. The E2 Cavg for Divigel 0.25 mg, 0.5 mg and 1.0 mg/day after two weeks of once daily administration to the skin of the upper thigh were 9.8 pg/mL, 23.1 pg/mL, and 30.5 pg/mL, respectively. Although, there has not been a concentration-response relationship established for this product, lower estradiol serum concentrations may result in less efficacy. Therefore, the benefit/risk ratio of Divigel 0.25 mg/day should be evaluated for MSVS.

1.3 Phase IV Commitments

None.

1.4 Summary of Clinical Pharmacology Findings

Divigel® (estradiol gel 0.1%) is a smooth, clear to opalescent gel (alcohol-based) in which the active ingredient, estradiol, is dissolved. E2 has been widely used as hormone replacement therapy in postmenopausal women. Divigel® is being proposed for once daily topical administration to skin (right or left upper thigh) of postmenopausal women with/without uterus for the treatment of moderate to severe vasomotor symptoms (b) (4) The sponsor's proposed starting dose is 0.5 g (equivalent to 0.5 mg of E2) daily. The dose can be increased to 1.0 g (eq. to 1 mg of E2) /day or decreased to 0.25 g (eq. to 0.25 mg) of E2/day depending on clinical response, in order to achieve the lowest effective dose.

Numerous estradiol transdermal delivery systems are available in the United States for the treatment of MSVS. The recently approved products (Climara®, Vivelle-Dot® and Esclim™) provide systemic delivery rates ranging from 0.025 to 0.1 mg/day. The Cavg levels from these products at the approved doses range from 22 pg/mL to 104 pg/mL[1]. Generally, the recommended starting systemic delivery rate for these products is 0.025 mg/day with increasing dose if needed to control symptoms while achieving the lowest effective dose for the patient. Topical therapies that provide systemic delivery of estradiol from 0.52 mg/day to 7.5 mg/day are

[1] Data taken from PDR online.

available including EstroGel®, Elestrin, and Estrasorb™. The estradiol Cavg following multiple administration of these products range from 15-44 pg/mL.

In the present submission, the sponsor, Upsher-Smith included the results of Study P04-001 to support the efficacy and safety of Divigel® for the treatment of MSVS (b) (4) Pivotal Study P04-001 was designed to identify the minimum effective dose of Divigel® 0.1% among three doses tested: 0.25 mg, 0.5 mg, and 1.0 mg/day. A total of 437 postmenopausal women, each with/without uterus, completed this randomized, multi-center, placebo-controlled, 12-week study. The primary efficacy endpoints were the change in mean daily frequency and mean daily severity of MSVS from baseline to week 4 and baseline to week 12.

The mean and median change in daily frequency and daily severity of hot flushes decreased statistically significantly from placebo ($p \leq 0.01$) for the Divigel® 0.5 mg and 1.0 mg from baseline to week 4 through week 12. The median change in daily frequency of hot flushes compared to placebo range from 2.1 to 3.87 units above placebo. While the 0.25 mg treatment group also demonstrated a greater reduction from baseline, the mean daily frequency of MSVS (-5.66 episodes) when compared to placebo (-4.56 episodes) was not statistically different at week 4. The 0.25 mg group treatment showed a statistically significant decrease ($p<0.038$) in the mean change from baseline in daily frequency and daily severity of hot flashes at week 5 through week 12 (except week at 6 for daily severity).

There was a trend for dose-response relationship in the mean change from baseline in the daily frequency and daily severity of hot flushes to week 4 and week 12; No clear differences were observed, however, in the mean change from baseline in daily frequency of hot flashes between the 0.25 mg and the 0.5 mg doses at week 5 through week 12. The 1.0 mg treatment group showed the greatest response (both severity and frequency) compared to the 0.5 mg and the 0.25 mg treatment groups (see MO's review for more details).

An analysis of dose-response for safety reveals a trend for a dose-response relationship for some adverse events such as vaginal discharge, breast tenderness, nipple pain, metrorrhagia, and fungal infections with the 1.0 mg dose showing higher percentage of patients having these adverse events. No clear trend in dose-response relationship was observed for the change from baseline in endometrial thickness to visit 6 (week 15). The 1 mg treatment group showed the highest change from baseline in endometrial thickness (mean: 4.38 mm ranged: 1-12 mm) (see MO's review for more details).

The mean change (min, max) from baseline in QTF (calculated using Fridericia's correction formula) following multiple dose administration of Divigel®, 0.25 mg, 0.5 mg, and 1.0 mg/day for 12 weeks increased proportionally to the dose as follows: placebo (PLB): -2.8 msec (-85 to 80 msec); 0.25 mg: -2.4 msec (-123 to 53); 0.5 mg: 3.6 msec (-68 to 90); 1.0 mg: 6.6 (-55 to 70 msec). Although there was a trend for dose-QTF relationship with a mean delta QTF of 6.6 msec observed at the maximum dose evaluated (1 mg/day), these data should be interpreted with caution since the study was not designed prospectively to address the potential effect of the drug on QTc: there was only one ECG value taken at baseline and one value of ECG taken after drug administration (the ECG collection time in regards to drug administration was not mentioned) at the end of week 13; in addition no positive control was included.

Although there was a trend for a dose-response relationship in terms of mean change from baseline in the daily frequency and daily severity of hot flushes, these changes were not statistically significant from placebo at week 4 (primary endpoint) for the 0.25 mg/day dose. In addition, an analysis of the E2 Cavg across several estradiol products (gels/emulsions) approved for MSVS showed that the Cavg for these products range from 15 pg/mL to 44 pg/mL following multiple administration. The E2 Cavg for Divigel 0.25 mg, 0.5 mg, and 1.0 mg/day after two

weeks of once daily administration to the skin of the upper thigh were 9.8 pg/mL, 23.1 pg/mL, and 30.5 pg/mL, respectively. Although, there has not been a concentration-response relationship established for this product, lower estradiol serum concentrations may result in less efficacy. Therefore, the benefit/risk ratio of Divigel 0.25 mg/day should be evaluated for the treatment of MSVS.

This submission also contains five Clinical Pharmacology Studies as follows: **Study P04-003** was conducted to assess the linearity of the pharmacokinetic (PK) profile as the applied topical dose was increased from 0.25 to 1.0 mg estradiol; **Study P04-002** evaluated the potential for transferability from the patient to a non-dosed individual; **Study P04-005** evaluated the effects of washing the site of application at selected times post-administration. In addition to these Phase 1 studies, limited PK samples were collected in the Phase 3 study (**Study P04-001**) to determine serum estradiol and its metabolites concentrations and attempted a population PK analysis. The potential effects of demographic and baseline characteristics and concomitant medications on estradiol and its metabolites PK were investigated using this population PK analysis. A bioequivalence study conducted by Orion Pharma (**Study FR00.037.2**) compared a new formulation (EFI08; now referred to as USL-221 which is the to-be-marketed formulation) to the original formulation (the original formulation was used in clinical studies sponsored by Orion and was available commercially outside of the U.S. from 1994 until 2002/2003).

In summary, there are no clinical pharmacology issues. Below is a summary of the clinical pharmacology of Divigel®.

Absorption

Single Dose Administration

Following single dose administration of Divigel® 0.1% to the skin of either the right or left upper thigh, E2 absorption through the skin is relatively slow with a median Tmax of 10 hrs. E2 (uncorrected for baseline) reached mean (%CV) peak serum concentrations of about 15 pg/mL (159), 17 pg/mL (98) and 38 pg/mL (90) at the doses of 0.25-, 0.5- and 1.0 mg, respectively. High variability (CV ranged from 76-176%) in the PK parameters (AUC and Cmax) was observed. E2 peak serum concentrations and AUC_{24hrs} (corrected for baseline) increased more than proportionally to the dose. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 147% in mean corrected $AUC_{0\text{-}24h}$ was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 219% was observed. Proportional increases in mean Cmax and AUC_{24hrs} from 0.5 mg to 1 mg were observed for uncorrected values. No changes in Cmax and AUC_{24hrs} were observed when comparing uncorrected values for the 0.25 mg and 0.5 mg doses.

The degree of transferability of Divigel to non-dosed subjects is inconclusive. Following single dose administration of Divigel®, 1 mg to the skin of the upper thigh of postmenopausal women, the percentage mean increase in E2 Cmax and AUCt in non-dosed patients who had skin contact with unclothed or clothed application site 60 minutes or 8 hrs after dose administration appears to be about 30% to 35% % and 9% to 13%, respectively compared to baseline (Table 1). Similar findings were observed for the metabolites. These data should be interpreted with caution due to uncertainty in the procedure used to calculate E2 change from baseline values across the treatments. Baseline was calculated as the average of 3 endogenous compound values determined at -12 hr, -6hr and prior drug administration. Change from baseline was then calculated as the AUC of individual values minus the mean of baseline. Patients will be advised to restrain from direct contact for at least 1 hr. after application of the gel and to cover the area of application after the gel is completely dry.

Table 1. Percentage of Estradiol Transfer in Nondosed Subjects Who Had Skin Contact With Dosed Subjects following single dose administration of Divigel 0.1%, 1 mg

Parameter	Contact with clothed application site 60 minutes after dosing (Treatment A) N=23		Contact with unclothed application site 60 minutes after dosing (Treatment B) N=22		Contact with unclothed application site 8 Hours after dosing (Treatment C) N=24	
	**Baseline	*Mean (SD) Percentage of transfer	**Baseline	*Mean (SD) Percentage of transfer	**Baseline	*Mean (SD) Percentage of transfer
AUC0-t (pg*hr/mL)	1633.1 (436)	13.2 (11.5)	1696 (509)	9.05 (6.4)	1683 (522)	11.8 (15.4)
Cmax (pg/mL)	23.6 (6.9)	34.3 (20.1)	25.09 (10)	36.3 (15.5)	25.5 (8.9)	30.4 (26.5)

* Calculated as uncorrected value / baseline value*100. **Baseline was calculated as the mean of the difference between reported uncorrected-corrected values.

Washing the application site one hour after single application of Divigel® 1.0 mg resulted in a decrease in the mean baseline-corrected and uncorrected E2 Cmax and AUC by 30 to 38% compared to no washing. Washing the application site one hour after single application of Divigel® 1.0 mg resulted in a decrease in the mean AUC baseline-corrected and uncorrected estrone (E1) by 15 to 53%. The mean baseline-corrected and uncorrected estrone sulfate (ES) Cmax and AUCt were decreased by 32 to 50% after washing the application site one hr post-application of Divigel®, 1.0 mg. Therefore, patients may be advised to restrain from washing the application site for at least one hr. after application. After a single topical application of Divigel® 1.0-mg estradiol, washing the application site for 3 minutes after 60 minutes of skin application removed all detectable amounts of estradiol on the application site.

The time it takes for the product to dry at the application site was not studied by the sponsor. In addition, the effect of sunscreens and other topical lotions on the systemic exposure of Divigel® was not studied by the sponsor.

Multiple Dose Administration

Following multiple dose administration of Divigel® 0.1%, E2 (uncorrected for baseline) reached peak serum concentrations of about 14.7 pg/ml, 28.4 pg/mL and 51.7 pg/mL at the doses 0.25-, 0.5-, and 1.0 mg/day, respectively with a mean Tmax of 8 to 16 hours. AUC and Cmax were highly variable; CV % ranged from 84 to 149%. Mean (%CV) Cavg were 9.8 pg/mL (92), 23.1 pg/mL (148), and 30.5 pg/mL (81) for the 0.25-, 0.5, and 1.0 mg, respectively. The accumulation factor based on AUC_{24hrs} was about 1.75 to 2.1, to 1.1 to 2.1, and 1.41 to 2.7 for E2, E1 and ES, respectively. The mean E2/E1 ratio ranged from 0.45 to 0.65 across Divigel® doses.

E2 Cmax and AUC increased roughly proportionally to the dose. Increases in dose from 0.25 mg to 0.5 mg produced an increase of approximately 114% and 93% in mean uncorrected E2 AUC_{0-24} and Cmax, respectively. Increases in dose from 0.5 mg to 1.0 mg produced an increase of approximately 45% and 93% in the mean uncorrected E2 AUC_{0-24} and Cmax, respectively. Based on the power model, E2 AUCss increased roughly proportional to the dose with a slope of 0.8. E1 and ES AUCss values increased less than proportionally to the dose following multiple administration of the treatments.

Elimination

Based on literature information, the half-life of 17 ß-E2 is approximately (b) (4) It circulates bound to sex hormone binding globulin (SHBG) (37%) and to albumin (61%), while only approximately 1-2% remains unbound in the circulation.

Metabolism of 17 ß-E2 occurs mainly in the liver and gut but also in target organs, and involves the formation of less active or inactive metabolites, including E1, catecholestrogens, and several estrogen sulphates and glucuronides. Estrogens are excreted with the bile, where they are hydrolyzed and reabsorbed (enterohepatic circulation), and mainly in urine in biologically inactive form.

Effect of Age

Based on population PK analysis, age (34 to 89 years) did not affect the PK of E2 and its metabolites.

Effect of Race

Based on population PK analysis, race did not affect the PK of E2 and its metabolites. This finding should be interpreted with caution since there were 287 White patients and only 40 Non-White patients (31 Black, 4 Asian, and 5 others) included in the population PK analysis.

Effect of Renal Impairment

The effect of renal impairment on the PK of E2 and its metabolites was not formally evaluated. Based on population PK analysis, renal impairment (mild or moderate; measured as a function of CrCL) did not affect the PK of E2 and its metabolites. The effect on severe renal impairment on the PK of the drug is unknown since no patients with this condition were included in the study.

Effect of Liver Impairment

The effect of hepatic function on the PK of E2 and its metabolites was not formally evaluated. Based on population PK analysis, hepatic impairment (mild or moderate; AST, ALT, alkaline phosphatase, and total bilirubin levels were used as indicators of hepatic function) did not affect the PK of E2 and its metabolites. The effect on severe hepatic impairment on the PK of the drug is unknown since no patients with this condition were included in the study.

Drug-Drug Interactions (DDI)

The effect of Divigel® on the PK of other drugs has not been evaluated by the sponsor. No formal studies were conducted to evaluate the effect of other drugs on the PK of Divigel®. Based on population PK analysis using data from pivotal clinical study P04-001, there were 50 concomitant medications taken by at least 6 patients each. The median CL/F of E2 for patients on miconazole (n = 7) was about 30% lower than the median of the whole population. These results were in disagreement with the findings for fluconazole, another CYP3A4 inhibitor. The median CL/F of E2 for patients on fluconazole (n = 6) was about the same as the median of the whole population. Therefore, no final conclusions on the effect of concomitant administration can be made from the population PK analysis.

Reviewer
Sandra Suarez-Sharp, Ph.D.
Office of Clinical Pharmacology
Division of Clinical Pharmacology III

Final version signed by Myong-Jin Kim , Pharm.D. Team leader
Office of Clinical Pharmacology
Division of Clinical Pharmacology III

cc:
OCP/DCPIII: Bashaw, Kim, Suarez-Sharp
HFD-580: Lyght, Gassman, Slaughter

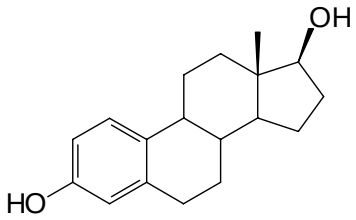
A briefing for this NDA held on February 7, 2007 at 1:00 PM was attended by D. Bashaw, HY Ahn, MJ Kim, D. Tran, J. Bai, S. Al-Habet, A. Adebowale, S. Monroe, and A. Gassman.

2. QUESTION BASED REVIEW

2.1 General Attributes

2.1.1 What are the highlights of the chemistry and physico-chemical properties of the drug substance and formulation of the drug product?

The active component of the topical gel is estradiol. Estradiol is a white or almost white crystalline powder. Its chemical name is estra-1, 3, 5 (10)-triene-3, 17β-diol hemihydrate with the empirical formula of $C_{18}H_{24}O_2$, ½ H_2O and a molecular weight of 281.4. The structural formula of E_2 is as follows:



FORMULATION

Estradiol Gel, 0.1% is a smooth, clear to opalescent gel in which the active ingredient, estradiol, is dissolved. Estradiol is absorbed following application of the gel to the skin. The 0.1 % bulk gel is packaged into three different weight single-dose foil-laminate packets (0.25, 0.5 and 1 g, corresponding to 0.25, 0.5 and 1.0 mg of estradiol, respectively). The 0.1 % bulk gel is manufactured, packaged, tested and released at Orion Pharma in Turku, Finland and is distributed by the NDA holder, Upsher-Smith Laboratories, Inc. in Minneapolis, MN. The formulation is provided in Table 2.1.1.1.

Table 2.1.1.1. Compositions of Test Product used in Clinical Trials

INGREDIENT	COMPLIES WITH USP/NF SPECIFICATIONS	FUNCTION	FORMULATION USL-221/ EF108 0.1% GEL (MG/G)	AMOUNT PER 0.25 G DOSE	AMOUNT PER 0.5 G DOSE	AMOUNT PER 1.0 G DOSE
Estradiol	Estradiol, USP	Active ingredient	1.0*	0.25 mg	0.5mg	1.0mg
Carbomer (b) (4)						(b) (4)
Triethanolamine						
Propylene Glycol						
Ethanol (b) (4)						
Purified Water						
						(b) (4)

*Quantity adjusted according to assay and water content

2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?
Mechanism of Action:

Endogenous estrogens are largely responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. The primary source of estrogen in normally cycling adult women is the ovarian follicle, which secretes 70 to 500 mcg of estradiol daily, depending on the phase of the menstrual cycle. After menopause, most endogenous estrogen is produced by conversion of androstenedione, secreted by the adrenal cortex, to estrone by peripheral tissues. Thus, estrone and the sulfate conjugated form, estrone sulfate, are the most abundant circulating estrogens in postmenopausal women.

Estrogens act through binding to nuclear receptors in estrogen-responsive tissues. To date, two estrogen receptors have been identified which vary in proportion from tissue to tissue. Circulating estrogens modulate the pituitary secretion of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) through a negative feedback mechanism. Estrogens act to reduce the elevated levels of these hormones seen in postmenopausal women.

INDICATION (as per proposed label)
Divigel® is indicated for usage in postmenopausal women for the following:

- Treatment of Moderate to Severe Vasomotor Symptoms Associated with Menopause

(b) (4)

2.1.3 What are the proposed dosage(s) and route(s) of administration?
DOSAGE AND ADMINISTRATION (as per proposed label)

The recommended starting dose is 0.5 g (0.5 mg estradiol) daily. The dose can be increased to 1.0 g/day or decreased to 0.25 g/day depending on clinical response, in order to achieve the lowest effective dose. Individual patients should be maintained on the lowest effective dose, taking into consideration the frequency and severity of symptoms. Therefore, a trial dose-reduction to 0.25 g/day should be considered for patients who achieve an adequate response with 0.5 g/day.

Divigel® should be applied once daily on the skin of either the right or left upper thigh. The application surface area should be about 5 by 7 inches (approximately the size of two palm prints). The entire contents of a unit dose packet should be applied each day. To avoid potential skin irritation, Divigel® should be applied to the right or left upper thigh on alternating days.

2.2 General Clinical Pharmacology
2.2.1 What efficacy and safety information (e.g., biomarkers, surrogate endpoints, and clinical endpoints) contribute to the assessment of clinical pharmacology study data? How was it measured?

Study P04-001 was considered as the primary efficacy study in this submission. Study P04-001 was a randomized, parallel, placebo-controlled, double-blind, multicenter study in postmenopausal women with moderate to severe vasomotor symptoms (MSVS). Patients (495 enrolled patients; about 120 /group) received treatment with USL-221 (Estradiol Gel, 0.1%) or placebo for 12 weeks. Additionally, the study evaluated postmenopausal women with complaints

(b) (4)

Study P04-01 consisted of a screening period, four study visits (Visits 2-5) for patients without an intact uterus, and five study visits (Visit 2-6) for patients with a uterus. Patients who met the eligibility criteria during the screening evaluations

were randomized to one of the following four treatment groups: 0.25 g, 0.5 g, 1.0 g Divigel 0.1%, or matching placebo gel.

The primary objective was to compare the change from baseline in mean daily frequency and severity of moderate to severe vasomotor symptoms at weeks 4 and 12 between USL-221 and placebo. The secondary objective was to assess the effect of USL-221 versus placebo on

(b) (4)

The primary efficacy analyses in the ITT population compared the change in mean daily frequency and severity of MSVS from baseline to week 4 and to week 12 using the LOCF (last observation carried forward) approach for invalid weeks. These parameters were analyzed by an analysis of covariance (ANCOVA) including treatment group, pooled center, and baseline values as covariates. The number and severity of symptoms were obtained from the weekly patient diaries.

Subjects were asked to record the frequency of each severity category of MSVS (hot flashes) representing menopausal symptoms, in the diary card throughout the trial. The severity of hot flashes was assessed using the following categories: (1) Mild: a transient sensation of hotness without sweating, (2) Moderate: a sensation of hotness with sweating, which allows continuation of current activity, and (3) Severe: a sensation of hotness with sweating that prohibits continuation of current activity, including any night sweats that result in awakening.

(b) (4)

Safety assessments included incidence and severity of AEs, vital signs and body weight, physical and breast examinations, gynecological examination, 12-lead ECGs, clinical safety laboratory assessments (hematology, blood chemistry, lipid metabolism, carbohydrate metabolism, coagulation parameters, sex hormone binding globulin [SHBG], and urinalysis). Other safety-related patient assessments included serum pregnancy test (for women with an intact uterus), skin tolerability assessment (Draize scale), cervical Pap smear, endometrial biopsy, and transvaginal ultrasound (TVU).

Blood samples for measuring serum concentrations of E2, E1, and ES to allow definition of population PK were collected at Visits 2, 3, 4, and 5. The baseline (Visit 2) blood sample was collected prior to the application of the study drug. After start of treatment, blood samples were to be collected one to 10 hours after the dose application.

2.2.2 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The measurement of serum concentrations of estradiol, estrone, and estrone sulfate (in a separate assay) was accomplished by LC/MS/MS. Both methods were developed and validated at (b) (4). The lower limits of quantification (LLOQ) were 2.5, 5.0 and

50.0 pg/mL for estradiol, estrone and estrone sulfate, respectively. An assay was also developed and validated at (b) (4) to assess the residual concentrations of estradiol remaining on skin by swabbing a proscribed skin surface area at various times after dose application. An assay range of 50 to 1000 ng/swab was established for use in the analytical method for swab analysis and the method was determined by the sponsor to be acceptable for quantifying estradiol samples via HPLC with MS/MS detection for use in subsequent studies using USL-221. The analytical method used for the measurement of estradiol in human serum samples from the Orion-conducted bioequivalence study (Study FR00.037.2) was a radioimmunoassay (RIA) that was developed and validated at (b) (4). The methods were linear, precise, and accurate.

2.2.3 Exposure Response

2.2.3.1 What are the characteristics of the dose-systemic exposure relationships for efficacy?

The applicant did not attempt to correlate E2 or its metabolites serum concentrations to the primary efficacy and safety endpoints for MSVS. The pivotal study P04-001 was designed to identify the minimum effective dose of Divigel® 0.1% among three doses tested: 0.25 mg, 0.5 mg, and 1.0 mg/day. A total of 437 postmenopausal women, each with an intact uterus, completed this randomized, multi-center, placebo-controlled, 12-week study. The primary efficacy endpoint was the change in mean daily frequency and mean daily severity of MSVS from baseline to week 4 and baseline to week 12.

Daily Frequency of Hot Flushes

According to the sponsor, the mean change in daily frequency of hot flashes decreased statistically significantly compared to placebo ($p \leq 0.01$) for the Divigel® 0.5 mg and 1.0 mg from baseline to week 4 through week 12. While the 0.25 mg treatment group also demonstrated a greater reduction from baseline the mean daily frequency of MSVS (-5.66 episodes) when compared to placebo (-4.56 episodes), this difference was not statistically different at week 4 (Table 2.2.3.1.1 and Figure 2.2.3.1.1). The 0.25 mg group treatment showed a statistically significant decreased ($p=0.005$) in the mean change in daily frequency of hot flashes from baseline to week 5 through week 12. Each of the three treatment groups showed statistically significant reductions in the mean daily frequency of MSVS from baseline to week 12 when compared to placebo ($p<0.001$) (see MO and statistician reviews for more details).

There was a trend for a dose-response relationship in the mean change from baseline in the daily frequency of hot flushes to week 4; however, from week 5 through week 12, no apparent differences in the mean change from baseline in daily frequency of HF could be observed between the 0.25 mg and the 0.5 mg doses (Figure 2.2.3.1.1). The 1.0 mg treatment group showed the greatest response (-7.63 episodes at week 4 and -8.92 episodes at week 12) compared to the 0.5 mg (-6.17 episodes at week 4 and -7.48 episodes at week 12) and 0.25 mg (-5.66 episodes at week 4 and -7.83 episodes at week 12) treatment groups (Table 2.2.3.1.1).

Table 2.2.3.1.1.Summary of mean and median daily frequency of moderate to severe vasomotor symptoms at baseline and change from baseline at weeks 4 and 12*.

	Divigel 0.1%			Placebo
	1.0 mg n=124	**0.5 mg n=119**	**0.25 mg n=121**	**n=124**
Mean daily frequency of moderate to severe vasomotor symptoms				
Baseline				
n	124	119	121	124
Mean (SD)	10.69 (4.083)	10.86 (4.356)	12.11 (9.942)	10.79 (5.815)
Median	9.64	9.24	9.72	9.32
Week 4 change from baseline				
n	124	119	121	124
Mean (SD)	−7.63 (4.729)	−6.17 (5.232)	−5.66 (5.877)	−4.56 (6.420)
Median	−7.20	−5.73	−5.00	−3.63
p-value[1]	<0.001	0.011	0.132	-
Week 12 change from baseline				
n	124	119	121	124
Mean (SD)	−8.92 (4.860)	−7.48 (5.126)	−7.83 (8.486)	−5.27 (6.506)
Median	−8.35	−7.29	−6.88	−4.48
p-value[1]	<0.001	<0.001	<0.001	-

[1] Comparison significant if $p<0.05$; p-value from ANCOVA model of treatment group, pooled center, and baseline covariate. *Table taken from sponsor's reported data in Study P04-001 with minor modifications

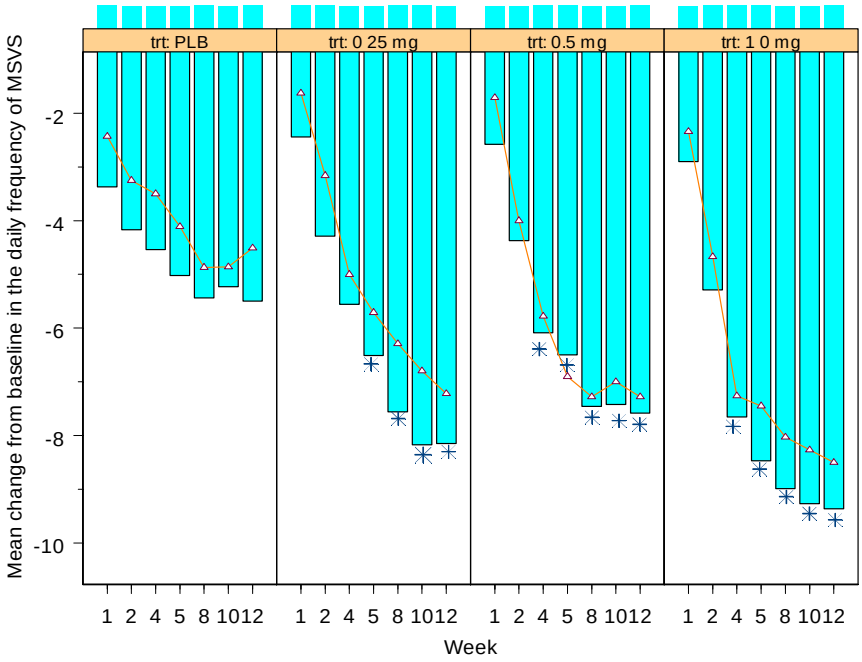


Figure 2.2.3.1.1. Mean (▵ median) change from baseline in the daily ***frequency*** of hot flushes in postmenopausal women taken Divigel doses of 0.25mg, 0.5 mg and 1.0 mg (using LOCF for ITT population). N=119 to 124 patients per treatment group. * Statistically significant at the 0.05 level compared to placebo (p<0.001) from week four through week twelve for the 0.5 mg and 1 mg and from week five through week twelve for the 0.25 mg dose. Data from Study P04-001.

Daily Severity of Hot Flushes

The mean and median change from baseline in daily severity of hot flushes decreased statistically significantly compared to placebo ($p < 0.001$) for the Divigel® 0.5 mg and 1.0 mg from week 4 through week 12. For the 0.25 mg this difference was not significant from placebo at week 4 (Figure 2.2.3.1.2). The 0.25 mg group treatment showed a statistically significant decreased ($p<0.038$) in the mean change from baseline in daily severity of hot flashes at week 5 through week 12, except at week 6. Each of the three treatment groups showed statistically significant reductions in the mean daily severity of MSVS from baseline to week 12 when compared to placebo ($p<0.001$).

There was a dose-response relationship in the mean change from baseline in the daily severity of hot flushes to week 4; however, from week 5 through week 12, no clear differences in the mean change from baseline in daily severity of HF can be observed between the 0.25 mg and the 0.5 mg doses (Figure 2.2.3.1.2). When considering the median values, these changes were more apparent across doses. The 1.0 mg treatment group showed the greatest response (-0.87 at week 4 and -1.39 at week 12), compared to the 0.5 mg (-0.65 at week 4 and -1.00 at week 12) and the 0.25 mg (-0.34 at week 4 and -0.84 at week 12) treatment groups.

Table 2.2.3.1.2.Summary of mean and median daily SEVERITY of moderate to severe vasomotor symptoms at baseline and change from baseline at weeks 4 and 12*.

	Divigel 0.1%			**Placebo**
	1.0 g n=124	**0.5 g n=119**	**0.25 g n=121**	**n=124**
Mean daily severity of MSVS				
Baseline				
n	124	119	121	124
Mean (SD)	2.52 (0.209)	2.52 (0.226)	2.53 (0.202)	2.53 (0.243)
Median	2.52	2.51	2.52	2.54
Week 4 change from baseline				
n	124	119	121	124
Mean (SD)	−0.87 (0.961)	−0.65 (0.931)	−0.34 (0.704)	−0.25 (0.621)
Median	−0.47	−0.18	−0.07	−0.04
p-value[1]	<0.001	<0.001	0.283	-
Week 12 change from baseline				
n	124	119	121	124
Mean (SD)	−1.39 (1.087)	-1.00 (1.085)	−0.84 (1.055)	−0.47 (0.863)
Median	−1.69	−0.56	−0.33	−0.13
p-value[1]	<0.001	0.002	0.021	-

[1] Comparison significant if $p<0.05$; p-value from ANCOVA model of treatment group, pooled center, and baseline covariate.
*Table taken from sponsor's reported data in Study P04-001 with minor modifications

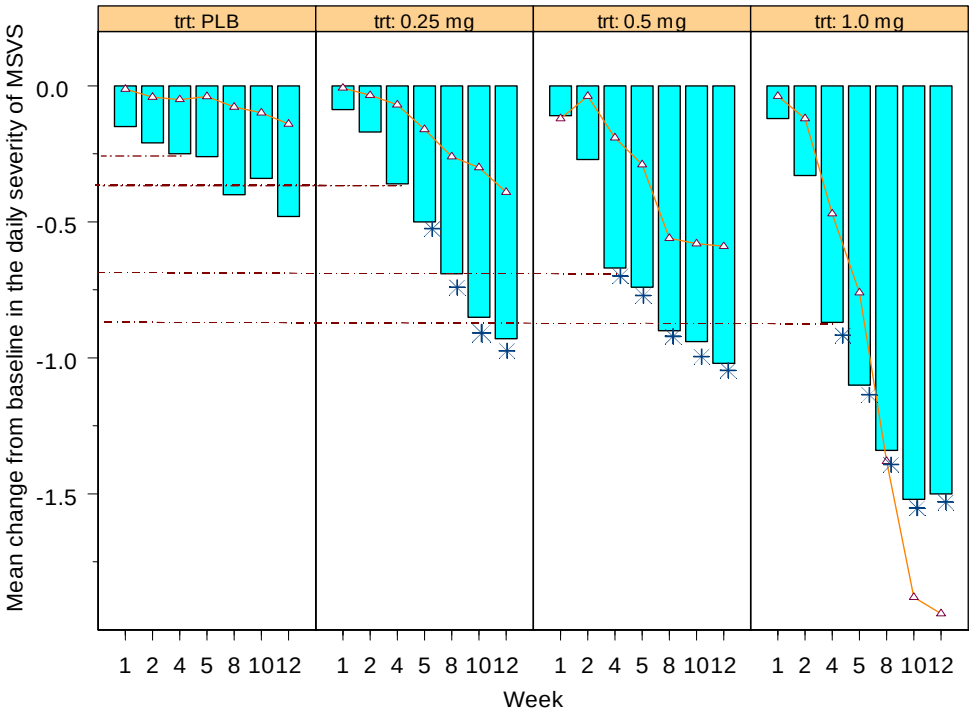


Figure 2.2.3.1.2. Mean (▵ median) change from baseline in the daily SEVERITY of hot flushes in postmenopausal women taken Divigel doses of 0.25mg, 0.5 mg and 1.0 mg (using LOCF for ITT population). N=119 to 124 patients per treatment group. * Statistically significant at the 0.05 level compared to placebo ($p<0.001$) at week four through week 12 for the 0.5 mg and 1 mg. The 0.25 mg treatment group showed significance ($p<0.038$) at week 5 through week 12 except at week 6. Data from Study P04-001.

In summary, the 0.5 mg and 1.0 mg treatment groups show statistically significant difference compared to placebo in the mean and median change from baseline daily frequency and severity of hot flashes at week 4. These statistically significant differences from placebo were maintained at each subsequent time point for the duration of treatment through week 12. The 0.25 mg group treatment showed statistically significant decreased in the mean change from baseline in daily frequency and severity of hot flashes at week 5 through week 12 (except at week 6 for severity).

There was a trend for dose-response relationship in the mean change from baseline in the daily frequency and severity of hot flushes to week 4 and week 12; However, no clear differences were observed in the mean change from baseline in daily frequency of HF between the 0.25 mg and the 0.5 mg doses at week 5 through week 12. The 1.0 mg treatment group showed the greatest response compared to the 0.5 mg and the 0.25 mg treatment groups.

2.2.3.2 What are the characteristics of the dose-systemic exposure relationships for safety?

Exposure-response analysis for safety was not attempted by the applicant. Safety information to support the approval of Divigel® 0.1% comes from pivotal clinical trial P04-001 and from safety data generated with the original formulation of Divigel® (Orion formulation).

Safety assessments included incidence and severity of AEs, vital signs and body weight, physical and breast examinations, gynecological examination, 12-lead ECGs, clinical safety laboratory assessments (such as hematology, lipid metabolism, carbohydrate metabolism, SHBG

and urinalysis). Other safety-related patient assessments included skin tolerability assessment (Draize scale), cervical Pap smear, endometrial biopsy, and TVU.

There appears to be a dose-response relationship in the percentage of patients experiencing some treatment related adverse events such as vaginal discharge, breast tenderness, nipple pain, methrorrhagia, and fungal infections with the 1.0 mg dose showing higher percentage of patients having these adverse events (Figure 2.2.3.2.1).

At the end of the treatment period or early discontinuation, patients with an intact uterus who had applied study drug for at least six weeks were given a 14-day treatment with medroxyprogesterone, followed by a TVU. Figure 2.2.3.2.2 shows the mean change from baseline in endometrial thickness to week 15 as a function of dose. No clear trend in dose-response relationship was observed for this safety variable. However, the 1 mg treatment group showed the highest change from baseline in endometrial thickness (mean: 4.38 mm ranged:1-12 mm). If double-wall endometrial thickness based on ultrasound assessment was greater than four millimeters, then a follow-up endometrial biopsy was to be obtained. The evaluable endometrial biopsies did not reveal any cases of hyperplasia or carcinoma (see MO review for more details).

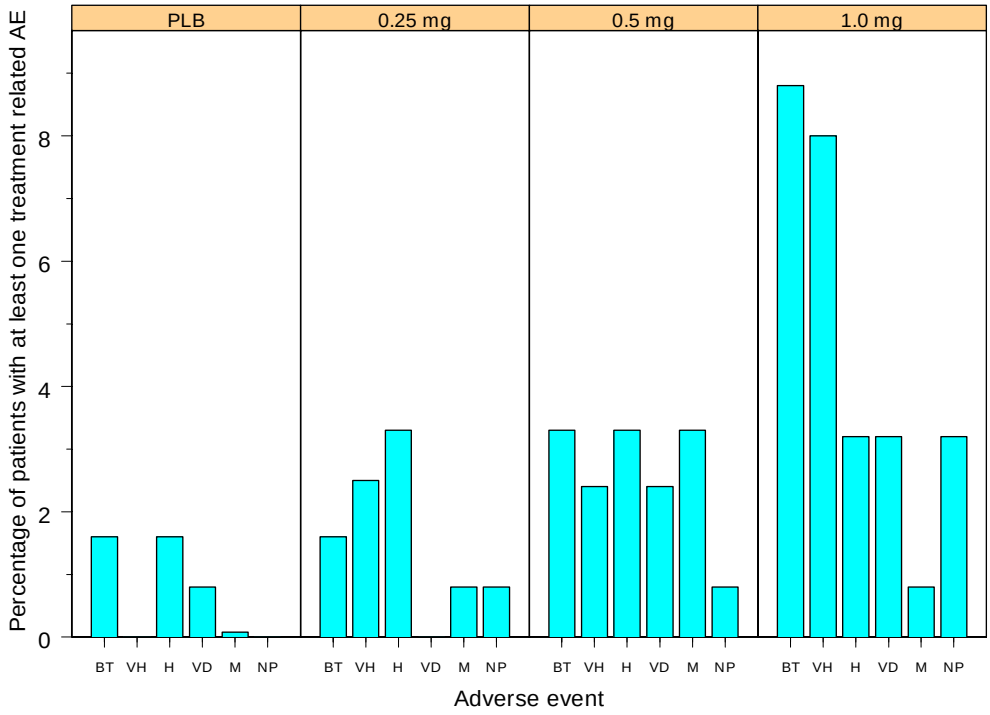


Figure 2.2.3.2.1. Percent of patients with at least one treatment-related AE. BT= Breast tenderness; VH:Vaginal Hemorrhage; H: Headache; VD: Vaginal Discharge; M:Metrorrhagia; NP: Nipple Pain. Data from Study P01-001. N=15 to 43.

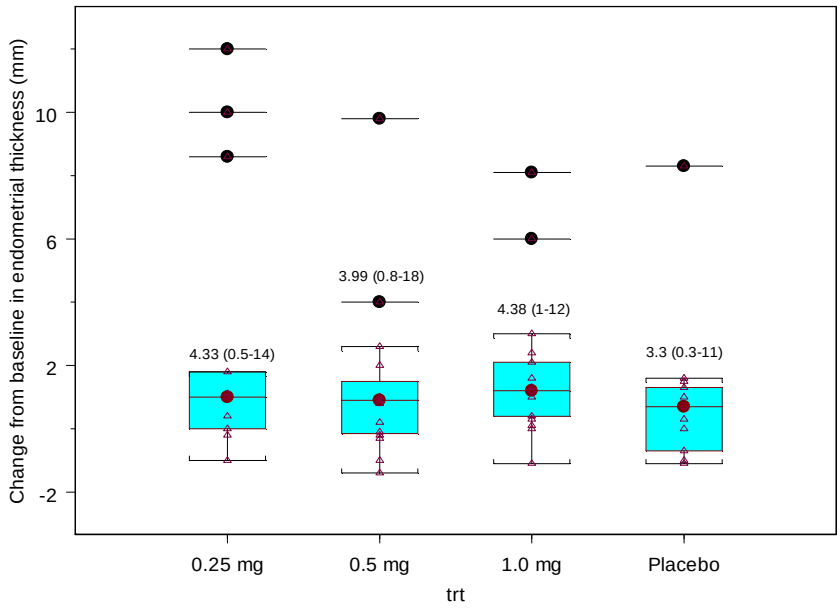


Figure 2.2.3.2.2. Endometrial thickness change from baseline to week 15 as a function of dose. Labels represent mean (min-max).

2.2.3.3 Does this drug prolong the QT or QTc interval?

The mean change (min, max) from baseline in QTF following multiple dose topical administration of Divigel® 0.25 mg, 0.5 mg, and 1.0 mg/day increased proportionally to the dose as follows: PLB: -2.8 msec (-85 to 80 msec); 0.25 mg: -2.4 msec (-123 to 53); 0.5 mg: 3.6 msec (-68 to 90); 1.0 mg: 6.6 (-55 to 70 msec) (see Table 2.2.3.3.1). Although there appears to be a trend for dose-QTF response relationship with a mean delta QTF of 6.6 msec observed at the maximum dose evaluated (1 mg/day), these data should be interpreted with caution since the study was not designed prospectively to address the potential effect of the drug on QTc: there was only one baseline value of ECG taken at baseline and one value of ECG taken after drug administration (the ECG collection time in regards to drug administration was not mentioned); in addition no positive control was included.

The above mentioned findings come from Study P04-001. This study was a randomized, parallel, placebo-controlled, double-blind, multicenter study in postmenopausal women with MSVS. Patients (495 enrolled patients; about 120 /group) received treatment with Divigel® (Estradiol Gel, 0.1%) or placebo for 12 weeks.

Single 12-lead ECGs were performed at baseline (Day -2 to -1), and single 12-lead ECGs were performed after the end of drug treatment (week 13). The sponsor reported QTc values, however, the method of correction was not mentioned. The heart rate values were highly variable; there was one subject whose HR was 118 bpm. This reviewer corrected the QT interval for heart rate (HR) using two fixed-exponent correction formula ($QTc=QT/RR^{\alpha}$) where $\alpha=0.500$ (Bazett's, QTcB) or $\alpha=0.333$ (Fridericia's, QTcF). Mean steady-state changes from mean baseline were calculated for each subject. Categorical analysis of the Emax values into <30 msec, >30 to <60 msec and >60 msec for each subject were also reported. Serum samples for E2, E1 or ES determination were not obtained at the same time points as the ECG recordings.

Comparisons of the results of the analysis showed that Fridericia's correction formula (QTcF) yielded a slope closer to zero (0.007) than Bazett's (-0.068) (Figure 2.2.3.3.1). Table

2.2.3.3.1 shows the mean change from baseline in QTB, QTF, and QTc (reported by the sponsor and confirmed by this reviewer).

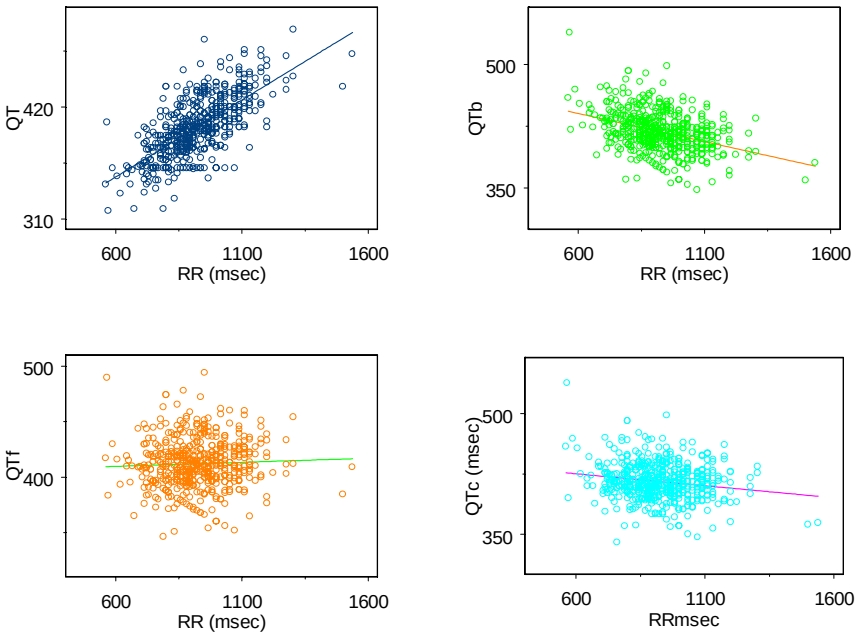


Figure 2.2.3.3.1. Individual QT, QTcB, QTcF, and QTc as a function of RR following multiple administration of Divigel®, 0.25 mg, 0.5 mg, and 1.0 mg/day to postmenopausal women.

Table 2.2.3.3.1. Mean, median, min and max Δ QTc change from baseline following multiple administration of the treatments

	Delta QTc (msec)				Delta QTF (msec)				Delta QTB (msec)			
TRT	**PLB**	**0.25**	**0.5**	**1.0**	**PLB**	**0.25**	**0.5**	**1.0**	**PLB**	**0.25**	**0.5**	**1.0**
minimum	-85	-123	-68	-55	-82.5	-51.2	-44	-31	-84.3	-95.4	-67	-47
mean	-2.8	-2.4	3.6	6.6	-1.76	-0.6	3.3	7.9	-2.0	-1.03	2.8	7.1
median	-3.0	1.0	3	3.0	-1.6	-2.4	3.5	5.7	-0.7	0.0	0.7	5.5
maximum	80	53.0	90	70	80	107.4	84	69.2	80	159.2	86	69.6
SD	20.6	24.7	20	20	19.3	21.2	19	17.5	20.5	28.2	21	20.22
N	110	109	107	111	110	109	107	111	110	109	107	111
95% CI	-6.7 to 1.1	-7 to 2.3	-0.35 to 7.6	2.8 to 10.4	-5.4 to 1.9	-4.6 to 3.4	-0.37 to 7	4.6 to 11.2	-6 to 1.8	-6.4 to 4.3	-1.28 to 6.9	3.3 to 10.9

Several subjects (25 to 30 subjects) had QTF values higher than 450 msec. Four subjects had QTF values between 480 and 500 msec, and 2 subjects had QTF values higher than 500 msec (504 msec) (Figure 2.2.3.3.2). Twenty two subjects had delta QTF values between 30 and 60 msec (4 after the 0.25 mg dose; 6 after the 0.5 mg dose; 10 after the 1 mg dose; and 2 after PLB) and 4 subjects delta QTF values above 60 msec (one for each treatment). The highest delta QTF value was 107.37 msec for a subject who received the 0.25 mg/day dose) (Figure 2.2.3.3.3).

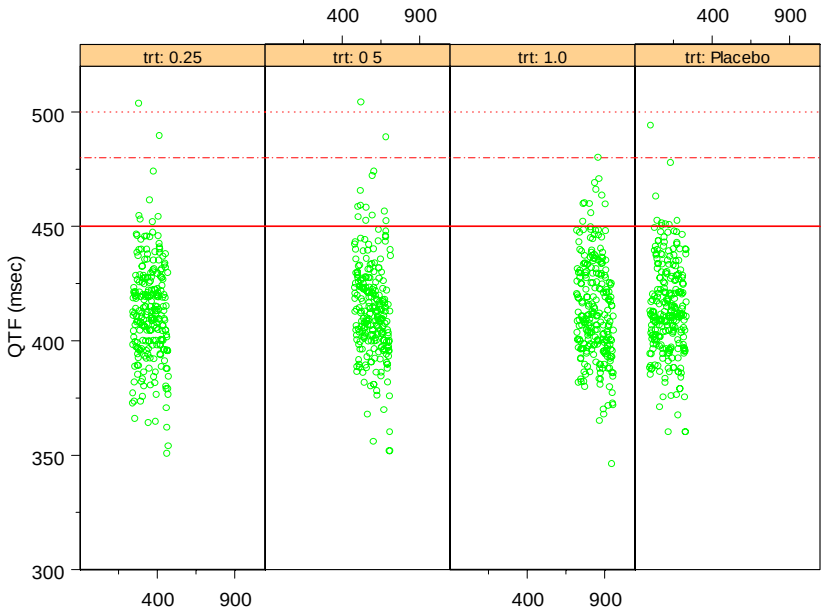


Figure 2.2.3.3.2. QTF (msec) for Divigel as a function of treatment.

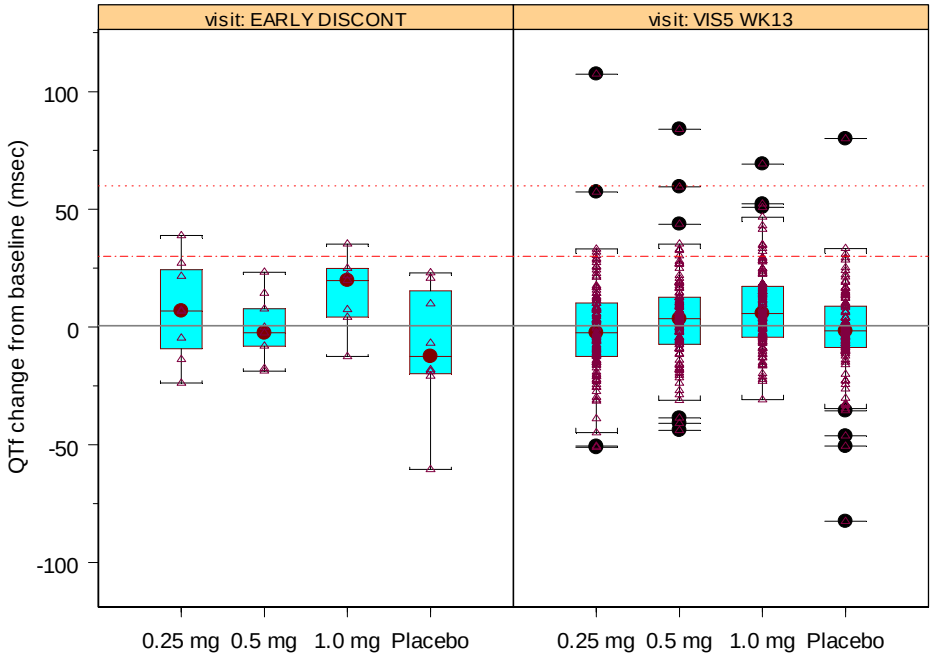


Figure 2.2.3.3.3. Mean change from baseline in QTF (msec) for Divigel as a function of treatment.

2.2.4 What are the PK characteristics of the drug?

2.2.4.1 What are the single and multiple dose PK parameters of E2 and its metabolites? How do the PK parameters change with time following chronic dosing?

Following single dose administration of Divigel® 0.1% to the upper thigh, slow absorption occurs from the skin. The E2 and its metabolites concentration and therefore, the PK parameters were highly variable. E2 (corrected for baseline) reaches peak serum concentrations of approximately 3.3 pg/ml, 9.2 pg/mL and 32.7 pg/mL following single doses of 0.25-, 0.5-, and 1.0 mg, respectively (CV ranged from 90-176%) within 10 hours (Table 2.2.4.1.1). Based on literature information, the half-life of 17 ß-E2 is approximately 15 hours. It circulates bound to sex hormone binding globulin SHBG (37%) and to albumin (61%), while only approximately 1-2% remains unbound in the circulation. The PK parameters of E1 and ES are summarized in Tables 2.4.2.1.2 and 2.2.4.1.3, respectively.

Table 2.2.4.1.1. Summary of Pharmacokinetic Parameters of Estradiol (Arithmetic Mean [%CV]) After a Single Dose of Divigel 0.1% on Day 1

Parameter (units)	Divigel 0.1% 0.25 mg		Divigel 0.1% 0.5 mg		Divigel 0.1% 1.0 mg	
	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)
AUC0-24 (pg•h/mL)	293 (159)	45 (126)	288 (106)	111 (98)	488 (80)	354 (76)
Cmax (pg/mL)	15.0 (159)	3.3 (176)	16.7 (98)	9.2 (98)	38.2 (88)	32.7 (90)
tmax* (h)	10 (0, 24)	10 (4, 24)	10 (0, 24)	10 (0, 24)	10 (5, 24)	10 (5, 24)

*Median (Min, Max).

Table 2.2.4.1.2. Summary of Pharmacokinetic Parameters of Estrone (Arithmetic Mean [%CV]) After a Single Dose of Divigel 0.01% on Day 1

Parameter	Divigel 0.1% 0.25 mg		Divigel 0.1% 0.5 mg		Divigel 0.1% 1.0 mg	
	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
(units)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-24 (pg•h/mL)	501 (53)	22 (67)	469 (52)	51 (84)	534 (37)	128 (84)
Cmax (pg/mL)	27.4 (48)	2.4 (115)	25.7 (50)	4.6 (105)	30.6 (30)	11.1 (73)
Tmax* (h)	24 (0, 24)	24 (0, 24)	24 (0, 24)	24 (0, 24)	24 (0, 24)	24 (0, 24)

*Median (Min, Max).

Table 2.2.4.1.3. Summary of Pharmacokinetic Parameters of Estrone Sulfate (Arithmetic Mean [%CV]) After a Single Dose of Divigel 0.01% on Day 1

Parameter	Divigel 0.1% 0.25 mg		Divigel 0.1% 0.5 mg		Divigel 0.1% 1.0 mg	
	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
(units)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-24 (pg•h/mL)	6552 (79)	335 (94)	7451 (78)	1684 (90)	9022 (56)	4239 (60)
Cmax (pg/mL)	392.9 (94)	45.5 (109)	422.6 (76)	129.7 (100)	540.3 (52)	292.2 (70)
tmax* (h)	5 (0, 24)	7 (1, 24)	11 (0, 24)	14 (2, 24)	14 (0, 24)	14 (1, 24)

*Median (Min, Max).

Multiple Dose Administration

Following multiple dose administration of Divigel® 0.1%, E2 (uncorrected for baseline) reached peak serum concentrations of approximately 14.7 pg/ml, 28.4 pg/mL and 51.7 pg/mL at the doses 0.25-, 0.5-, and 1.0 mg/day, respectively with a median tmax of 8 to 16 hours (Table 2.2.4.1.4). PK parameters were highly variable; CV % ranged from 84 to 149. The accumulation factor based on AUC_{24hrs} was about 1.75 to 2.1, 1.1 to 2.1, and 1.41 to 2.7 for E2, E1 and ES, respectively. The PK parameters for E1 and ES are summarized in Tables 2.2.4.1.5 and 2.2.4.1.6. The mean E2/E1 ratio ranged from 0.45 to 0.65 across Divigel® doses.

The single and multiple PK information presented above come from Study P04-003. This study was a Phase 1, randomized, open-label, multiple-dose study conducted according to a 3-way crossover design. Twenty-one subjects were randomized to 1 of 3 treatment sequences in which each subject received the following treatments over 3 study periods: Treatment A: 0.25 g of estradiol gel 0.1% (0.25 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days; Treatment B: 0.5 g of estradiol gel 0.1% (0.5 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days; Treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

Steady state, tested by regressing trough level concentrations collected on Days 12, 13, and 14 onto day, resulted in slope values that were not significantly different from 0 indicating that multiple doses of USL-221 resulted in the achievement of steady state for each of the 3 doses administered in this study (data not shown).

Table 2.2.4.1.4. Summary of Pharmacokinetic Parameters of Uncorrected Estradiol (Arithmetic Mean [%CV]) After Multiple Doses of Divigel 0.1% on Day 14

Parameter (units)	**Divigel 0.1% 0.25 mg** Mean (%CV)	**Divigel 0.1% 0.5 mg** Mean (%CV)	**Divigel 0.1% 1.0 mg** Mean (%CV)
AUC0-24 (pg•h/mL)	236 (94)	504 (149)	732 (81)
AUC0-72 (pg•h/mL)	717 (106)	1262 (144)	1424 (83)
AUC0-t (pg•h/mL)	712 (107)	1260 (145)	1421 (83)
Cmax (pg/mL)	14.7 (84)	28.4 (139)	51.5 (86)
Cavg (pg/mL)	9.8	21	30.5
Cmin (pg/mL)	10.6 (103)	21.5 (149)	19.6 (64)
Cflux (%)	79 (216)	47 (116)	166 (124)
tmax* (h)	16 (0, 72)	10 (0, 72)	8 (0, 48)

*Median (Min, Max).

Table 2.2.4.1.5. Summary of Pharmacokinetic Parameters of Uncorrected Estrone (Arithmetic Mean [%CV]) After Multiple Doses of Divigel 0.1% on Day 14

Parameter (units)	**Divigel 0.1% 0.25 mg** Mean (%CV)	**Divigel 0.1% 0.5 mg** Mean (%CV)	**Divigel 0.1% 1.0 mg** Mean (%CV)
AUC0-24 (pg•h/mL)	555 (36)	771 (38)	1122 (46)
AUC0-72 (pg•h/mL)	1759 (33)	2273 (37)	3045 (41)
AUC0-t (pg•h/mL)	1759 (33)	2273 (37)	3045 (41)
Cmax (pg/mL)	30.2 (27)	39.7 (38)	58.9 (45)
Cmin (pg/mL)	26.8 (35)	36.8 (37)	48.7 (46)

Cflux (%)	18 (122)	8 (121)	24 (130)
tmax* (h)	24 (0, 48)	4 (0, 48)	8 (0, 72)

*Median (Min, Max).

Table 2.2.4.1.6. Summary of Pharmacokinetic Parameters of Uncorrected Estrone Sulfate (Arithmetic Mean [%CV]) After Multiple Doses of Divigel 0.1% on Day 14

Parameter	**Divigel 0.1% 0.25 mg**	**Divigel 0.1% 0.5 mg**	**Divigel 0.1% 1.0 mg**
(units)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-24 (pg•h/mL)	9220 (62)	13586 (47)	24089 (67)
AUC0-72 (pg•h/mL)	27688 (57)	40382 (49)	61029 (64)
AUC0-t (pg•h/mL)	27688 (57)	40382 (49)	61029 (64)
Cmax (pg/mL)	616.9 (60)	861.0 (47)	1465.6 (70)
Cmin (pg/mL)	398.1 (59)	621.5 (48)	980.4 (75)
Cflux (%)	61 (83)	46 (104)	59 (61)
tmax* (h)	8 (0, 72)	8 (0, 48)	5 (0, 72)

*Median (Min, Max).

Based on population PK analysis on data from the pivotal Phase III clinical trial (P04-001) where multiple dose administration of Divigel® 0.25 mg, 0.5 mg, 1.0 mg/day, and placebo for 12 weeks to postmenopausal women, the predicted AUC values are presented in Table 2.2.4.1.7. The uncorrected AUCss values from population PK analysis were similar to those AUC_{72hrs} reported in Study P04-003.

Table 2.2.4.1.6. Comparison of model predicted E2 and E1 average concentrations and AUCss following multiple administration of Divigel 0.25, 0.5 and 1.0 mg/day to postmenopausal women (Data from Study P04-001)

Variable	**Mean reported by sponsor**	**Mean calculated by this reviewer**
	0.25 mg estradiol (n =109)	
CL/F of El (L/hr)	964	961
CL/F of E2 (L/hr)	597	596
AUC of El (hr*pg/mL)	922.2	931
AUC of E2 (hr*pg/mL)	613.2	616
Cavg of E1 (pg/mL)	38.4	38.8
Cavg of E2 (pg/mL)	25.6	26
Cavg of E2/ Cavg of E1	0.68	0.67
	0.5 mg estradiol (n =106)	
CL/F of El (L/hr)	989	987
CL/F of E2 (L/hr)	768	765
AUC of El (hr*pg/mL)	12056	1229
AUC of E2 (hr*pg/mL)	1188.1	1193
Cavg of E1 (pg/mL)	50.2	51.22
Cavg of E2 (pg/mL)	50	50
Cavg of E2/ Cavg of E1	0.96	0.99
	1.0 mg estradiol (n =112)	
CL/F of El (L/hr)	955	953
CLIF of E2 (L/hr)	637	637
AUC of El (hr*pg/mL)	1839.7	1843

AUC of E2 (hr*pg/mL)	2155	2163
Cavg of E1 (pg/mL)	77	77
Cavg of E2 (pg/mL)	89.8	90
Cavg of E2/ Cavg of E1	1.23	1.17

2.2.4.2 Are the PK of Divigel® and its metabolites linear and dose-proportional?

Dose-proportionality following single and multiple administration of Divigel®, 0.25 mg, 0.5 mg and 1.0 mg was evaluated in as part of Study P04-003 (Phase I, multiple dose study.

Following single dose administration, E2 peak serum concentrations and AUC24hrs increased more than proportionally to the dose. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 147% in mean corrected AUC0-24 was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 219% was observed.

Following multiple dose administration, E2 peak serum concentrations and AUC increased roughly less than proportionally to the dose. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 114% in mean uncorrected AUC_{0-24} was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 45% was observed. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 93% in mean uncorrected Cmax was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 81% was observed. Based on the power model, E2 increase roughly proportional to the dose with a slope of 0.8 (Figure 2.2.4.1.1). E1 and ES AUC values increased less than proportionally to the dose following multiple administration of the treatments (see Tables 2.2.4.1.5, 2.2.4.1.6 and Figure 2.2.4.1.1).

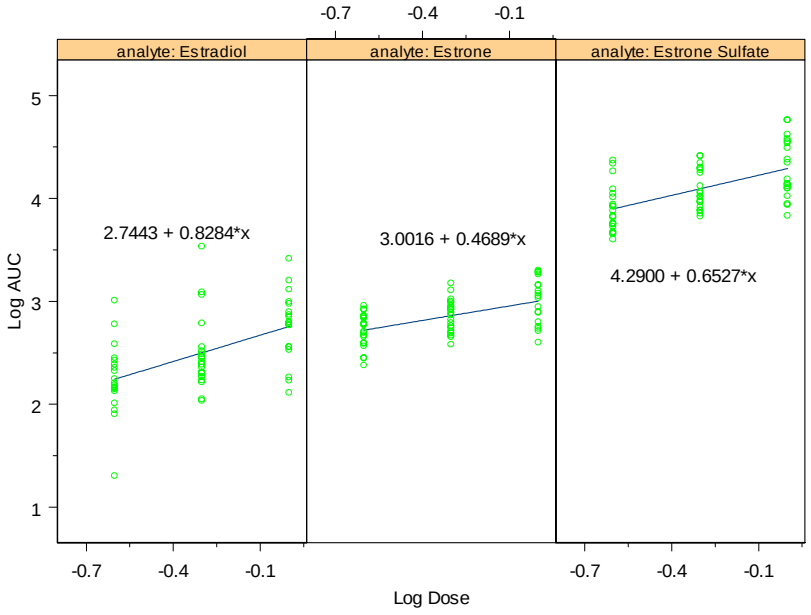


Figure 2.2.4.1.1. Individual E2, E1 and ES AUC (log values) as a function of log-Dose (fitted line from power model: $AUCE2 = e^{-2.7} * (dose)^{0.8}$; $AUCE2 = e^{-3} * (dose)^{0.5;}$ $AUCE2 = e^{-4.2} * (dose)^{0.7}$) following multiple administration of the treatments. Data from Study P04-003 (Phase I multiple dose study).

Based on data from population PK analysis the mean serum concentrations and the predicted AUCss increased proportionally to the dose: two fold increased in the dose from 0.25 mg to 0.5 mg and from 0.5 mg to 1.0 mg produced two-fold increased in the predicted AUCss

values (613-, 1188-, and 2155 pg*hr/mL, for the 0.25-, 0.5- and 1.0 mg/day, respectively (see Table 2.2.4.1.6 and Figure 2.2.4.1.2).

Figure 2.2.4.1.2. Box plot of individual E2 serum concentrations following multiple administration of Divigel 0.25, 0.5, 1.0 mg and PLB (data from Phase III study P01-001).

2.2.4.3 What is the degree of estradiol transferability from subjects dose with Divigel® to nondosed subjects?

Following single dose administration of Divigel®, 1 mg to the skin of the upper thigh (200-cm^2 area) of postmenopausal women, the percentage mean increase in E2 Cmax (33.95 pg/mL) compared to mean baseline (25.01 pg/mL) in non-dosed patients who had skin contact with **unclothed** application site **60 minutes** after dose was about 35%. The percentage mean increase in E2 AUCt (1803.21 pg*hr/mL) compared to mean baseline (1663.9 pg*hr/mL) in non-dosed patients who had skin contact with **unclothed** application site **60 minutes** after dose was about 9% (Table 2.2.4.3.1 and 2.2.4.3.2)

The percentage mean increase in E2 Cmax and AUCt compared to mean baseline in non-dosed patients who had skin contact with **unclothed** application site **8 hrs** after dose was about 30% and 12%, respectively. The percentage mean increase in Cmax and AUC of E2 in nondosed subjects who had contact with **clothed** application site **60 minutes** after application was about 34% and 13%, respectively (Figure 2.2.4.3.1, Table 2.2.4.3.2).

The clinical relevance of about 10% increase in systemic exposure (AUC) of in non-dosed subjects (i.e. male volunteers) in unknown.

Table 2.2.4.3.1. Summary of Pharmacokinetic Parameters of Estradiol (Arithmetic Mean [%CV]) in Nondosed Subjects Who Had Skin Contact With Dosed Subjects

Parameter (units)	**Contact with clothed application site 60 minutes after dosing (Treatment A) N=23**		**Contact with unclothed application site 60 minutes after dosing (Treatment B) N=22**		**Contact with unclothed application site 8 Hours after dosing (Treatment C) N=24**	
	Uncorrected	**Corrected**	**Uncorrected**	**Corrected**	**Uncorrected**	**Corrected**
	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-t (pg•hr/mL)	1751.82 (31)	203.83 (83)	1803.21 (31)	139.26 (69)	1793.76 (33)	165.72 (99)
Cmax (pg/mL)	31.84 (34)	8.24 (63)	33.95 (37)	8.89 (57)	31.49 (34)	6.89 (62)
Cavg (pg/mL)	24.3 (7.6)	2.83 (2.3)	25.04 (7.8)	1.93 (1.33)	24.9 (8.2)	2.3 (2.2)
tmax* (h)	10.0 (0, 72)	10.0 (0, 72)	4.0 (0, 48)	4.0 (0, 48)	9.0 (1, 24)	7.0 (1, 24)

*Median (Min, Max).

Table 2.2.4.3.2. Percentage of Estradiol Transfer in Nondosed Subjects Who Had Skin Contact With Dosed Subjects

Parameter	**Contact with clothed application site 60 minutes after dosing (Treatment A) N=23**		**Contact with unclothed application site 60 minutes after dosing (Treatment B) N=22**		**Contact with unclothed application site 8 Hours after dosing (Treatment C) N=24**	
	**Baseline	*Mean (SD) Percentage of transfer	**Baseline	*Mean (SD) Percentage of transfer	**Baseline	*Mean (SD) Percentage of transfer
AUC0-t (pg*hr/mL)	1633.1 (436)	13.2 (11.5)	1696 (509)	9.05 (6.4)	1683 (522)	11.8 (15.4)
Cmax (pg/mL)	23.6 (6.9_	34.3 (20.1)	25.09 (10)	36.3 (15.5)	25.5 (8.9)	30.4 (26.5)

* Calculated as uncorrected value / baseline value*100. **Baseline was calculated as the mean of the difference between reported uncorrected-corrected values.

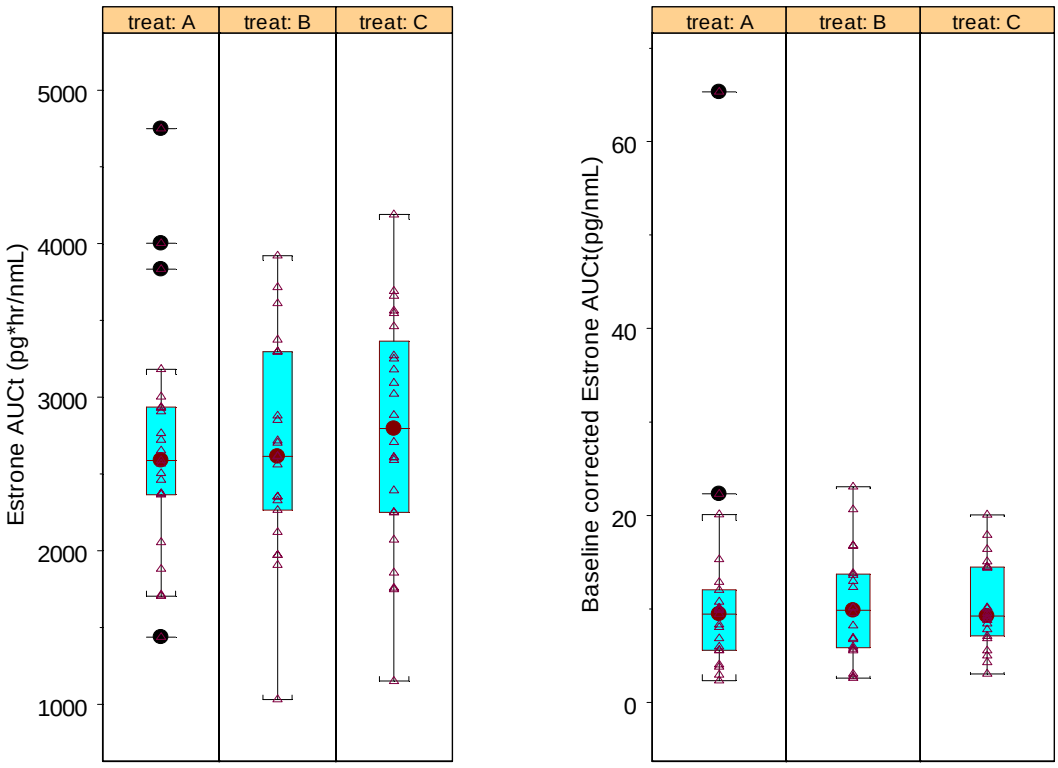


Figure 2.2.4.3.1. Individual E1 AUCt corrected and non-baseline corrected values following single administration of the treatments: Treatment: A = Skin contact with clothed application site 60 minutes after dose; B = Skin contact with unclothed application site 60 minutes after dose; C = Skin contact with unclothed application site 8 hours after dose to 24 healthy postmenopausal women. N=22 to 24 subjects.

These results come from study P04-002. This was a randomized, open-label, single-dose study conducted according to a 3-way crossover design. Subjects were assigned to pairs in which 1 subject was dosed and one was not. Each pair of subjects was randomized to 1 of 3 treatment sequences in which the treatments were received over 3 study periods.

- These data should be interpreted with caution due to the uncertainty on the procedure used to calculate E2 and its metabolites baseline values across the treatments. In several NDAs containing estradiol gel, baseline was determined based on 24 hrs blood sampling. In this NDA, baseline values were calculated as the average of 3 endogenous compound values determined at -12 hr, -6hr and prior drug administration. Change from baseline was then calculated as the AUC of individual values minus the mean of baseline. This reviewer considers that this procedure for calculating baseline and change from baseline of estrogens levels is not appropriate.

The labeling will reflect this uncertainty on the degree of transferability. Patients will be advised to restrain from direct contact for at least 1 hrs. after application of the gel and to cover the area of application after the gel is completely dry.

2.2.4.4 What is the effect of washing the application site (skin) on the systemic exposure of Divigel®?

Washing the application site one hour after single application of Divigel® 1.0 mg resulted in a decrease in total exposure (Cmax and AUC) of mean baseline-corrected and uncorrected estradiol by 30 to 38% (Table 2.2.4.4.1, Figure 2.2.4.4.1). Baseline-corrected values were calculated by subtracting the mean of the 3 predose values for each subject (-12-hour, -1-hour, and 0-hour samples) from all subsequent values.

Washing the application site one hour after single application of Divigel® 1.0 mg resulted in a decrease in total exposure of mean baseline-corrected and uncorrected estrone by 15 to 53% (Table 2.2.4.4.1). The mean baseline-corrected and uncorrected estrone sulfate Cmax and AUCt were decreased by 32 to 50% after washing the application site one hr post-application of Divigel®, 1.0 mg. The time it takes for the majority of the drug to be absorbed from the application site is unknown. This reviewer considers that since the amount of Divigel remaining in the site of application after one hour post application is significant, patients may be advised to restrain from washing the application site for at least one hr. after application.

Washing the application site for 3 minutes after 60 minutes of single skin application of Divigel 0.1% removed all detectable amounts of estradiol from the application site.

Table 2.2.4.4.1. Summary of Pharmacokinetic Parameters of Estradiol (Arithmetic Mean [%CV]) After a Single Dose of Divigel 0.1% With and Without Washing 1 Hour After Application

Parameter (units)	**Washed 1 Hour After Application (Treatment A) N=16**		**Not Washed (Treatment B) N=16**	
	Uncorrected Mean (%CV)	**Corrected Mean (%CV)**	**Uncorrected Mean (%CV)**	**Corrected Mean (%CV)**
AUC0-t (pg•h/mL)	1422 (133)	568 (122)	2304 (182)	773 (87)
AUC0-24 (pg•h/mL)	547 (95)	233 (74)	1059 (152)	477 (81)
Cmax (pg/mL)	52 (64)	41 (70)	98 (110)	66 (84)
tmax* (h)	5.5 (0.5, 36)	5.5 (0.5, 36)	8.0 (0.0, 48)	8.0 (0.0, 48)

*Median (Min, Max).

Treatment A = USL-221 1.0 mg, after 60 minutes wash with mild hypoallergenic soap and washcloth for 30 seconds and rinse with warm water for 2.5 minutes; Treatment B = USL-221 1.0 mg.

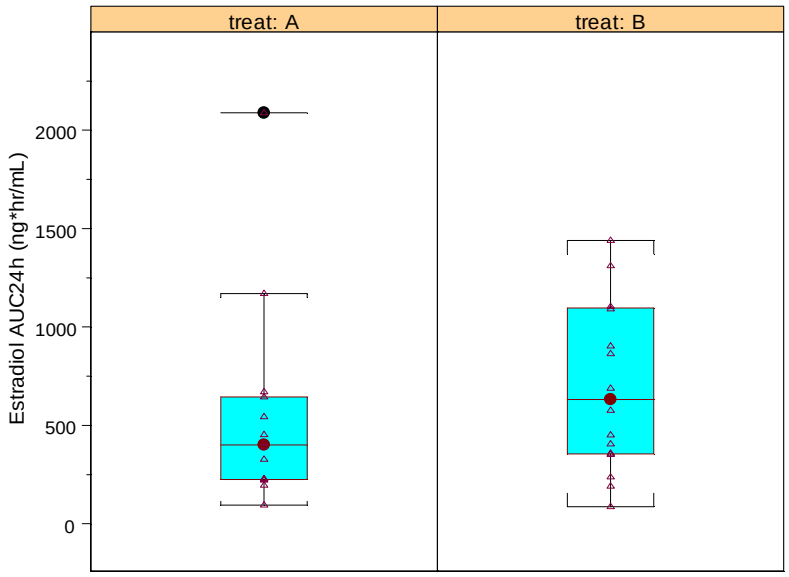


Figure 2.2.4.4.1. Individual E2 AUCt non-baseline corrected values following single administration of the treatments: Treatment A: washing 1 hr after single dose administration of Divigel 1 mg (n=16).; Treatment B: no washing (n=16).

Table 2.2.4.4.2. Summary of Pharmacokinetic Parameters of Estrone (Arithmetic Mean [%CV]) After a Single Dose of Divigel 0.1% With and Without Washing 1 Hour After Application

Parameter (units)	Washed 1 Hour After Application (Treatment A) N=16		Not Washed (Treatment B) N=16	
	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)
AUC0-t (pg•h/mL)	2042 (57)	353 (78)	2568 (91)	501 (55)
AUC0-24 (pg•h/mL)	654 (52)	87 (91)	875 (103)	155 (81)
Cmax (pg/mL)	34 (53)	9 (91)	45 (88)	14 (56)
tmax* (h)	24 (0, 72)	24 (0, 72)	24 (7, 72)	24 (16, 72)

These results come from Study P04-005. This was a Phase 1, randomized, open-label, single-dose study conducted according to a 3-way crossover design. The study consisted of 3 periods. Sixteen subjects were randomized to 1 of 2 treatment sequences in which each subject received the following treatments over the first 2 study periods:

Treatment A: 1.0 mg of Divigel® applied to a 200-cm^2 area on the thigh. The application site was washed with soap and water 60 minutes after study drug was applied. Treatment B: 1.0 mg of Divigel® applied to a 200-cm^2 area on the thigh. There was a 14-day washout period between treatments. After completion of treatment period, subjects were crossed over to the other study treatment. During Period 3, subjects were randomized to receive Treatment C and D as follows: Treatment C: 1.0 g of Divigel® applied to a 200-cm^2 area on the thigh. After 60 minutes, a 10-cm^2 area was swabbed for analysis of residual levels of estradiol at the application site. The area was then washed, and a second swab collection was taken 15 minutes after the start of washing. Treatment D: 1.0 mg of Divigel® applied to a 200-cm^2 area on the thigh. After 8 hours, a 10-cm^2 area was swabbed for analysis of residual levels of estradiol at the application

site. The area was then washed, and a second swab collection was taken 15 minutes after the start of washing.

2.3 Intrinsic Factors

2.3.1 Does age, WT, race, or disease state affect the PK of the drug? What dosage regimen adjustments are recommended for the subgroups?

Uterus status (absence or presence), sex hormone binding globulin (SHBG) levels at screening, FSH levels at screening, estradiol dose, race, age (34 to 89 years), and body weight were evaluated as covariates in the population PK analysis. None of the covariates evaluated had a significant effect on the CL/F estimates of E2 and its metabolites (Table 2.3.1.1 and Figures 2.3.1.1 and 2.3.1.2). NO effect of race should be interpreted with caution since there were 287 White patients and only 40 Non-White patients (31 Black, 4 Asian, and 5 others).

Table 2.3.1.1. Listing of PK model in NONMEM analysis for E2 in chronological order

Test	Reference	OF	Change in OF	Description of the Model Tested	Test Results
Mod 1	-	1879.08	-	Base model	-
Mod 2	Mod 1	1875.67	-4.13	WT on CL	SIG
Mod 3	Mod 1	1879.65	-0.14	Dose on CL	NS
Mod 4	Mod 1	1879.20	-0.59	CrCL on CL	NS
Mod 5	Mod 1	1879.77	-0.02	ALKP on CL	NS
Mod 6	Mod 1	1879.16	-0.64	AST on CL	NS
Mod 7	Mod 1	1879.51	-0.28	Race on CL	NS
Mod 8	Mod 1	1879.76	-0.03	Age on CL	NS
Mod 9	Mod 1	1879.05	-0.75	Uter on CL	NS
Mod 2	-	1875.67	-	Full model	-
Mod 1	Mod 2	1879.8	4.13	Remove WT on CL	NS
Mod1cov	Mod 1	1840.0	-39.8	Covariance of CL and V	SIG
Mod1cov				Final Model	

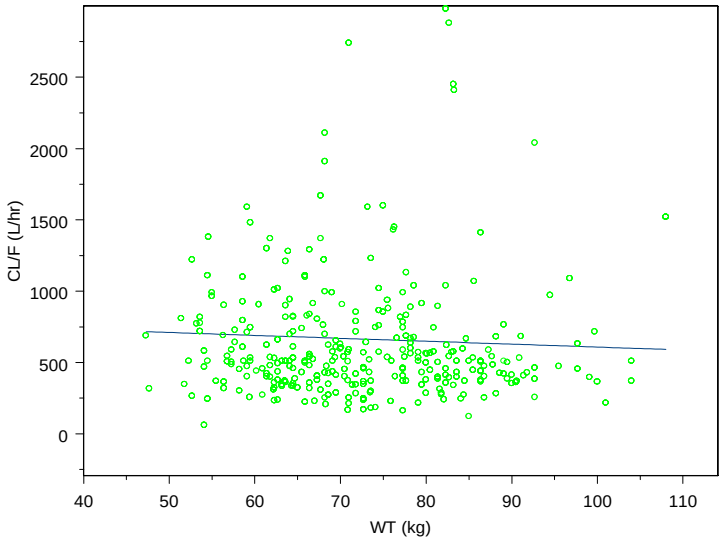


Figure 2.3.1.1. Final Model-Predicted Individual Bayesian Estimates of E2 CL/F versus WT.

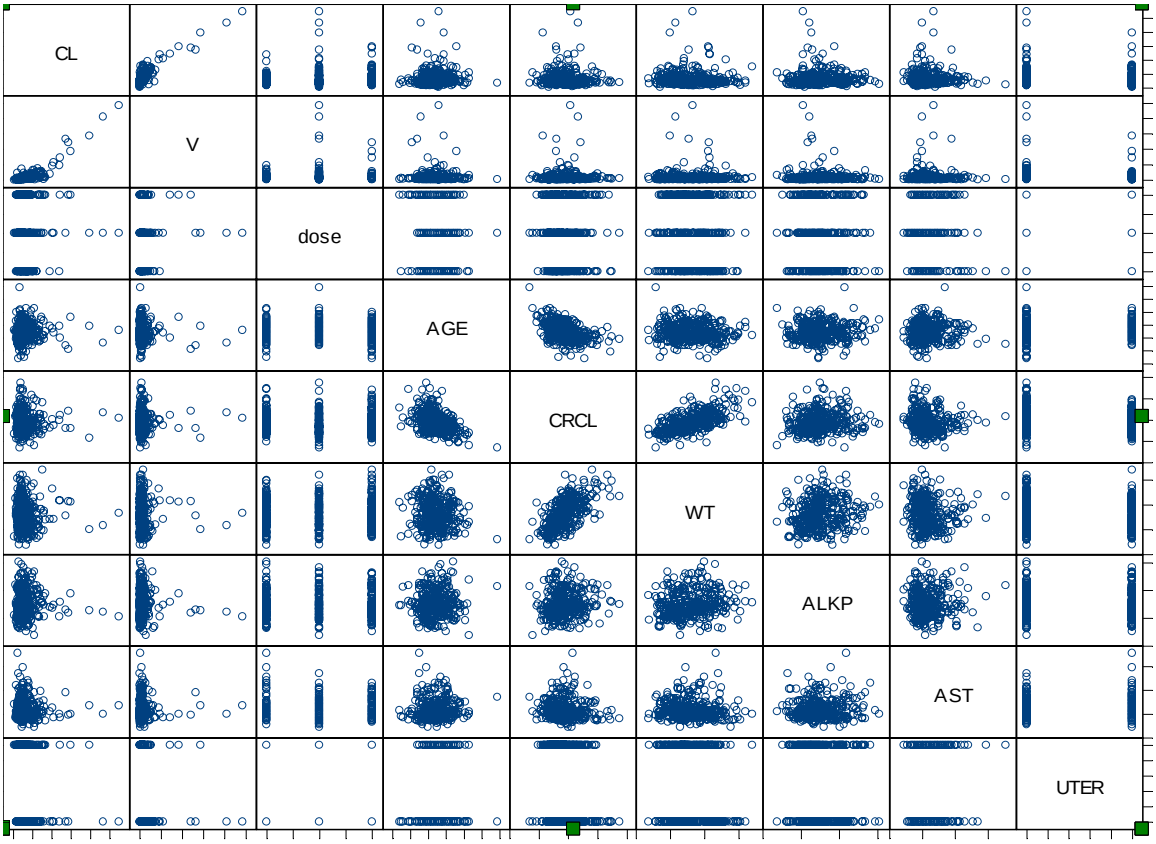


Figure 2.3.1.2. Matrix plots of E2 CL (L/hr) versus demographic variables: AGE (years), WT (kg), Dose (mg), CrCL (mL/min), presence/absence of Uterus, Hepatic function (ALKP, AST), and Race (Caucasian, Black, Asian, Other).

The above mentioned population PK analysis of Divigel® and its metabolites included data from pivotal clinical trial P04-001 (Phase 3 trial in postmenopausal female patients). Basic structural population PK models for E2 and its two metabolites were developed using data from Divigel® phase I studies. These models were then applied to estimate the population and individual PK parameters and steady-state concentrations of E2, E1, and ES in postmenopausal patients following once daily application of USL-221 at three estradiol dose amounts (0.25 mg, 0.5 mg, and 1.0 mg). The potential effects of demographic and baseline characteristics and concomitant medications on E2, E1, and ES PK following Divigel® application were also investigated using the population PK analysis.

The data sets available for the population PK analysis of E2, El, and ES profiles consisted of 1,291 serum samples collected from 327 female postmenopausal patients at weeks 0, 4, 8, and 12, from Protocol P04-001. Samples were collected at baseline and then within 1-10 hours of the morning dose at the time of each patient's routine. There were approximately 80 samples collected after 10 hrs of drug administration.

An open one-compartmental model with linear disposition and sequential zero-order and first-order absorption incorporating lag time was found to best describe the data in this analysis for each analyte. The evaluation of covariates was performed in a sequential approach. Identification of relevant covariates was based on step-wise forward and backward elimination method. The model was validated using the bootstrap technique**.**

2.3.2 Does renal impairment affect the PK of the drug? Is dosage regimen adjustment recommended?

The effect of renal impairment on the PK of E2 and its metabolites was not formally evaluated. Based on population PK analysis, renal impairment (mild or moderate; measured as a function of CrCL) did not affect the PK of E2 and its metabolites. The effect on severe renal impairment on the PK of the drug is unknown since no patients with this condition were included in the study.

2.3.3 Does liver impairment affect the PK of the drug? Is dosage adjustment recommended?

The effect of hepatic function on the PK of E2 and its metabolites was not formally evaluated. Based on population PK analysis, hepatic impairment (mild or moderate; AST, ALT, alkaline phosphatase, and total bilirubin levels were used as indicators of hepatic function) did not affect the PK of E2 and its metabolites. The effect on severe hepatic impairment on the PK of the drug is unknown since no patients with this condition were included in the study.

2.3.4 What pregnancy and lactation use information is there in the application?

Estrogen administration to nursing mothers has been shown to decrease the quantity and quality of the milk. Detectable amounts of estrogens have been identified in the milk of mothers receiving estrogen therapy. Caution should be exercised when DIVIGEL is administered to a nursing woman.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of any differences in exposure on pharmacodynamics?

The effects of herbal products, diet, smoking and alcohol use were not evaluated.

2.4.2 Drug-Drug Interactions (DDI)

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

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 the uterine bleeding profile. Inhibitors of CYP3A4 such as erythromycin, clarithromycin, ketoconazole, itraconazole, ritonavir, and grapefruit juice may increase plasma concentrations of estrogens and result in side effects.

2.4.2.2 Is the drug a substrate of CYP enzymes?

Estrogens are metabolized partially by cytochrome P450 3A4. Metabolism of 17 ß-E2 occurs mainly in the liver and gut but also in target organs, and involves the formation of less active or inactive metabolites, including E1, catecholestrogens, and several estrogen sulphates and glucuronides. Estrogens are excreted with the bile, where they are hydrolyzed and reabsorbed (enterohepatic circulation), and mainly in urine in biologically inactive form.

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

The potential inhibitor/inducer effect of Divigel® on CYP enzymes has not been reported by the sponsor.

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

This was not evaluated by the sponsor.

2.4.2.5 What is the effect of Divigel® on the PK of other drugs? What is the effect of other drugs on the PK of Divigel®?

The effect of Divigel® on the PK of other drugs has not been evaluated by the sponsor. No formal studies were conducted to evaluate the effect of other drugs on the PK of Divigel®. Based on population PK analysis using data from pivotal clinical study P04-001, there were 50 concomitant medications taken by at least 6 patients each. The median CL/F of E2 for patients on miconazole (n = 7) was about 30% lower than the median of the whole population. These results were in disagreement with the findings for fluconazole, another CYP3A4 inhibitor. The median CL/F of E2 for patients on fluconazole (n = 6) was about the same as the median of the whole population. Therefore, no final conclusions on the effect of concomitant administration can be made from this population PK analysis.

2.5 General Biopharmaceutics

2.5.1 What is the BCS Class classification for Divigel®?

This information was not provided by the sponsor.

2.5.2 Was the to-be-marketed formulation used in the PK/clinical trials?

YES. The history of the proposed U.S. commercial formulation consists of two main formulations. The original formulation was used in clinical studies sponsored by Orion and was available commercially outside of the U.S. from 1994 until 2002/2003. (b) (4)

Three sponsors currently have clinical development programs in place for Estradiol Gel, 0.1 %: (b) (4) Orion Pharma (Europe) and USL (U.S.). Thirty-four of the clinical studies conducted by Orion, (b) (4) and USL are included in this NDA submission as supporting information. The majority of the supporting studies included in this submission (conducted by Orion (b) (4)) utilized the original formulation of Estradiol Gel, 0.1 %, although both sponsors also conducted a few clinical studies with the (b) (4) formulation. In contrast, USL's clinical development program (four Phase 1 studies and one Phase 3 study) used only the (b) (4) formulation of Estradiol Gel, 0.1 % (USL-221), which is the same as the to-be marketed formulation.

2.5.3 Was the to-be-marketed formulation equivalent to the clinical trial formulation?

The to-be marketed formulation and the formulation used in the PK and pivotal clinical trials are the same. However, as mentioned above, the sponsor conducted a BE study (Study FR00.037.2) between the current Estradiol Gel, 0.1 % (b) (4)

formulation) and the original Estradiol Gel, 0.1 % used in the clinical studies sponsored by Orion.

Study FR00.037.2 was a multiple dose, fasting, three-way, cross-over, BE study with 3 formulations of Divigel®. Patients (twenty-four healthy postmenopausal women) received the following treatments without a washout period:

- Treatment A (reference product, Orion formulation): Divigel® formulation A 1 mg of gel applied on a skin area of 400 cm^2 (thigh) once daily for 14 days.
- Treatment B (Test formulation 1): Divigel® formulation B 1 mg of gel applied on a skin area of 400 cm^2 (thigh) once daily for 14 days.
- Treatment C (Test formulation 2: to-be marketed formulation of Divigel® 0.1%): Divigel® formulation C 1 mg of gel applied on a skin area of 400 cm^2 (thigh) once daily for 14 days.

Single oral doses of Provera® 10 mg tablets for 14 days were given to all subjects (except hysterectomised subjects) at the end of the Divigel® treatment.

The arithmetic mean (%CV) of E2 PK parameters are shown in Table 2.5.3.1. Substantial inter-subject variability was observed for E2 levels across treatments. The results of the BE study can be summarized as follows:

- The Divigel® Reference Product (Formulation A, Orion formulation) was bioequivalent to the Test product C (to-be marketed formulation) in terms of AUCt following multiple dose administration via skin. The 90% CI (89.7 to 123) were within BE standards. However, the two formulations were not BE in terms of Cmax. The 90% CI were within 87.9 128 (Table 2.5.3.2)

Table 2.5.3.1 Mean (%CV) uncorrected E2 PK parameters following administration of the treatments

Parameter	Treatment A	Treatment B	Treatment C
AUCt (pg*hr/mL)	592.6 (42)	974.22 (133)	757.6 (87)
Cmax (pg/mL)	41.6 (80)	63.78 (104)	44.11 (82)
Cmin (pg/mL)	18.9 (28)	28.66 (133)	23.04 (85)
Cavg (pg/mL)	24.8 (42)	27.84 (132)	31.472 (49)

Table 2.5.3.2. Point estimates and 90% confidence intervals based on uncorrected E2 levels

Comparison	**PK parameter**	**Point estimates**	**90% confidence intervals**
C/A*	Cmax	106	87.9-128
	AUCτ	105	89.7-123
B/A	Cmax	133	110-160
	AUCτ	126	108-148

*to-be marketed formulation versus original Orion formulation

Conclusion

- The current estradiol Gel, 0.1 % formulation (USL-2211EFI08, benzene free formulation) and the original Estradiol Gel, 0.1 % used in the clinical studies sponsored by Orion are not bioequivalent. The 90% CI for Cmax (87.9 to 128) were out of BE standards.
- This increase in Cmax in the to-be marketed formulation compared to the original Divigel® formulation may not be clinically relevant, considering the large variability observed for E2 PK parameters.
- The to-be marketed formulation and the formulation used in the PK and pivotal clinical trials are the same.

2.5.4 Are the method and dissolution specifications supported by the data provided by the sponsor?

A method for evaluation of in vitro release, using an automated (b) (4) a polysulfone synthetic membrane, was developed and validated by (b) (4). The amount of drug released per unit area ($\mu g/cm^2$) is plotted against the square root of time and the release rate is determined by calculating the slope of the line. An average of six determinations (slopes) is used for each sample.

In vitro release tests were carried out for different Estradiol Gel, 0.1 % batches and formulations. According to the sponsor, studies with different formulations of Estradiol Gel, 0.1 % showed a slight difference in estradiol release rate between the Original Formulation and the USL-221/EF108 formulation, whereas no difference was seen between the non-equivalent formulations of EFI07 and EFI08. SUPAC-SS comparisons showed no statistical differences between the three formulations tested. The sponsor stated that freshly manufactured Estradiol Gel, 0.1 % batches showed minimal variation on the release rate of estradiol as a function of viscosity. Aging of the product had no effect on the release rate since the release rates of the long-term stability samples were similar to the results for the freshly manufactured batches.

According to the sponsor, the proposed specification limits are intended to encompass the variability between both the batches of product and between different runs of the in vitro test. Using data from the study and three times standard deviation -around the minimum and maximum observed values, the proposed acceptance criteria for the average slope is between (b) (4). The average slope will be based on a minimum of five out of six individual determinations. For a detail information on the in vitro release testing and specifications for this product, refer to the Chemistry review done by Dr. Maria Ysern.

2.5.5 What is the effect of food on the BA of the drug?

Not applicable.

2.6 Analytical Section

2.6.1 Was the suitability of the analytical method supported by the submitted information?

The concentrations of 17ß-estradiol, estrone, and estrone sulfate in serum were determined by means of LC/MS/MS-methods. The lower limit of quantification for 17ß-estradiol was 2.5 pg/mL, for estrone was 5 pg/mL and for estrone sulfate was 50 pg/mL. In general, the sponsor provided enough information to show that the methods used were precise, accurate, specific, and sensitive for the measurement of the relevant moieties (see Tables 2.6.1 – 2.6.2).

Table 2.6.1. Summary of Study Performance (In Study Validation) for estradiol, estrone, and estrone sulfate

Parameter		Result
Calibration range	Estradiol	2.5-250 pg/mL
	Estrone	5.0-500 pg/mL
	Estrone sulfate	50-5000 pg/mL
Define LOQ	Estradiol	2.5 pg/mL
	Estrone	5.0 pg/mL
	Estrone sulfate	50 pg/mL
Linearity (mean r^2)	Estradiol	0.998
	Estrone	0.998
	Estrone sulfate	0.999

Table 2.6.2. Summary of Quality Control Performance for Estradiol and Estrone (data from Phase III clinical trial P04-001)

PPD Method	LCMSC 248.1			
Analytes	Estrone and 17-ß-Estradiol			
Matrix	Human Serum (modified and unmodified)			
Sample Volume	500 µL			
Estrone Validated Range	5.00 to 500 pg/mL			
17-ß-Estradiol Validated Range	2.50 to 250 pg/mL			
Internal Standard	(b) (4)			
Sample Storage Condition	-20 oC			
Assay Validation and Performance in Modified Human Serum				
	Intra-Assay Quality Control Samples		Inter-Assay Quality Control Samples	
Analyte	Precision (%CV)	Accuracy (% Diff from Nominal)	Precision (%CV)	Accuracy (% Diff from Theo)
Estrone	(b) (4)			
17-ß-Estradiol				
	Assay Validation Performance in Nonstripped Human Serum			
	Intra-Assay Quality Control Samples		Inter-Assay Quality Control Samples	
Analyte	Precision (%CV)	Accuracy (% Diff from Nominal)	Precision	Accuracy
Estrone			(%CV) ≤5.79%	(% Diff from Theo)
17-ß-Estradiol	(b) (4)			

Data on long term stability, stock stability, bench top stability, freeze-thaw cycle stability, percentage of recovery were not provided.

3. Labeling Comments

The following changes (strikethrough and double underlined) are recommended comments for the Description, Clinical Pharmacology, and Precaution Sections of the label:

DESCRIPTION

DIVIGEL® (Estradiol Gel) 0.1% is a clear, colorless gel, which is odorless when dry. It is designed to deliver sustained circulating concentrations of estradiol when applied once daily to

5 Page(s) of Draft Labeling have been Withheld in Full following this page as B4 (CCI/TS)

4. APPENDIX
4.1 Individual Study Reports

" Randomized, Open-Label, Multiple-Dose, 3-Way Cross-over Pharmacokinetic Study Evaluating Three Dose Levels of USL-221"

Study no.:	P04-003
Development Phase of Study:	Phase I
Principal investigator:	Soran Hong, MD
Study Dates:	Aug 17th, 2004 to Nov 11th, 2004

Objectives

Primary:

- To evaluate single and multiple dose pharmacokinetic (PK) profiles of 3 dose levels of USL-221 when given for 14 days to postmenopausal women.

Study Population

STUDY DESIGN, TREATMENT AND ADMINISTRATION

This Phase 1, randomized, open-label, multiple-dose study was conducted according to a 3-way crossover design. Twenty-one subjects were randomized to 1 of 3 treatment sequences in which each subject received the following treatments over 3 study periods:

Treatment A: 0.25 g of estradiol gel 0.1% (0.25 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

Treatment B: 0.5 g of estradiol gel 0.1% (0.5 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

Treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

Each study period was 17 days long, with a 14-day washout period between treatments. After completion of 1 treatment period, subjects were crossed over to another study treatment. During each study period, subjects were confined to the study site on Days 1, 2, and 14 (for dosing, safety assessments, and PK sampling). On Day 7 subjects presented to the clinic for dosing and safety assessments. On Days 12 and 13 subjects presented to the clinic for dosing, safety assessments, and trough levels. On Days 15, 16, and 17 subjects presented to the clinic for safety assessments and PK sampling.

At the end of the study, all women with an intact uterus received progestin for 14 days, after which they returned to the study site for safety assessments.

FORMULATION

The following drug product was used in this study:

USL-221	**Dose**	**Lot Number**	**Manufacturing Date**
Estradiol gel,	0.1% 1.0 g (1 mg estradiol)	1053375	03/2004

Study drug was packaged in individual, unit-dose foil sachets.

PHARMACOKINETIC MEASUREMENTS

In each period, 10-mL venous blood was collected in plain red-top (no gel) Vacutainer® tubes at -12 hours, -1 hour, immediately prior to dosing on Day 1 (0), and at the following nominal times after dosing: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, and 24 hours. Prior to dosing on Days 12, 13, and 14, samples were collected for analysis of trough serum concentrations. On Day 14 in each study period, samples were collected at the following times after dosing: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36, 48, and 72 hours.

Analytical Method

(b) (4)

Table 1. Summary of Study Performance for estradiol and estrone,

PPD Method	LCMSC 248.1			
Analytes	Estrone and 17-β-Estradiol			
Matrix	Human Serum			
Sample Volume	500 µL			
Estrone Validated Range	5.00 to 500 pg/mL			
17-β-Estradiol Validated Range	2.50 to 250 pg/mL			
Internal Standard	(b) (4)			
Sample Storage Conditions	-20°C			
Assay Validation Performance in Modified Serum				
	Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
	Precision	**Accuracy**	**Precision**	**Accuracy**
Analyte	(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
Estrone 17-β-Estradiol	(b) (4)			
Assay Validation Performance in Nonstripped Human Serum				
	Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
	Precision	**Accuracy**	**Precision**	**Accuracy**
Analyte	(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
Estrone 17-β-Estradiol	(b) (4)			

Estrone Sulfate

(b) (4)

Table 2. Method Description for Estrone Sulfate

PPD Method	LCMS 27.1 V2		
Analyte	Estrone Sulfate		
Matrix	Human Serum		
Sample Volume	500 µL		
Validated Range	50.0 to 5000 pg/mL		
Internal Standard	(b) (4)		
Sample Storage Conditions	-80°C		
Assay Validation Performance			
Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
Precision	**Accuracy**	**Precision**	**Accuracy**
(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)

(b) (4)

Data on long term stability, stock stability, bench top stability, freeze-thaw cycle stability, percentage of recovery were not provided.

SAFETY MEASUREMENTS

Safety assessments included clinical laboratory evaluations (hematology, serum chemistry, and urinalysis), physical and breast examinations, 12-lead electrocardiogram tracings, vital signs, Draize scale analysis on the test application site, and AE reporting.

DATA ANALYSIS

Pharmacokinetic Data Analysis and Statistical Analysis

For the PK analysis, both uncorrected and baseline-corrected serum concentrations were evaluated for all 3 analytes following the single dose on Day 1. Baseline-corrected values for PK analysis were calculated by subtracting the mean of the 3 predose values (samples taken at -12 hour, -1 hour, and 0 hour) for each subject from all subsequent values. Any postdose-corrected calculation that had a negative value was to be considered as 0.00 pg/mL for the purposes of the PK analysis. Baseline correction was not performed for serum concentrations collected after multiple dosing. The multiple-dose PK population consisted of all subjects in the single-dose PK population who were compliant with the dosing schedule and achieved steady state before dosing on Day 14. Steady state was assessed by fitting the linear regression model, Cmin = intercept + slope * day + error, where Cmin is the 3 trough level concentrations collected on Days 12, 13, and 14. Statistical evidence that steady state was not achieved was assumed if the slope was positive and significantly different from zero at the 5% level.

RESULTS

Pharmacokinetic Results

Twenty-one subjects were enrolled and nineteen completed the study. Two subjects (Subjects 008 and 015) discontinued study participation prematurely. All 21 subjects were included in the safety and single-dose PK analysis populations. All subjects were included in the multiple-dose PK analysis with the exception of Subject 008 who failed to achieve steady state.

Additional statistical analyses were performed on corrected and uncorrected PK parameters on Day 1 and uncorrected PK parameters on Day 14 for estradiol, estrone, and estrone sulfate that excluded 3 subjects (Subjects 003, 014, and 020) who had baseline estradiol concentrations >20 pg/mL.

Tables 3 to 8 summarize the PK parameters for E2, E1 and ES following single and multiple administration of the treatments. Individual E2, and E1, Cmax and AUCt box plots non-baseline corrected values are shown in Figures 1 to 4.

Table 3. Summary of PK Parameters of Estradiol (Arithmetic Mean [%CV]) After a Single Dose of USL-221 on Day 1

Parameter (units)	USL-221 0.25 mg		USL-221 0.5 mg		USL-221 1.0 mg	
	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)
AUC0-24 (pg•h/mL)	293 (159)	45 (126)	288 (106)	111 (98)	488 (80)	354 (76)
Cmax (pg/mL)	15.0 (159)	3.3 (176)	16.7 (98)	9.2 (98)	38.2 (88)	32.7 (90)
tmax* (h)	10 (0, 24)	10 (4, 24)	10 (0, 24)	10 (0, 24)	10 (5, 24)	10 (5, 24)

*Median (Min, Max)

Table 4. Summary of Pharmacokinetic Parameters of Uncorrected Estradiol (Arithmetic Mean [%CV]) After Multiple Doses of USL-221 on Day 14

Parameter (units)	USL-221 0.25 mg Mean (%CV)	USL-221 0.5 mg Mean (%CV)	USL-221 1.0 mg Mean (%CV)
AUC0-24 (pg•h/mL)	236 (94)	504 (149)	732 (81)
AUC0-72 (pg•h/mL)	717 (106)	1262 (144)	1424 (83)
AUC0-t (pg•h/mL)	712 (107)	1260 (145)	1421 (83)
Cmax (pg/mL)	14.7 (84)	28.4 (139)	51.5 (86)
Cmin (pg/mL)	10.6 (103)	21.5 (149)	19.6 (64)
Cflux (%)	79 (216)	47 (116)	166 (124)
tmax* (h)	16 (0, 72)	10 (0, 72)	8 (0, 48)

*Median (Min, Max)

Table 5. Summary of Pharmacokinetic Parameters of Estrone (Arithmetic Mean [%CV]) After a Single Dose of USL-221 on Day 1

Parameter	USL-221 0.25 mg		USL-221 0.5 mg		USL-221 1.0 mg	
	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
(units)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC_{0-24} (pg•h/mL)	501 (53)	22 (67)	469 (52)	51 (84)	534 (37)	128 (84)

Cmax (pg/mL)	27.4 (48)	2.4 (115)	25.7 (50)	4.6 (105)	30.6 (30)	11.1 (73)
tmax* (h)	8 (0, 24)	24 (0, 24)	24 (0, 24)	24 (0, 24)	24 (0, 24)	24 (0, 24)

Table 6. Summary of Pharmacokinetic Parameters of Uncorrected Estrone (Arithmetic Mean [%CV]) After Multiple Doses of USL-221 on Day 14

Parameter (units)	**USL-221 0.25 mg** Mean (%CV)	**USL-221 0.5 mg** Mean (%CV)	**USL-221 1.0 mg** Mean (%CV)
AUC0-24 (pg•h/mL)	555 (36)	771 (38)	1122 (46)
AUC0-72 (pg•h/mL)	1759 (33)	2273 (37)	3045 (41)
AUC0-t (pg•h/mL)	1759 (33)	2273 (37)	3045 (41)
Cmax (pg/mL)	30.2 (27)	39.7 (38)	58.9 (45)
Cmin (pg/mL)	26.8 (35)	36.8 (37)	48.7 (46)
Cflux (%)	18 (122)	8 (121)	24 (130)
tmax* (h)	24 (0, 48)	4 (0, 48)	8 (0, 72)

*Median (Min, Max)

Table 7. Summary of Pharmacokinetic Parameters of Estrone Sulfate (Arithmetic Mean [%CV]) After a Single Dose of USL-221 on Day 1

Parameter	**USL-221 0.25 mg**		**USL-221 0.5 mg**		**USL-221 1.0 mg**	
	Uncorrected	**Corrected**	**Uncorrected**	**Corrected**	**Uncorrected**	**Corrected**
(units)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-24 (pg•h/mL)	6552 (79)	335 (94)	7451 (78)	1684 (90)	9022 (56)	4239 (60)
Cmax (pg/mL)	392.9 (94)	45.5 (109)	422.6 (76)	129.7 (100)	540.3 (52)	292.2 (70)
tmax* (h)	5 (0, 24)	7 (1, 24)	11 (0, 24)	14 (2, 24)	14 (0, 24)	14 (1, 24)

Table 8. Summary of Pharmacokinetic Parameters of Uncorrected Estrone Sulfate (Arithmetic Mean [%CV]) After Multiple Doses of USL-221 on Day 14

Parameter	**USL-221 0.25 mg**	**USL-221 0.5 mg**	**USL-221 1.0 mg**
(units)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-24 (pg•h/mL)	9220 (62)	13586 (47)	24089 (67)
AUC0-72 (pg•h/mL)	27688 (57)	40382 (49)	61029 (64)
AUC0-t (pg•h/mL)	27688 (57)	40382 (49)	61029 (64)
Cmax (pg/mL)	616.9 (60)	861.0 (47)	1465.6 (70)
Cmin (pg/mL)	398.1 (59)	621.5 (48)	980.4 (75)
Cflux (%)	61 (83)	46 (104)	59 (61)
tmax* (h)	8 (0, 72)	8 (0, 48)	5 (0, 72)

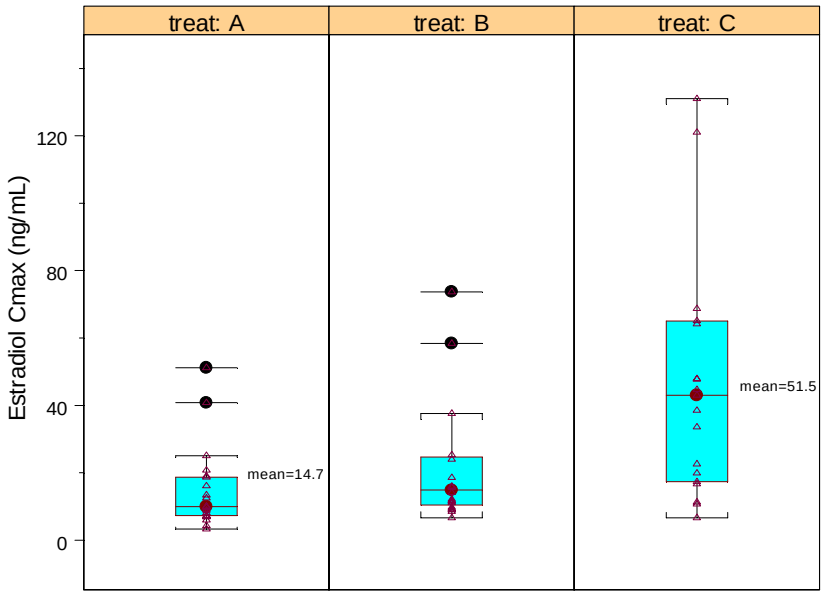


Figure 1. Individual E2 Cmax non-baseline corrected values following single administration of the treatments: Treatment A: 0.25 g of estradiol gel 0.1% (0.25 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.; treatment B: 0.5 g of estradiol gel 0.1% (0.5 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days; treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

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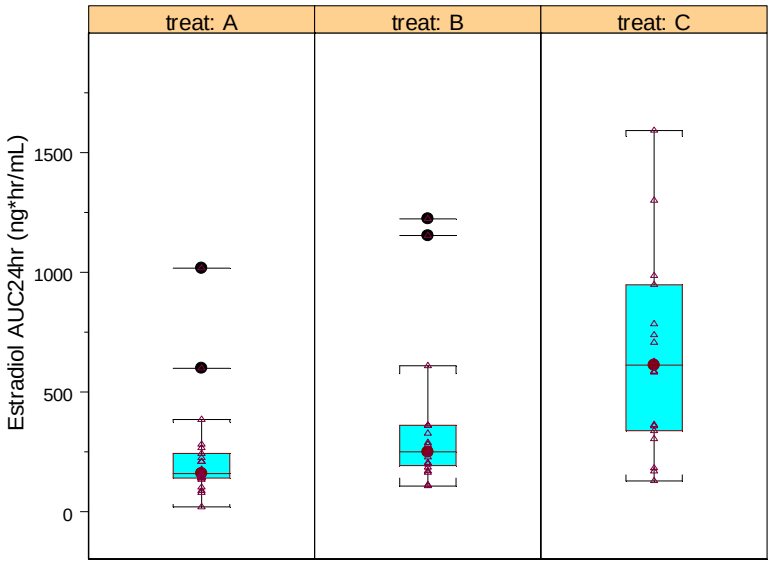

Figure 2. Individual E2 AUC24hr non-baseline corrected values following single administration of the treatments: Treatment A: 0.25 g of estradiol gel 0.1% (0.25 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.; treatment B: 0.5 g of estradiol gel 0.1% (0.5 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days; treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

Figure 3. Individual E1 Cmax non-baseline corrected values following single administration of the treatments: Treatment A: 0.25 g of estradiol gel 0.1% (0.25 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.; treatment B: 0.5 g of estradiol gel 0.1% (0.5 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days; treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

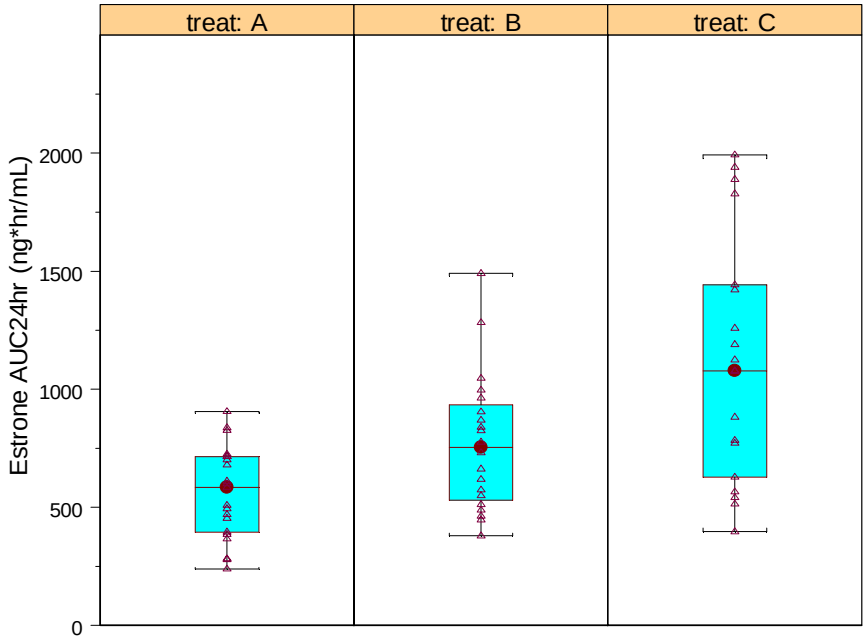

Figure 4. Individual E1 AUC24hr non-baseline corrected values following single administration of the treatments: Treatment A: 0.25 g of estradiol gel 0.1% (0.25 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.; treatment B: 0.5 g of estradiol gel 0.1% (0.5 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days; treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area of the thigh once daily for 14 days.

Dose proportionality

Dose-proportionality following single and multiple skin administration of Divigel, 0.25 mg, 0.5 mg and 1.0 mg was evaluated in as part of this study.

Following single dose administration, E2 peak serum concentrations and AUC (uncorrected for baseline) increased more than proportionally to the dose. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 147% in mean corrected AUC0-24 was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 219% was observed.

Following multiple dose administration, E2 peak serum concentrations and AUC (uncorrected) increased roughly less than proportionally to the dose. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 114% in mean uncorrected AUC0-24 was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 45% was observed. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 93% in mean uncorrected Cmax was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 81% was observed. Based on the power model, E2 increase roughly proportional to the dose with a slope of 0.8 (Figure 5).

Steady state, tested by regressing trough level concentrations collected on Days 12, 13, and 14 onto day, resulted in slope values that were not significantly different from 0 indicating that multiple doses of USL-221 resulted in the achievement of steady state for each of the 3 doses administered in this study (data not shown).

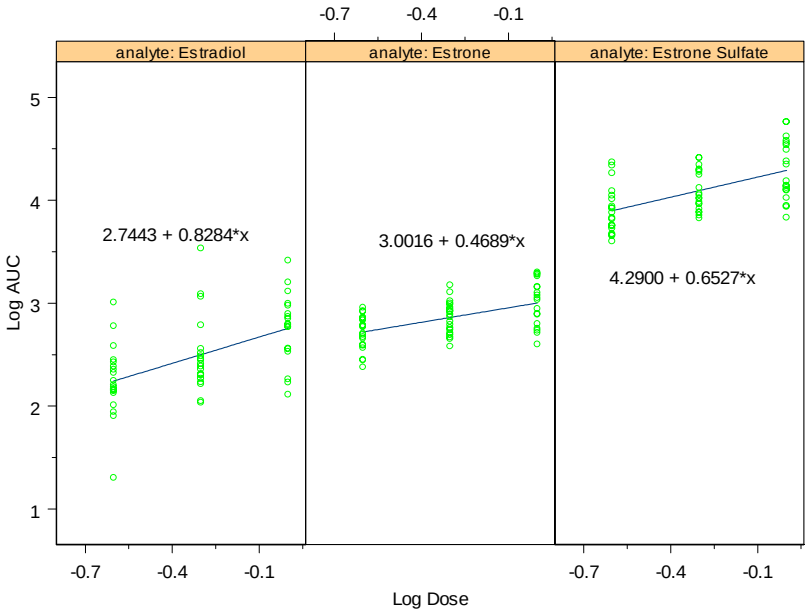

Figure 5. Individual E2, E1 and ES AUC (uncorrected from baseline; log values) as a function of log-Dose (fitted line from power model: $AUCE2 = e^{-2.7} * (dose)^{0.8}$; $AUCE2 = e^{-3} * (dose)^{0.5;}$ $AUCE2 = e^{-4.2} * (dose)^{0.7}$) following multiple administration of the treatments.

CONCLUSION

Pharmacokinetic Conclusions

- Following single dose administration, E2 peak serum concentrations and AUC (uncorrected for baseline) increased more than proportionally to the dose. After increases

in dose from 0.25 mg to 0.5 mg, an increase of approximately 147% in mean corrected AUC0-24 was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 219% was observed.

- Following multiple dose administration, E2 peak serum concentrations and AUC (uncorrected) increased roughly less than proportionally to the dose. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 114% in mean uncorrected AUC0-24 was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 45% was observed. After increases in dose from 0.25 mg to 0.5 mg, an increase of approximately 93% in mean uncorrected Cmax was observed, and from 0.5 mg to 1.0 mg, an increase of approximately 81% was observed.
- Steady state, tested by regressing trough level concentrations collected on Days 12, 13, and 14 onto day, resulted in slope values that were not significantly different from 0 indicating that multiple doses of USL-221 resulted in the achievement of steady state for each of the 3 doses administered in this study.

" Randomized, Open-Label, Single-Dose, 3-Way Cross-over Study of the Transferability of USL-221 During Skin-to-Skin Contact With and Without Clothing"

Study no.:	P04-002
Development Phase of Study:	Phase I
Principal investigator:	Lawrence Galitz, MD
Study Dates:	April 15th, 2005 to July 11th, 2005

Objectives

Primary:

- To determine the extent of skin-to-skin estradiol transfer from postmenopausal female subjects dosed with USL-221 to nondosed male or postmenopausal female subjects, both in the presence and absence of clothing.

Study Population

Forty-two (42) subjects were planned, and 54 subjects were enrolled for this study to account for dropouts. Twenty-seven (27) nondosed subjects, who completed a minimum of 1 treatment were included in the pharmacokinetic (PK) analysis, 54 subjects were included in the safety analysis, and 40 subjects completed the study. Overall, demographic data were similar for dosed and nondosed subjects. The majority of the subjects enrolled in the study were Hispanic or Latino (48/54; 88.9%), and there was a larger percentage of black subjects in the nondosed group compared with the dosed group (29.6% vs 3.7%).

STUDY DESIGN, TREATMENT AND ADMINISTRATION

This was a randomized, open-label, single-dose study conducted according to a 3-way crossover design. Subjects were assigned to pairs in which 1 subject was dosed and one was not. Each pair of subjects was randomized to 1 of 3 treatment sequences in which the following treatments were received over 3 study periods:

Treatment A: A postmenopausal female subject was dosed with 1.0 g of estradiol gel 0.1% (1.0 mg) to a 200-cm^2 area of the thigh. After 60 minutes, the nondosed subject rubbed the anterior portion of his/her forearm over the dosed subject's clothed application site for 5 minutes (10-15 rubs per minute) and then maintained contact with the same forearm at the application site for another 10 minutes without the rubbing motion.

Treatment B: A postmenopausal female subject was dosed with 1.0 g of estradiol gel 0.1% (1.0 mg) to a 200-cm^2 area of the thigh. After 60 minutes, the nondosed subject rubbed the anterior portion of his/her forearm over the dosed subject's unclothed application site for 5 minutes (10-15 rubs per minute) and then maintained contact with the same forearm at the application site for another 10 minutes without the rubbing motion.

Treatment C: A postmenopausal female subject was dosed with 1.0 g of estradiol gel 0.1% (1.0 mg) to a 200-cm^2 area of the thigh. After 8 hours, the nondosed subject rubbed the anterior portion of his/her forearm over the dosed subject's unclothed application site for 5 minutes (10-

15 rubs per minute) and then maintained contact with same forearm at the application site for another 10 minutes without the rubbing motion.
Each study period was 4 days long, with at least a 14-day washout period between treatments. After completion of 1 treatment period, subjects were crossed over to another study treatment.

FORMULATION

The following drug product was used in this study:

USL-221	**Dose**	**Lot Number**	**Manufacturing Date**
Estradiol gel,	0.1% 1.0 g (1 mg estradiol)	1053363	03/2004

Study drug was packaged in individual, unit-dose foil sachets.

PHARMACOKINETIC MEASUREMENTS

Eighteen blood samples were obtained from only the nondosed subjects at -12, -1, and 0 hours prior to initiation of contact and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36, 48, and 72 hours after subject-to-subject contact and analyzed for estradiol, estrone, and estrone sulfate during each study period.

Analytical Method

(b) (4)

Table 1. Summary of Study Performance (In Study Validation) for estradiol and estrone,

PPD Method	LCMSC 248.1
Analytes	Estrone and 17-β-Estradiol
Matrix	Human Serum
Sample Volume	500 µL
Estrone Validated Range	5.00 to 500 pg/mL
17-β-Estradiol Validated Range	2.50 to 250 pg/mL
Internal Standard	(b) (4)
Sample Storage Conditions	-20°C

Assay Validation Performance in Modified Serum				
	Intra-Assay Quality Control Samples		Inter-Assay Quality Control Samples	
	Precision	Accuracy	Precision	Accuracy
Analyte	(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
Estrone 17-β-Estradiol	(b) (4)			

Assay Validation Performance in Nonstripped Human Serum				
	Intra-Assay Quality Control Samples		Inter-Assay Quality Control Samples	
	Precision	Accuracy	Precision	Accuracy
Analyte	(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
Estrone 17-β-Estradiol	(b) (4)			

Estrone Sulfate

(b) (4)

Table 2. Method Description for Estrone Sulfate

PPD Method	LCMS 27.1 V2		
Analyte	Estrone Sulfate		
Matrix	Human Serum		
Sample Volume	500 µL		
Validated Range	50.0 to 5000 pg/mL		
Internal Standard	(b) (4)		
Sample Storage Conditions	-80°C		
Assay Validation Performance			
Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
Precision	**Accuracy**	**Precision**	**Accuracy**
(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
(b) (4)			

Data on long term stability, stock stability, bench top stability, freeze-thaw cycle stability, percentage of recovery was not provided.

SAFETY MEASUREMENTS

Safety was assessed based on AE monitoring, electrocardiogram (ECG) tracings, clinical laboratory results, physical examination findings, evaluation of skin irritation potential (Draize scale), and vital sign monitoring

DATA ANALYSIS

Pharmacokinetic Data Analysis and Statistical Analysis

Continuous data were summarized using descriptive statistics (n, mean, standard deviation [SD], standard error of the mean [SEM], median, minimum value, and maximum value, unless otherwise specified). Categorical data were summarized by presenting the number (frequency) and percentage of subjects at each level of response. Baseline was defined as the

latest nonmissing result prior to dosing on Study Day 1. Therefore, Baseline could have been defined as Screening or Study Day -1 for each period, depending on scheduled procedures. For the PK analysis, both uncorrected and baseline-corrected serum concentrations were evaluated for all 3 analytes. Baseline-corrected values for PK analysis were calculated by subtracting the mean of the 3 predose values for each subject (samples taken at -12, -1, and 0 hours) from all subsequent values. Any postdose baseline-corrected calculations that had a negative value were considered to be 0.00 pg/mL for the purposes of the PK analysis.

RESULTS

Pharmacokinetic Results

Forty-two subjects were initially randomized, and 14 of these subjects (7 of whom were dosed subjects and 7 of whom were nondosed subjects) prematurely discontinued study participation. Twelve additional subjects were enrolled as replacements, and a total of 40 subjects completed all 3 periods of the study. The summary of PK parameters of E2, E1 and Es corrected and uncorrected for baseline for nondosed subjects is presented in Tables 3 to 5. Individual E2, E1, E1S Cmax and AUCt non-baseline corrected values following the administration of the treatments are shown in Figures 1 to 4, respectively.

Table 3. Summary of Pharmacokinetic Parameters of Estradiol (Arithmetic Mean [%CV]) in Nondosed Subjects Who Had Skin Contact With Dosed Subjects

Parameter (units)	**Contact with Clothed Application Site 60 Minutes After Dosing (Treatment A) N=23**		**Contact with unclothed Application Site 60 Minutes After Dosing (Treatment B) N=22**		**Contact with unclothed Application Site 8 Hours After Dosing (Treatment C) N=24**	
	Uncorrected	**Corrected**	**Uncorrected**	**Corrected**	**Uncorrected**	**Corrected**
	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-t (pg•hr/mL)	1751.82 (31)	203.83 (83)	1803.21 (31)	139.26 (69)	1793.76 (33)	165.72 (99)
Cmax (pg/mL)	31.84 (34)	8.24 (63)	33.95 (37)	8.89 (57)	31.49 (34)	6.89 (62)
tmax* (h)	10.0 (0, 72)	10.0 (0, 72)	4.0 (0, 48)	4.0 (0, 48)	9.0 (1, 24)	7.0 (1, 24)
AUC0-24 (pg•hr/mL)	573.91 (31)	-	620.22 (35)	-	587.48 (33)	-
Cavg0-24 (pg/mL)	23.91 (31)	-	25.84 (35)	-	24.48 (33)	-
Cavg0-t (pg/mL)	24.33 (31)	-	25.05 (31)	-	24.92 (33)	-

*Median (Min, Max).

$Cavg_{0\text{-}24} = AUC_{0\text{-}24}/24$ and $Cavg_{0\text{-}t} = AUC_{0\text{-}t}/t$.

Treatment A = Skin contact with clothed application site 60 minutes after dose.

B = Skin contact with application site 60 minutes after dose.

C = Skin contact with application site 8 hours after dose.

Table 4. Summary of Pharmacokinetic Parameters of Estrone (Arithmetic Mean [%CV]) in Nondosed Subjects Who Had Skin Contact With Dosed Subjects

Parameter (units)	Contact with Clothed Application Site 60 Minutes After Dosing (Treatment A) N=23		Contact with Application Site 60 Minutes After Dosing (Treatment B) N=22		Contact with Application Site 8 Hours After Dosing (Treatment C) N=24	
	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-t (pg•hr/mL)	2683.09 (28)	305.58 (99)	2657.25 (26)	338.71(78)	2783.48 (27)	166.48 (88)
Cmax (pg/mL)	48.37 (38)	11.98 (111)	47.28 (24)	10.37 (56)	49.02 (29)	10.37 (45)
tmax* (h)	6.0 (0, 72)	6.0 (0, 72)	14.0 (0, 72)	24.0 (0, 72)	11.0 (0, 48)	12.0 (0, 48)
AUC0-24 (pg•hr/mL)	856.79 (26)	-	873.56 (27)	-	889.73 (29)	-
Cavg0-24 (pg/mL)	35.70 (26)	-	36.40 (27)	-	37.07 (29)	-
Cavg0--t (pg/mL)	37.27 (28)	-	37.23 (24)	-	38.67 (27)	-

*Median (Min, Max).

$Cavg_{0\text{-}24} = AUC_{0\text{-}24}/24$ and $Cavg_{0\text{-}t} = AUC_{0\text{-}t}/t$.

Treatment: A = Skin contact with clothed application site 60 minutes after dose.

B = Skin contact with application site 60 minutes after dose.

C = Skin contact with application site 8 hours after dose.

Table 5. Summary of Pharmacokinetic Parameters of Estrone Sulfate (Arithmetic Mean [%CV]) in Nondosed Subjects Who Had Skin Contact With Dosed Subjects

Parameter (units)	Contact with Clothed Application Site 60 Minutes After Dosing (Treatment A) N=23		Contact with Application Site 60 Minutes After Dosing (Treatment B) N=22		Contact with Application Site 8 Hours After Dosing (Treatment C) N=24	
	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)	Mean (%CV)
AUC0-t (pg•hr/mL)	46753.24 (65)	3637.41 (144)	43011.60 (59)	3457.93 (106)	44440.26 (61)	4739.26 (97)
Cmax (pg/mL)	928.74 (60)	261.58 (64)	852.41 (55)	210.75 (69)	878.17 (60)	262.84 (67)
tmax* (h)	8.0 (1, 36)	8.0 (1, 36)	8.0 (3, 72)	9.0 (3, 72)	10.0 (0, 48)	12.0 (2, 48)
AUC0-24 (pg•hr/mL)	16501.05 (64)	-	15492.60 (57)	-	15846.75 (60)	-
Cavg0-24 (pg/mL)	687.54 (64)	-	645.53 (57)	-	660.28 (60)	-
Cavg0--t (pg/mL)	649.35 (65)	-	597.38 (59)	-	617.28 (61)	-

*Median (Min, Max).

Note: BLQ values were set to zero for summary statistics.

$Cavg_{0\text{-}24} = AUC_{0\text{-}24}/24$ and $Cavg_{0\text{-}t} = AUC_{0\text{-}t}/t$.

Treatment: A = Skin contact with clothed application site 60 minutes after dose.

B = Skin contact with application site 60 minutes after dose.

C = Skin contact with application site 8 hours after dose.

Figure 1. Individual E2 Cmax corrected and non-baseline corrected values following single administration of the treatments: Treatment: A = Skin contact with clothed application site 60 minutes after dose; B = Skin contact with application site 60 minutes after dose; C = Skin contact with application site 8 hours after dose to 24 healthy postmenopausal women.

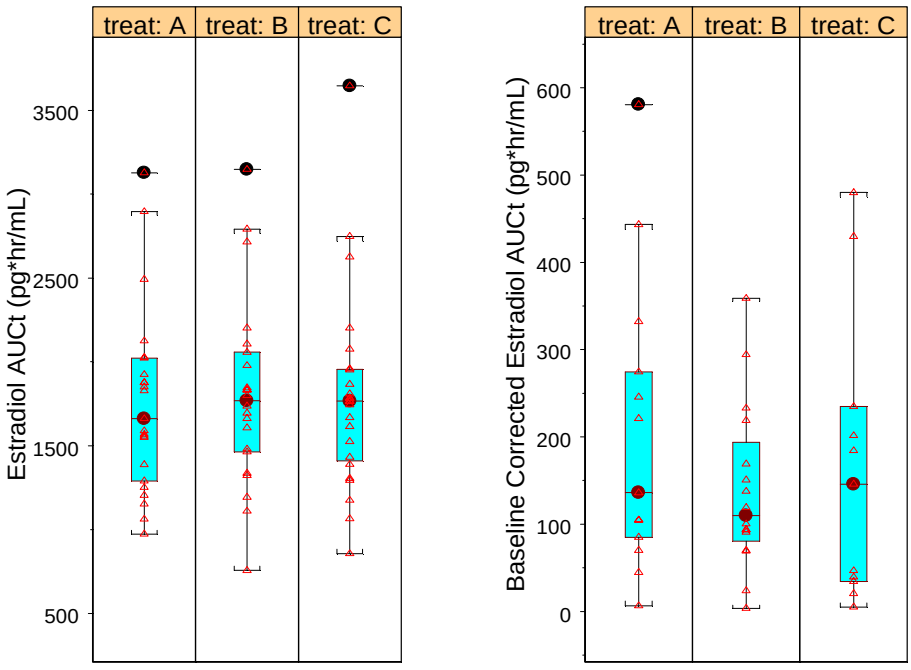

Figure 2. Individual E2 AUCt corrected and non-baseline corrected values following single administration of the treatments: Treatment: A = Skin contact with clothed application site 60 minutes after dose; B = Skin contact with application site 60 minutes after dose; C = Skin contact with application site 8 hours after dose to 24 healthy postmenopausal women.

(b) (4)

Figure 3. Individual E1 Cmax corrected and non-baseline corrected values following single administration of the treatments: Treatment: A = Skin contact with clothed application site 60 minutes after dose; B = Skin contact with application site 60 minutes after dose; C = Skin contact with application site 8 hours after dose to 24 healthy postmenopausal women.

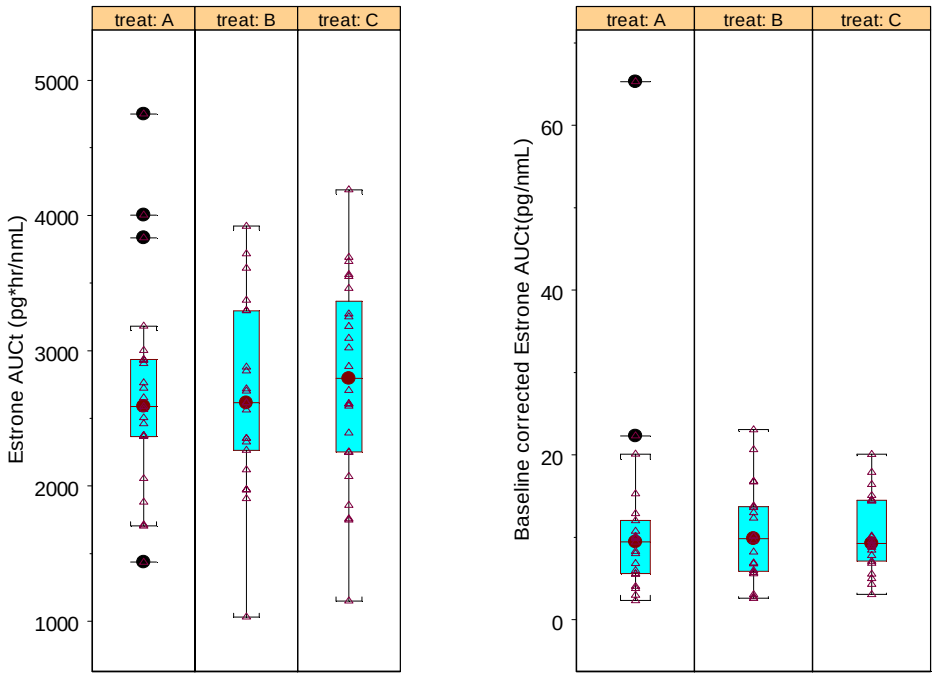

Figure 4. Individual E1 AUCt corrected and non-baseline corrected values following single administration of the treatments: Treatment: A = Skin contact with clothed application site 60 minutes after dose; B = Skin contact with unclothed application site 60 minutes after dose; C = Skin contact with unclothed application site 8 hours after dose to 24 healthy postmenopausal women.

CONCLUSION

- The percentage mean increase in E2 Cmax (33.95 pg/mL) compared to mean baseline (25.01 pg/mL) in non-dosed patients who had skin contact with **unclothed** application site 60 minutes after dose was about 25%.
- The percentage mean increase in E2 AUCt (1803.21 pg*hr/mL) compared to mean baseline (1663.9 pg*hr/mL) in non-dosed patients who had skin contact with **unclothed** application site 60 minutes after dose was about 10%.
- The percentage mean increase in E1 Cmax and AUCt compared to mean baseline in non-dosed patients who had skin contact with **unclothed** application site 60 minutes after dose was about 21% and 13%, respectively.
- The clinical relevance of about 10 to 25% increase in systemic exposure of E2 and E1 in non-dosed subjects (i.e. male volunteers) in unknown.
- The percentage mean increase in E2 Cmax (31.49 pg/mL) and E2 AUCt (1793.8 pg*hr/mL) compared to mean baseline (25.5 pg/mL) and 1682.1 pg*hr/mL in non-dosed patients who had skin contact with **unclothed** application site 8 hrs after dose was about 23% and 7%, respectively.
- The percentage mean increase in E2 Cmax (31.84 pg/mL) and E2 AUCt (1751.08 pg*hr/mL) compared to mean baseline (23.6 pg/mL) and 1633.1 pg*hr/mL in non-dosed patients who had skin contact with **clothed** application site 60 minutes after dose was about 34% and 7%, respectively.
- These data should be interpreted with caution due to uncertainty in the procedure used to calculate E2 change from baseline values across the treatments. Baseline was calculated as the average of 3 endogenous compound values determined at -12 hr, -1hr and prior drug administration. Change from baseline was then calculated as the AUC of individual values minus the mean of baseline.
- The degree of transferability across treatments was similar. Therefore, the results from this study are inconclusive.

" Randomized, Open-Label, Single-Dose, 3-Way Crossover Study of the Washability of USL-221"

Study no.:	P04-005
Development Phase of Study:	Phase I
Principal investigator:	Soran Hong, MD
Study Dates:	March 19th, 2005 to Apr 16th, 2005

Objectives

Primary:

- to determine the effect that washing had on the absorption of USL-221 (estradiol gel 0.1%) in postmenopausal women.

Secondary:

- to determine if measurable concentrations of USL-221 were detectable on the skin before and after washing the application site 1 and 8 hours after dosing.

STUDY DESIGN, TREATMENT AND ADMINISTRATION

This Phase 1, randomized, open-label, single-dose study was conducted according to a 3-way crossover design. The study consisted of 3 periods. Sixteen subjects were randomized to 1 of 2 treatment sequences in which each subject received the following treatments over the first 2 study periods:

Treatment A: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area on the thigh. The application site was washed with soap and water 60 minutes after study drug was applied.

Treatment B: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area on the thigh.

Each of the first 2 study periods was 4 days long with a 14-day washout period between treatments. After completion of 1 treatment period, subjects were crossed over to the other study treatment. Blood samples were obtained before and up to 72 hours after dosing and analyzed for estradiol, estrone, and estrone sulfate during both of these periods.

Treatment Periods 2 and 3 were separated by another 14-day washout period. During Period 3, half of the subjects were randomized to receive Treatment C and the other half were randomized to receive Treatment D as follows:

Treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area on the thigh. After 60 minutes, a 10-cm^2 area was swabbed for analysis of residual levels of estradiol at the application site. The area was then washed, and a second swab collection was taken 15 minutes after the start of washing.

Treatment D: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area on the thigh. After 8 hours, a 10-cm^2 area was swabbed for analysis of residual levels of estradiol at the application site. The area was then washed, and a second swab collection was taken 15 minutes after the start of washing.

During Period 3, only swab samples were collected to determine residual levels of estradiol at the application site (no blood samples were collected). In addition to the swab samples collected

after dosing, baseline swab samples (prior to dose application) were collected for both Treatments C and D.

FORMULATION

The following drug product was used in this study:

USL-221	**Dose**	**Lot Number**	**Manufacturing Date**
Estradiol gel,	0.1% 1.0 g (1 mg estradiol)	1053363	03/2004

Study drug was packaged in individual, unit-dose foil sachets.

PHARMACOKINETIC MEASUREMENTS

In Periods 1 and 2, 10-mL venous blood was collected at -12 hours, -1 hour, immediately prior to dosing on Day 1 (0), and at the following nominal times after dosing: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36, 48 and 72 hours. At each time point, serum concentrations of estradiol, estrone and estrone sulfate were measured.

In Period 3, swab samples were collected to determine residual levels of estradiol at the application site (no blood samples were collected). Baseline swab samples (prior to dose application) were collected for all subjects. Thereafter, swab samples were taken 1 and 8 hours after application and then again 15 minutes after the start of washing.

Analytical Method

(b) (4)

Table 1. Summary of Study Performance for estradiol and estrone,

PPD Method	LCMSC 248.1			
Analytes	Estrone and 17-β-Estradiol			
Matrix	Human Serum			
Sample Volume	500 µL			
Estrone Validated Range	5.00 to 500 pg/mL			
17-β-Estradiol Validated Range	2.50 to 250 pg/mL			
Internal Standard	(b) (4)			
Sample Storage Conditions	-20°C			
Assay Validation Performance in Modified Serum				
	Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
	Precision	**Accuracy**	**Precision**	**Accuracy**
Analyte	(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
Estrone 17-β-Estradiol	(b) (4)			
Assay Validation Performance in Nonstripped Human Serum				
	Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
	Precision	**Accuracy**	**Precision**	**Accuracy**
Analyte	(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
Estrone 17-β-Estradiol	(b) (4)			

Estrone Sulfate

(b) (4)

Table 2. Method Description for Estrone Sulfate

PPD Method	LCMS 27.1 V2		
Analyte	Estrone Sulfate		
Matrix	Human Serum		
Sample Volume	500 µL		
Validated Range	50.0 to 5000 pg/mL		
Internal Standard	(b) (4)		
Sample Storage Conditions	-80°C		
Assay Validation Performance			
Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
Precision	**Accuracy**	**Precision**	**Accuracy**
(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
(b) (4)			

Data on long term stability, stock stability, bench top stability, freeze-thaw cycle stability, percentage of recovery were not provided.

Estradiol Swab Samples

(b) (4)

Table 3. Method Description for Estradiol Swab Samples

PPD Method	LCMSC 353			
Analytes	Estradiol			
Matrix	Swab sample			
Sample Volume	One swab sample			
Estradiol Validated Range	50.0 to 1000 ng/swab			
Internal Standard	(b) (4)			
Sample Storage Conditions	-20°C			
Assay Validation Performance				
	Intra-Assay Quality Control Samples		**Inter-Assay Quality Control Samples**	
Analyte	**Precision**	**Accuracy**	**Precision**	**Accuracy**
	(%CV)	(% Diff from Theo)	(%CV)	(% Diff from Theo)
Estradiol	(b) (4)			

SAFETY MEASUREMENTS

Safety assessments included clinical laboratory evaluations (hematology, serum chemistry, and urinalysis), physical and breast examinations, 12-lead electrocardiogram tracings, vital signs, Draize scale analysis on the test application site, and AE reporting.

DATA ANALYSIS

Pharmacokinetic Data Analysis and Statistical Analysis

Both uncorrected and baseline-corrected serum concentrations were evaluated for all 3 analytes following dosing on Day 1 in Periods 1 and 2. Baseline-corrected values for PK analysis were calculated by subtracting the mean of the 3 predose values (samples taken at -12 hour, -1 hour, and 0 hour) for each subject from all subsequent values. Any postdose baseline-corrected calculation that had a negative value was considered as 0.00 pg/mL for the purposes of the PK analysis. If any subject had fewer than 4 continuous measurable serum concentrations for any analyte in any one period, their data set for analysis of AUC0-t and AUC0-inf were considered incomplete for that analyte.

An analysis of variance (ANOVA) with fixed effects for sequence, period, treatment, and random effect for subject nested within sequence, was performed on the natural logarithms of

AUC0-t, AUC0-inf, and Cmax for both uncorrected and baseline-corrected estradiol, estrone, and estrone sulfate. Point estimates and 90% confidence intervals (CIs) for differences between the least squares means on the log scale were exponentiated to obtain estimates for ratios of geometric means on the original scale. The percent change in washed versus not-washed treatment was calculated by the equation (1 - geometric mean ratio) x 100%. Formal equivalence comparisons were done using 90% CIs for the ratios of the averages (population geometric means) for the washed and not-washed treatments. Washing was considered to have no effect on the systemic absorption of USL-221 if the 90% CIs for the baseline-corrected ratios were completely contained within the limits of 80% to 125%.

Analysis of Swab Samples

Subjects were randomized to treatment in Period 3 in such a way that one half of the subjects had swab samples collected 1 hour after application and the other half had swab samples collected 8 hours after application. This population consisted of all randomized subjects who received study drug in Period 3 and had concentration data, either measurable or BLQ, for all 3 sampling time points. This population was used for the swab sample concentration analysis table.

Uncorrected and baseline-corrected estradiol concentrations obtained from skin swab samples were presented at each scheduled time point. Baseline-corrected residual levels of estradiol concentrations obtained from skin swab samples were summarized before washing and at 1 and 8 hours after dosing using the following descriptive statistics: n, mean, SD, coefficient of variation (%CV), median, minimum, and maximum. The percentage of estradiol removed from the skin surface after washing was calculated by the equation (1-[after]/[before]) x 100. The null hypothesis that the mean percent change is equal to zero was tested using a one-sample *t* test. The sign-test was used to test the null hypothesis that the median percent change is equal to zero.

RESULTS

Pharmacokinetic Results

A total of 16 subjects were enrolled and all 16 completed the first two periods of the study. Three subjects (106, 109, and 110) prematurely discontinued study participation before Period 3. Two subjects had baseline estradiol concentrations >20 pg/mL (Subjects 106 and 115). Additional statistical analyses were performed on baseline-corrected and uncorrected serum PK parameters for estradiol, estrone, and estrone sulfate with data from these 2 subjects excluded.

Tables 4 to 6 summarized the PK parameters for E2, E1 and ES following administration of the treatments. Tables 7 to 9 show a statistical analysis of E2, E1 and ES after a single dose of USL-221 With and Without Washing 1 hr after application. Individual E2, E1, and ES Cmax and AUCt box plots non-baseline corrected values following the administration of the treatments are shown in Figures 1 to 4, respectively.

Table 4. Summary of Pharmacokinetic Parameters of Estradiol (Arithmetic Mean [%CV]) After a Single Dose of USL-221 With and Without Washing 1 Hour After Application

Parameter (units)	Washed 1 Hour After Application (Treatment A) N=16		Not Washed (Treatment B) N=16	
	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)
AUC0-t (pg•h/mL)	1422 (133)	568 (122)	2304 (182)	773 (87)
AUC0-24 (pg•h/mL)	547 (95)	233 (74)	1059 (152)	477 (81)
Cmax (pg/mL)	52 (64)	41 (70)	98 (110)	66 (84)
tmax* (h)	5.5 (0.5, 36)	5.5 (0.5, 36)	8.0 (0.0, 48)	8.0 (0.0, 48)

*Median (Min, Max).
Note: Treatment A = USL-221 1.0 mg, after 60 minutes wash with mild hypoallergenic soap and washcloth for 30 seconds and rinse with warm water for 2.5 minutes; Treatment B = USL-221 1.0 mg.

Table 5. Summary of Pharmacokinetic Parameters of Estrone (Arithmetic Mean [%CV]) After a Single Dose of USL-221 With and Without Washing 1 Hour After Application

Parameter (units)	Washed 1 Hour After Application (Treatment A) N=16		Not Washed (Treatment B) N=16	
	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)
AUC0-t (pg•h/mL)	2042 (57)	353 (78)	2568 (91)	501 (55)
AUC0-24 (pg•h/mL)	654 (52)	87 (91)	875 (103)	155 (81)
Cmax (pg/mL)	34 (53)	9 (91)	45 (88)	14 (56)
tmax* (h)	24 (0, 72)	24 (0, 72)	24 (7, 72)	24 (16, 72)

Table 6. Summary of Pharmacokinetic Parameters of Estrone Sulfate (Arithmetic Mean [%CV]) After a Single Dose of USL-221 With and Without Washing 1 Hour After Application

Parameter (units)	Washed 1 Hour After Application (Treatment A) N=16		Not Washed (Treatment B) N=16	
	Uncorrected Mean (%CV)	Corrected Mean (%CV)	Uncorrected Mean (%CV)	Corrected Mean (%CV)
AUC0-t (pg•h/mL)	28381 (101)	8635 (113)	46139 (121)	14563 (69)
AUC0-24 (pg•h/mL)	8637 (98)	1953 (102)	14882 (144)	3655 (73)
Cmax (pg/mL)	526 (91)	245 (89)	933 (118)	472 (83)
tmax* (h)	24 (3, 72)	24 (7, 72)	36 (6, 48)	36 (6, 48)

Table 5. Statistical Analysis of Estradiol After a Single Dose of USL-221 With and Without Washing 1 Hour After Application

Parameter (Unit)	Baseline Correction	[1] Treatment	N	Geometric LS Means	Ratio of Geometric Means [A/B] [%]	90% Confidence Interval	[2] Percent Change A vs B
AUC0-t (pg•hr/mL)	Uncorrected	A	14	903.34	69.69	(52.78, 92.03)	30.31
		B	16	1296.18			
	Corrected	A	12	381.68	63.50	(45.50, 88.63)	36.50
		B	14	601.07			
Cmax (pg/mL)	Uncorrected	A	16	39.17	61.60	(38.46, 98.65)	38.40
		B	16	63.60			
	Corrected	A	16	29.05	62.30	(35.40, 109.63)	37.70
		B	16	46.63			

[1] Treatment: A=USL-221 1.0 mg, after 60 minutes wash with mild hypoallergenic soap and washcloth for 30 seconds and rinse with warm water for 2.5 minutes; B=USL-221 1.0 mg.
[2] Percent change = (1-Ratio of Geometric Means)*100%.

Table 7. Statistical analysis of Estrone After a Single Dose of USL-221 With and Without Washing 1 Hour After Application

Parameter (Unit)	Baseline Correction	[1] Treatment	N	Geometric LS Means	Ratio of Geometric Means [A/B] [%]	90% Confidence Interval	[2] Percent Change A vs B
AUC0-t (pg•hr/mL)	Uncorrected	A	16	1770.05	85.41	(74.14, 98.40)	14.59
		B	16	2072.29			
	Corrected	A	10	226.76	51.77	(27.44, 97.67)	48.23
		B	14	438.02			
Cmax (pg/mL)	Uncorrected	A	16	30.33	82.07	(70.62, 95.38)	17.93
		B	16	36.96			
	Corrected	A	16	5.81	46.95	(31.10, 70.87)	53.05
		B	15	12.38			

Table 8. Statistical Analysis of Estrone Sulfate After a Single Dose of USL-221 With and Without Washing 1 Hour After Application

Parameter (Unit)	Baseline Correction	[1] Treatment	N	Geometric LS Means	Ratio of Geometric Means [A/B] [%]	90% Confidence Interval	[2] Percent Change A vs B
AUC0-t (pg•hr/mL)	Uncorrected	A	16	19653.13	67.56	(53.91, 84.66)	32.44
		B	15	29091.32			
	Corrected	A	15	5613.14	49.16	(27.51, 87.87)	50.84
		B	15	11417.49			
Cmax (pg/mL)	Uncorrected	A	16	380.29	63.44	(50.27, 80.05)	36.56
		B	16	599.48			
	Corrected	A	15	178.78	50.86	(34.37, 75.28)	49.14
		B	16	351.50			

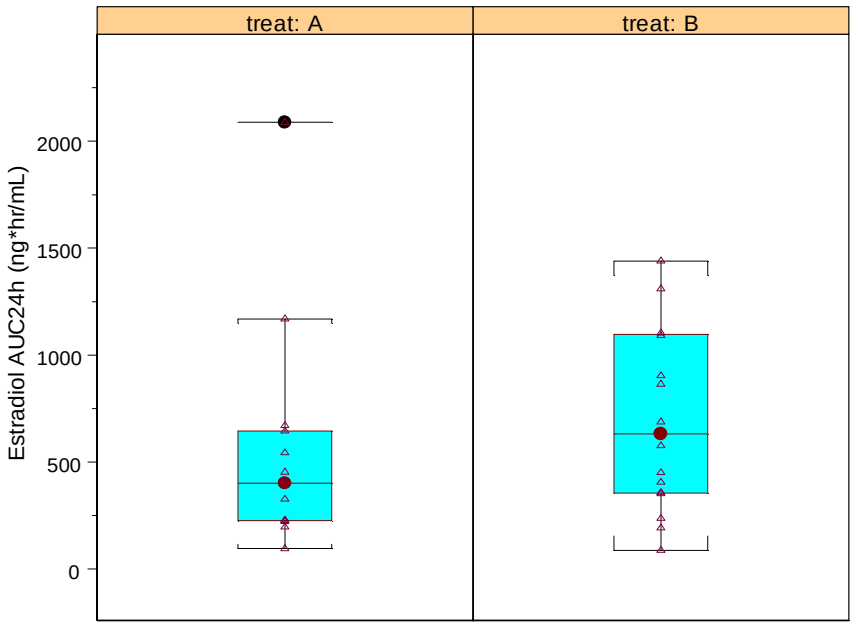

Figure 1. Individual E2 AUCt non-baseline corrected values following single administration of the treatments: Treatment A: washing 1 hr after single dose administration of Divigel 1 mg (n=16).; Treatment B: no washing (n=16).

Figure 2. Individual E2 Cmax non-baseline corrected values following single administration of the treatments: Treatment A: washing 1 hr after single dose administration of Divigel 1 mg (n=16).; Treatment B: no washing (n=16).

Figure 3. Individual E1 AUC non-baseline corrected values following single administration of the treatments: Treatment A: washing 1 hr after single dose administration of Divigel 1 mg (n=16).; Treatment B: no washing (n=16).

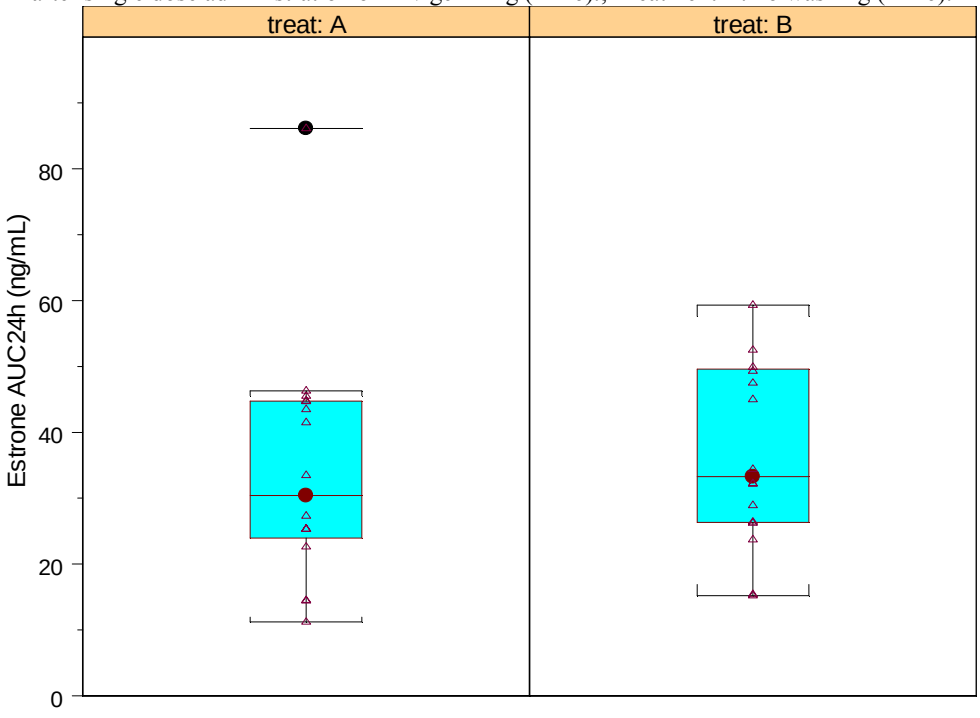

Figure 4. Individual E1 AUC non-baseline corrected values following single administration of the treatments: Treatment A: washing 1 hr after single dose administration of Divigel 1 mg (n=16).; Treatment B: no washing (n=16).

Analysis of Skin Swab Estradiol Concentrations

The amount of estradiol from application site swabs and percentage of dose detected in the swab samples are summarized in Table 9.

Table 9. Summary of Estradiol Swab Samples

[1] 1 Hour Swab Sample (Treatment C) N=6				
Statistic	**Baseline**	**Before Wash**		**After Wash**
		Amount	**[3] % of Dose**	
Mean (CV%)	0.00	36.72 (119.85)	0.07 (119.85)	0.00
Min, Max	0.00, 0.00	0.00, 106.00	0.00, 0.21	0.00, 0.00
[2] 8 Hour Swab Sample (Treatment D) N=7				
Statistic	**Baseline**	**Before Wash**		**After Wash**
		Amount	**[3] % of Dose**	
Mean (CV%)	0.00	536.57 (75.06)	1.07 (75.06)	0.00
Min, Max	0.00, 0.00	227, 1400.00	0.45, 2.80	0.00, 0.00

[1] Treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area on the thigh. After 60 minutes, a 10-cm^2 area was swabbed for analysis of residual levels of estradiol at the application site. The area was then washed with mild soap and a washcloth for 30 seconds and rinsed with warm water for 2.5 minutes. A second swab collection was taken 15 minutes after the start of washing.

[2] Treatment D: 1.0 g of estradiol gel 0.1% (1.0 mg) applied to a 200-cm^2 area on the thigh. After 8 hours, a 10-cm^2 area was swabbed for analysis of residual levels of estradiol at the application site. The area was then washed with mild soap and a washcloth for 30 seconds and rinsed with warm water for 2.5 minutes. A second swab collection was taken 15 minutes after the start of washing. In addition to the swab samples collected after dosing, baseline swab samples (prior to dose application) were collected for both Treatments C and D.

[3] Percentage of USL-221 applied to the skin that is detected in the swab assuming equal distribution of the dose (1 mg) over the application area (200cm2). Calculated as: [Amount collected (mg)/((1 mg/200 cm^2) * 10 cm^2)] * 100.

As shown in Table 9 greater amount of estradiol were observed 8 hours after dosing compared with amount observed 1 hour after dosing, most likely due to large variability in the residual percentage of dose. Therefore, this reviewer is of the opinion that the skin swab technique used by the sponsor is not reliable, and therefore, the values reported can't be used to make conclusions about the amount of estradiol left over time on the site of application after drug administration. However, the sponsor may claim that amount of estradiol on the skin after washing were BLQ, indicating that washing for 3 minutes removed all detectable amounts of estradiol.

CONCLUSION

- Washing the application site one hour after application resulted in a decrease in total exposure (Cmax and AUC) of mean baseline-corrected, uncorrected estradiol by 30 to 38%.
- Washing the application site one hour after application resulted in a decrease in total exposure of mean baseline-corrected, no baseline uncorrected estrone by 15 to 53%.
- After a single topical application of Divigel 1.0-mg estradiol, washing the application site for 3 minutes removed all detectable amounts of estradiol on the application site.

Population Pharmacokinetics of Estradiol, Estrone and Estrone Sulfate Following Once Daily Administration of USL-221 in Postmenopausal Female Patients

Protocol No: P04-001
Date of Final Report:
Phase: III

BACKGROUND

Basic structural population pharmacokinetics models for Estradiol (E2), and its two metabolites, Estrone (E1) and Estrone Sulfate (ES) were developed using data obtained following administration of Divigel, 0.1% (a topical estradiol preparation), in female postmenopausal patients (data from two Phase I studies). These models were then applied to estimate the population and individual PK parameters and steady-state concentrations of E2, E1, and ES in postmenopausal patients following once daily application of USL-221 at three estradiol dose amounts (0.25 mg, 0.5 mg, and 1.0 mg) in a Phase 3 trial in postmenopausal female patients. The potential effects of demographic and baseline characteristics and concomitant medications on E2, E1, and ES pharmacokinetics following Divigel application were also investigated using the population PK analysis.

OBJECTIVES

- To characterize the population pharmacokinetics of E2, E1, and ES after repeated, once daily topical administration of Divigel 0.1% in Phase III clinical trials.
- To investigate the potential effects of demographic and baseline characteristics and concomitant medications on E2, El, and ES pharmacokinetics following Divigel 0.1% application.
- To validate the population pharmacokinetic model established using bootstrapping techniques

METHODS

Subjects and Sample Size

Pharmacokinetic data obtained from postmenopausal women (about 40) from two Phase 1 studies (P04-003 and P04-005) which contain an intensive sampling schedule were used to develop the structural pharmacokinetic models for E2 and its two metabolites. The data sets available for the population PK analysis of E2, El, and ES profiles consisted of 1,291 serum samples collected from 327 female postmenopausal patients at weeks 0, 4, 8, and 12, in a Phase 3 trial, Protocol P04-001.

Study Design and Treatments

Protocol P04-001 was a randomized, parallel, placebo-controlled, double-blind, prospective multicenter Phase 3 study in postmenopausal women, presenting with moderate to severe vasomotor symptoms. Placebo or one of three Divigel doses (0.25, 0.5, and 1.0 mg estradiol) was administered topically once daily for a 12-week period. At the end of study or early termination, all women with an intact uterus, who had received at least 6 weeks of therapy, received oral progestin for 14 days to reverse any influence of the estradiol on endometrial tissue. Four hundred -ninety-five patients were enrolled in the study and randomized to one of the four

treatments. The demographics of subjects included in the population PK analysis are summarized in table 1.

Table 1. Summary of Demographic Parameters and Baseline Characteristics of Postmenopausal Female Patients Included in the Population PK Analysis

Parameter	1.0 mg Estradiol (N=112)	0.5 mg Estradiol (N=106)	0.25 mg Estradiol (N=109)	All (N=327)
Intact uterus	50	52	54	156
No uterus	62	54	55	171
Age group:				
18-45 yr	14	5	7	26
46-65 yr	94	94	97	285
>65 yr	4	7	5	16
Race:				
White	100	92	95	287
Non-White	12	14	14	40
Estradiol, pg/mL[b]	17.5 (<5-97)	16.7 (<5-113)	24.3 (<5-316)	19.5 (<5-316)
FSH, U/L	75	80.09	67.8	74.5
SHBG, nmole/L	45.8	50.6	46.5	47.6
Weight, kg	73.7 (53-108)	70.7 (47.7 - 103.6)	72.8 (47.3 - 99.7)	72.4 (47.3 - 108)
Age, yr	53.6	54.8	54.9	54.5 (34-89)
Creatinine Clearance, mL/min	96 (46.8 - 170)	90.8 (34.7 - 187)	96.1 (49 -173)	94.3 (34.7 - 187)

Blood Samples Collection

Blood samples from the Phase III trial were collected from all patients at weeks 0, 4, 8, and 12 to measure serum concentrations of E2, El and ES. Samples were collected at baseline and then within 1-10 hours of the morning dose at the time of each patient's routine. There were approximately 80 samples collected after 10 hrs of drug administration.

Assay Methods

Serum concentration of E2, El and ES were determined using a validated HPLC assay with mass spectrometric detection. The assay was conducted using calibration standards and quality controls prepared in modified serum from which all endogenous steroid-like compounds had been removed (stripped) by exposure to activated carbon. The results of the assay validation are summarized on Table 2.

Table 2. Results of analytical method validation

PPD Method	LCMSC 248.1			
Analytes	Estrone and 17-ß-Estradiol			
Matrix	Human Serum (modified and unmodified)			
Sample Volume	500 µL			
Estrone Validated Range	5.00 to 500 pg/mL			
17-ß-Estradiol Validated Range	2.50 to 250 pg/mL			
Internal Standard	(b) (4)			
Sample Storage Condition	-20 oC			
Assay Validation and Performance in Modified Human Serum				
	Intra-Assay Quality Control Samples		Inter-Assay Quality Control Samples	
Analyte	Precision (%CV)	Accuracy (% Diff from Nominal)	Precision (%CV)	Accuracy (% Diff from Theo)
Estrone	(b) (4)			

17-ß-Estradiol	(b) (4)			
	Assay Validation Performance in Nonstripped Human Serum			
	Intra-Assay Quality Control Samples		Inter-Assay Quality Control Samples	
Analyte	Precision (%CV)	Accuracy (% Diff from Nominal)	Precision	Accuracy
Estrone	(b) (4)			
17-ß-Estradiol				

Serum concentrations of ES were determined using another validated HPLC assay with mass spectrometric detection. The method was validated on the range of 50 to 5000 pg/mL. The inter- and intra-day precision and accuracy values were less than 12%.

DATA ANALYSIS

Population Pharmacokinetic Analysis

The NONMEM V level 1.1 (b) (4) software was used for all mixed-effect model fittings. Due to the sparse nature of the blood sampling in the Phase 3 study, it was decided, that the intensively sampled serum concentration-time data from two Phase 1 PK studies of Divigel (P04- 003 and P04-005) could be used to develop the structural pharmacokinetic models. The population pharmacokinetics analysis plan follow by the sponsor can be summarized as follows:

- A graphical exploratory analysis of the population PK data set was performed to detect potential outliers.
- A base population pharmacokinetic model was constructed to include the structural component as well as intra- and inter-individual variability in basic pharmacokinetic parameters.
- A graphical exploratory analysis was performed to evaluate the covariate factors and random, effects.
- The covariate models were developed to identify covariates that had significant effect on the CL/F estimates of E2 and its two metabolites. The covariates included in the models were: race (native Americans, Asians, Black of African Americans, Native Hawaiians, White, and others), age, age group (18-35 yrs, 34-46 yrs, 46- 65 yrs, and > 65yrs, body weight, CrCL, alanine aminotransferase (ALT), aspartate aminotransferase (AST), LBM, BSA, uterus vs. no uterus, concomitant medications, and others.
- Final models were validated using the bootstrap resampling technique.

Model Building

Structural Model

An open one-compartmental model with linear disposition and sequential zero-order and first-order absorption incorporating lag time was found to best describe the data in this analysis for each analyte. The model was parameterized in terms of apparent clearance (CL/F), apparent volume of distribution of the central compartment (V/F), first-order absorption rate constant (ka), duration of zero- order-absorption (Dl) and a lag time in absorption (alag). The RATE variable in the NONMEM input file was set to be -2 to allow estimation of the duration of zero-order input, D1, in the population PK analysis. Therefore, the zero-order absorption rate constant, k0 was dependent on the dose and D1.

All data were modeled without baseline value correction. The endogenous baseline concentrations were modeled by a zero-order formation rate (r0/F) as follow:

$$\text{Baseline} = (r0/F)/(CL/F)$$

The exponential error models were used to describe the between-subject variability in PK parameters, which were assumed to follow the lognormal distribution. The residual variability in log-transformed serum concentrations was modeled using the additive error. The same model as E2 was found adequate to characterize E1 and ES PK profiles.

Final Model

The last model with all significant covariates was considered the final model; after all non-significant covariates had been removed from the full model. Subsequently, a Bayesian post hoc analysis was performed on the final model to estimate the model-predicted PK parameters for each patient including the baseline and steady-state averaged concentration and AUC of E2 and E1 (uncorrected for the baseline). The E2/E1 ratios at baseline and at steady state were also calculated. The first order method (FO) was used in all analyses.

Model Validation

The model was validated using the bootstrap technique. This involved resampling from the original data and each individual subject as a sampling unit. About (b) (4) replicates of the data were generated by bootstrap for the NONMEM analysis to obtain the mean and %CV of the fixed-effect and random-effect parameters.

RESULTS

Nine PK samples were deleted from the analysis data set due to missing collection times. There were 78 PK samples from 43 patients excluded from the analysis data set because there was no baseline sample collected from these patients or because there was only one post-dose sample collected from that patient. There were 1291 remaining samples collected from 327 postmenopausal female patients. The majority of serum samples were collected between 0-10 hours post dose. There were 696 serum samples between 0 to 2 hour post-application, 318 serum samples between 2 to 4 hours post-application, 197 serum samples between 4 to 10 hours post-application, and 80 samples after 10 hours post-application. This sampling window was not prospectively determined for use in a population PK analysis, since the estimated time to maximum concentration from Phase I studies with Divigel was about 10hrs.

All stepwise tested models from base model to final model for E2 and its two metabolites are summarized in Table 3 to 5. In the initial tests against the base model, the effect of body weight on CL/F of E2 was the only one found to be significant with a decrease in OF > 3.84. However, the effect of body weight CL/F of E2 became non-significant in the model reduction step. For CL/F of E1, none of the covariates tested were significant.

Table 3. Listing of PK model in NONMEM analysis for E2 in chronological order

Test	Reference	OF	Change in OF	Description of the Model Tested	Test Results
Mod 1	-	1879.08	-	Base model	-
Mod 2	Mod 1	1875.67	-4.13	WT on CL	SIG
Mod 3	Mod 1	1879.65	-0.14	Dose on CL	NS
Mod 4	Mod 1	1879.20	-0.59	CrCL on CL	NS
Mod 5	Mod 1	1879.77	-0.02	ALKP on CL	NS
Mod 6	Mod 1	1879.16	-0.64	AST on CL	NS
Mod 7	Mod 1	1879.51	-0.28	Race on CL	NS
Mod 8	Mod 1	1879.76	-0.03	Age on CL	NS
Mod 9	Mod 1	1879.05	-0.75	Uter on CL	NS
Mod 2	-	1875.67	-	Full model	-
Mod 1	Mod 2	1879.8	4.13	Remove WT on CL	NS
Mod1cov	Mod 1	1840.0	-39.8	Covariance of CL and V	SIG
Mod1cov				Final Model	

Table 4. Listing of PK model in NONMEM analysis for E1 in chronological order

Test	Reference	OF	Change in OF	Description of the Model Tested	Test Results
Mod 1	-	361.01	-	Base model	-
Mod 2	Mod 1	359.73	-1.28	WT on CL	NS
Mod 3	Mod 1	360.28	-0.73	Dose on CL	NS
Mod 4	Mod 1	360.99	-0.02	CrCL on CL	NS
Mod 5	Mod 1	360.77	-0.25	ALKP on CL	NS
Mod 6	Mod 1	360.5	-0.51	AST on CL	NS
Mod 7	Mod 1	361	-0.01	Race on CL	NS
Mod 8	Mod 1	359.7	-1.31	Age on CL	NS
Mod 9	Mod 1	360.7	-0.31	Uter on CL	NS
Mod1cov	Mod 1	360.39	-0.62	Covariance of CL and V	NS
Mod 1				Final Model	

Table 5. Listing of PK model in NONMEM analysis for ES in chronological order

Test	Reference	OF	Change in OF	Description of the Model Tested	Test Results
Mod 1	-	1383	-	Base model	-
Mod 2	Mod 1	1379.12	-3.88	WT on CL	SIG
Mod 3	Mod 1	1381.19	-1.81	Dose on CL	NS
Mod 4	Mod 1	1382.69	-0.3	CrCL on CL	NS
Mod 5	Mod 1	1382.66	-0.34	ALKP on CL	NS
Mod 6	Mod 1	1378.51	-4.49	AST on CL	SIG
Mod 7	Mod 1	1377.54	-5.46	Race on CL	SIG
Mod 8	Mod 1	1382.09	-0.91	Age on CL	NS
Mod 9	Mod 1	1379.6	-3.4	Uter on CL	NS
Mod 10	-	1367.42	-	Full model	-
Mod 11	Mod 10	1374	6.58	Remove WT on CL	NS
Mod 12	Mod 10	1373	5.58	Remove AST on CL	NS
Mod 13	Mod 10	1373.46	6.04	Remove race on CL	NS
Mod1cov	Mod 1	1374.966	-8.03	Covariance of CL and V	SIG
Mod1cov				Final Model	

The Final E2 Pharmacokinetic Model

None of the covariates were found to be significant in estimating CL/F of E2. CL/F and V/F of E2 were found to be highly correlated with correlation coefficient of 0.69. Therefore, incorporation of covariance between CL/F and V/F was chosen to be the final E2 Model. The E2 population PK parameter estimates obtained from the final model are summarized in Table 6. The population means CL/F of E2 was 510 L/hr with large between-subject variability (78%). The goodness of fit plots (population predicted versus observed concentrations, individual predicted versus observed concentrations, and population weighted residuals versus population predicted for E2 population PK model are shown in Figures 1 to 3.

Table 6. Population PK parameters estimates of E2 in the postmenopausal female patients obtained from the final model

Parameters	Mean (%CV)[a]	BSV (%) [b]
CL/F (L/hr)[c]	510 (52)	78 (44)
V/F (L)[c]	10000 fixed	185 (101)
Ka (hr^{-1})	0.71 fixed	0 fixed
D2 (hr)	4.7 fixed	215 (64)
Alag (hr)	1.8 fixed	0 fixed
ro/F (μg/h)	1.59 (312)	67 (546)

Proportional residual error 49%

[a] Parameter precision is expressed as coefficient of variation

[b]BSV=between subject variability

[c]Correlation between CL/F and V/F was 69%

Figure 1. Scatter plot of the observed E2 serum concentrations versus individual predicted.

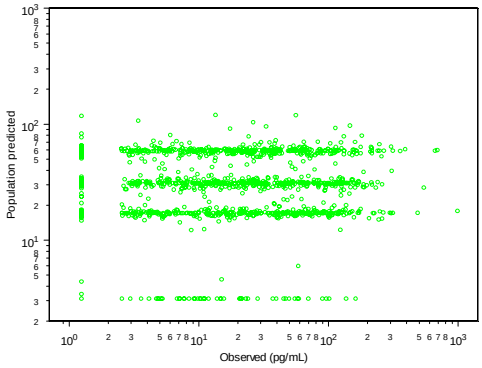

Figure 2. Scatter plot of the observed E2 serum concentrations versus population predicted.

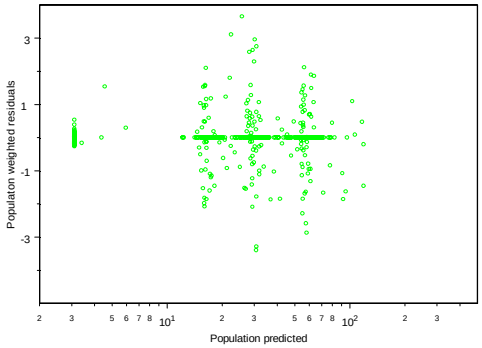

Figure 3. Scatter plot of the population predicted E2 serum concentrations versus population weighted residuals.

The Final E1 Pharmacokinetic Model

None of the covariates were found to have a significant effect on CL/F estimate of E1. There was no correlation between CL/F and V/F of E1, addition of covariance between CL/F and V/F did not improve the model. The E1 population PK parameter estimates obtained from the final model are summarized in Table 7. The population mean CL/F of E1 was 946 L/hr with between-subject variability of 46%. The goodness of fit plots (population predicted versus observed concentrations, individual predicted versus observed concentrations, population

weighted residuals versus population predicted, and individual weighted residuals versus population predicted) for E1 population PK model are shown in Figures 4 to 6.

Table 7. Population PK parameters estimates of E1 in the postmenopausal female patients obtained from the final model

Parameters	Mean (%CV)[a]	BSV (%) [b]
CL/F (L/hr)	946 (8)	46 (9)
V/F (L)	46400 fixed	422 (62)
Ka (hr^{-1})	0.12 fixed	0 fixed
D2 (hr)	4.7 fixed	32 fixed
Alag (hr)	3.39 fixed	0 fixed
ro/F (µg/h)	21.9 (15)	0 (>500)

Proportional residual error 29%

[a] Parameter precision is expressed as coefficient of variation

[b]BSV=between subject variability

Figure 4. Scatter plot of the observed E1 serum concentrations versus individual predicted.

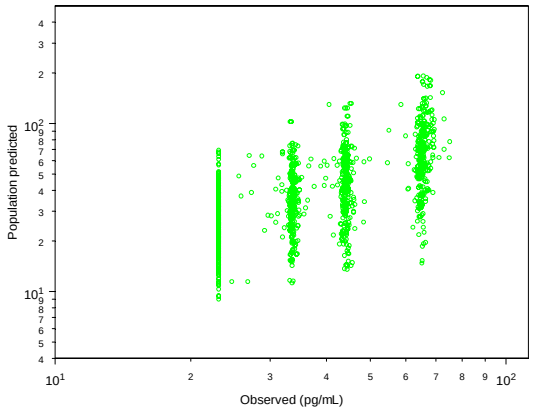

Figure 5. Scatter plot of the observed E1 serum concentrations versus population predicted.

Figure 6. Scatter plot of the population predicted E1 serum concentrations versus individual weighted residuals.

The Final ES Pharmacokinetic Model

None of the covariates were found to have a significant effect on CL/F estimate of

ES. There was some correlation between CL/F and V/F of ES, addition of covariance between CL/F and V/F in the final model after covariate analysis significantly improved the model. The ES population pharmacokinetic parameter estimates obtained from the final model are summarized in Table 8. The population mean CL/F of ES was 43.7 L/hr with large between-subject variability (77%). The goodness of fit plots (population predicted versus observed concentrations, individual predicted versus observed concentrations, population weighted residuals versus population predicted, and individual weighted residuals versus population predicted) for ES population PK model are shown in Figures 8 through 11.

Table 8. Population PK parameters estimates of ES in the postmenopausal female patients obtained from the final model

Parameters	Mean (%CV)[a]	BSV (%) [b]
CL/F (L/hr)[c]	43.7 (8)	77 (13)
V/F (L)[c]	904 fixed	562 (88)
Ka (hr^{-1})	0.047 fixed	0 fixed
D2 (hr)	4.7 fixed	32 fixed
Alag (hr)	1.77 fixed	0 fixed
ro/F (μg/h)	9.43 (21)	32 (276)

Proportional residual error 44%

[a] Parameter precision is expressed as coefficient of variation

[b]BSV=between subject variability

[c]Correlation between CL/F and V/F is 69%

Figure 7. Scatter plot of the observed ES serum concentrations versus individual predicted.

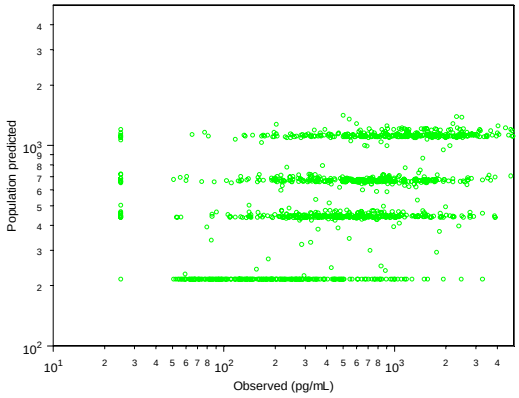

Figure 8. Scatter plot of the observed ES serum concentrations versus population predicted.

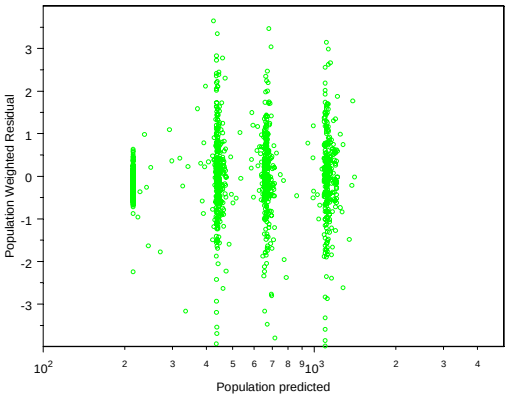

Figure 9. Scatter plot of the population predicted ES serum concentrations versus population weighted residuals.

Model Validation

The mean and %CV of the fixed effect and random-effect parameters of E2 obtained by bootstrap is presented in Table 9. Most of the estimates obtained by bootstrap were in good

agreement with the final estimates of population PK parameter obtained from the original data set with few exceptions in the estimates Of BSV of V/F. The results of the bootstrap validation demonstrated good stability in most of the estimates of the final models for each analyte.

Table 9. Comparison of Mean (%CV) population PK parameters estimates of E2 in the postmenopausal female patients

Parameters	Final estimates[a]	Bootstrap[c]
CL/F (L/hr)	510 (52)	540 (11)
ro/F (μg/h)	1.59 (312)	2.35 (57)
BSV of CL (%)[b]	78 (44)	77 (10)
BSV of V/F (%)[b]	185 (101)	181 (18)
BSV of D1 (%)[b]	215 (64)	209 (19)
BSV of ro/F (%)[b]	67 (546)	46 (89)

[a] Parameter precision is expressed as coefficient of variation
[b]BSV=between subject variability
[c] Mean and (%CV) from 500 patients

Effect of Covariates

Estradiol Dose

Estradiol dose did not have significant effect on CL/F estimates of E2 and its two metabolites. There was no correlation between dose and CL/F estimates of E2 and its two metabolites. The steady-state averaged concentration and AUC (uncorrected for the baseline concentration) for E2 and E1 were estimated for each patient based on the estimated CL/F values of E2 and El and are summarized on Table 10.

There were no significant differences in baseline E2 and El concentrations or E2/El ratio in patients between dose groups. The predicted E2 concentrations at baseline were low (3.3 to 3.5 pg/mL) and the E2/El ratios at baseline were all 0.13, across dose groups. The predicted steady state E2 concentrations increased dose proportionally from 0.25 to 1.0 mg estradiol doses.

Table 10. Model predicted E2 and E1 baseline concentrations and steady-state AUC and average concentrations in Postmenopausal female patients (data reported by the sponsor)

Variable	Mean	%CV	Median	Minimum	Maximum
	0 25 mg estradiol (n =109)				
CL/F of El (L/hr)	964	37 6	946	316	2439
CL/F of E2 (L/hr)	597	54 1	542	162	2410
E1 baseline (pg/mL)	26 0	39 2	23 1	9 0	69 1
E2 baseline (pg/mL)	3 4	57 7	2 8	0 6	10 7
Cavg of E 1 (pg/mL)	38 4	39 2	34 1	13 2	102 0
Cavg of E2 (pg/mL)	25 6	52 6	22 2	4 9	75 1
AUC of El (hr*pg/mL)	922 2	39 2	819 3	317 6	2449 0
AUC of E2 (hr*pg/mL)	613 2	52 6	532 2	117 5	1801 8
E2-to-E1 baseline	0 13	43 3	0 12	0 04	0 32
E2-to-E1 at SS	0 68	40 0	0 62	0 23	1 9
	0 5 mg estradiol (n =106)				
CL/F of El (L/hr)	989	39 3	946	325	2323
CL/F of E2 (L/hr)	768	106 2	510	61	5488
E1 baseline (pg/mL)	25 7	42 0	23 1	9 4	67 2
E2 baseline (pg/mL)	3 5	90 3	3 1	0 3	27 7

Cavg of E1 (pg/mL)	50 2	42 0	45 2	18 4	131
Cavg of E2 (pg/mL)	49 5	84 4	44 0	4 1	367 6
AUC of El (hr*pg/mL)	1205 8	42 0	1083 7	441 1	3151 2
AUC of E2 (hr*pg/mL)	1188 1	84 4	1056 1	97 5	8822 4
E2-to-E1 baseline	0 13	54 3	0 13	0 02	0 44
E2-to-E1 at SS	0 96	53 0	0 92	0 15	3 14
	1 0 mg estradiol (n =112)				
CL/F of El (L/hr)	955	37 8	910	331	2353
CL/F of E2 (L/hr)	637	73 6	495	181	2984
E1 baseline (pg/mL)	26 4	41 0	24 0	9 3	66 0
E2 baseline (pg/mL)	3 3	45 5	3 2	0 5	8 6
Cavg of E1 (pg/mL)	76 7	41 0	69 8	27 0	191 8
Cavg of E2 (pg/mL)	89 8	45 7	87 3	14 5	237 4
AUC of El (hr*pg/mL)	1839 7	41 0	1675 8	648 0	4603 5
AUC of E2 (hr*pg/mL)	2155 4	45 7	2096 3	347 5	5697 8
E2-to-E1 baseline	0 13	42 7	0 12	0 03	0 31
E2-to-El at SS	1 23	42 7	1 7	0 33	2 83

Body Weight and Body Mass Index

Body weight did not have significant effect on CL/F estimates of E2 and its two metabolites. Relatively large between subject variability in CL/F estimates of E2 and the metabolites and relatively small range of body weight distribution (47-108 kg) in the patient population might explain the lack of significant correlation between body weight and the CL/F estimates.

E2, FSH and SHGH at Screening

The observed E2 concentrations at screening ranged from 0.5 to 316 pg/mL. FSH level at screening ranged from 0.5 to 186 U/L. SHBG level at screening ranged from 9 to 243 nmole/L. E2, FSH and SHBG at screening and CL/F estimates of E2 and its two metabolites were found not to be correlated.

Renal Function

Creatinine clearance was estimated by the Cockcroft-Gault method as a measure of renal function of each patient, which ranged from 34.7 to 187 mL/min in the patient population. There were no patients with severe impairment of renal function. Creatinine clearance did not have a significant effect on CL/F estimates of E2 and its two metabolites.

Hepatic Function

AST, ALT, alkaline phosphatase, and total bilirubin levels were used as indicators of hepatic function. The relationships between CL/F and these lab measurements were explored graphically. None of the four measurements showed any correlation with CL/F estimates ofE2 and its metabolites. Only alkaline phosphatase was evaluated in the model building step and found to have no significant effect on CL/F estimates of E2 and its metabolites.

Age

Age of the patient population ranged from 34 to 89 years of age and only 16 of the 327 patients were older than 65 years of age. When age was evaluated as a continuous covariate graphically, age did not correlate with CL/F estimates of E2 or its two metabolites. When age

was evaluated as a categorical covariate, no significant difference in CL/F estimates of E2 or its two metabolites was found in patients between elderly (> 65 yr) and non-elderly (≤ 65 yr).

Race

There were 287 White, and 40 Non-White patients (31 Black, 4 Asian, and 5 others). No significant difference in CL/F estimates of E2 or its two metabolites was found between White and Non-White patients.

Uterus Status

There were 156 patients with intact uterus, and 171 patients without intact uterus. No significant difference in CL/F estimates of E2 or its two metabolites was found between patients with intact uterus and patients without intact uterus.

Concomitant Medications

There were 50 concomitant medications taken by at least 6 patients each. E2 undergoes extensive hepatic metabolism involving the CYP450 3A4 isoenzyme (CYP3A4). The median CL/F of E2 for patients on miconazole (n = 7) was about 30% lower than the median of the whole population. However, the median CL/F of E2 for patients on fluconazole (n = 6) was about the same as the median of the whole population. None of the 50 concomitant medications are inducers of CYP3A4. None of the other concomitant medications showed any meaningful effect on the CL/F of E2.

REVIEWER'S REMARKS

This reviewer used the final model developed by the sponsor for E2 and its metabolites to corroborate the predicted estimates of CL/F and V/F. The parameter estimates were very similar to those reported by the sponsor (See Table 11). Figure 10, 11, 12 and 13 show a scatter plots of E2, E1 and ES concentrations versus time since last dose, respectively for all subjects included in the population PK analysis. Figure 11 shows a box plot for individual E2 concentrations as a function of dose and visit. The goodness of fit plots for the E2, E1, and ES population pharmacokinetic models plotted by this reviewer using the data generated from the control files provided by the sponsor were shown in Figures 1 to 9. No apparent bias was found in these diagnostic plots. Figures 14 and 15 show a Final Model-Predicted Individual Bayesian Estimates of CL/F for E2 and E1, respectively. Figures 17 to 18 show a box plot of the predicted AUC for E2, E1 and ES, respectively as a function of Divigel dose. Table 12 shows a comparison (sponsor's reported versus this reviewer's calculated values) of model predicted E2 and E1 average concentrations and steady state AUCs following multiple administration of Divigel 0.25, 0.5 and 1.0 mg/day to postmenopausal women.

Figure 19 is a Matrix plot of E2 CL (L/hr) versus demographic variables: AGE (years), WT (kg), Race (Caucasian, Black, Asian, Other) presence of uterus.

Table 11. Comparison of population parameters estimates for E2

Parameter	Population Estimate reported by the sponsor	Population Estimate calculated by this reviewer
CL = THETA(1)	0.51 (0.26)	0.51 (0.26)
WT on CL=THETA(7)	0	0
Dose on CL=THETA(8)	0	0
CrCl on CL= THETA(9)	0	0
ALKP on CL= THETA(10)	0	0
AST on CL= THETA(11)	0	0
Race group on CL= THETA(12)	0	0
Age group on CL= THETA(13)	0	0
Uterus on CL= THETA(14)	0	0
V2 = THETA(2)	10.0	10.0
KA = THETA(3)	0.71	0.71
D1 = THETA(4)	4.7	4.7
ALAG1= THETA(5)	1.8	1.8
K0 = THETA(6)	1.59 (4.96)	1.59 (4.98)
THETA(15)	0.49 (0.0.5)	0.49 (0.035)
MOF	1840.724	1840.72

Figure 10. Scatter plot of individual E2 serum concentrations-time data from postmenopausal women (data from Phase III study P01-001).

Figure 11. Box plot of individual E2 serum concentrations following multiple administration of Divigel 0.25, 0.5, 1.0 mg and PLB (data from Phase III study P01-001).

Figure 12. Scatter plot of individual E1 serum concentrations-time data from postmenopausal women (data from Phase III study P01-001).

Figure 13. Scatter plot of individual ES serum concentrations-time data from postmenopausal women (data from Phase III study P01-001).

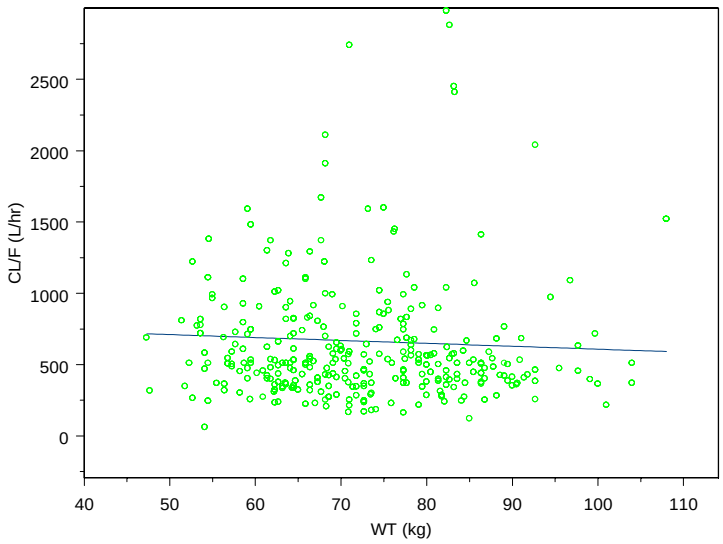

Figure 14. Final Model-Predicted Individual Bayesian Estimates of E2 CL/F versus WT.

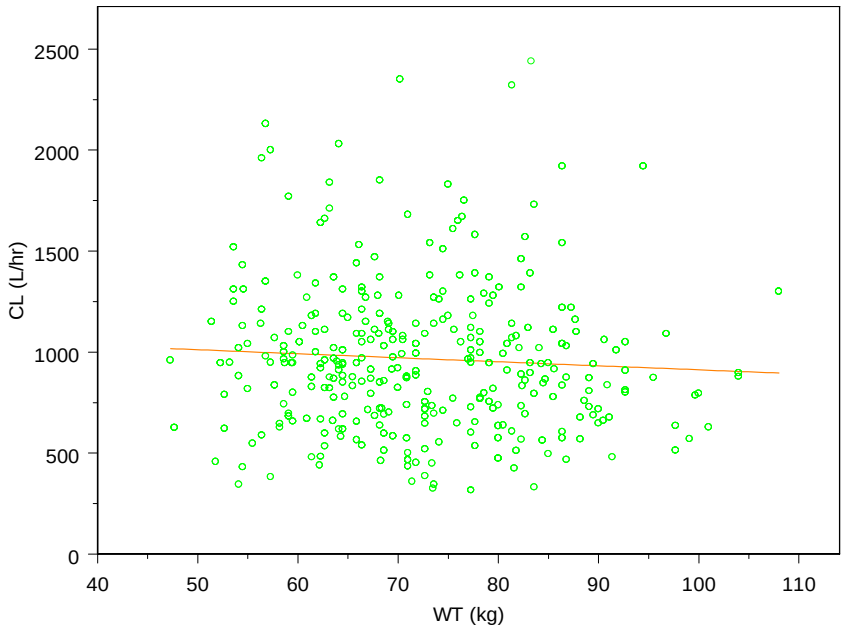

Figure 15. Final Model-Predicted Individual Bayesian Estimates of E1 CL/F versus WT.

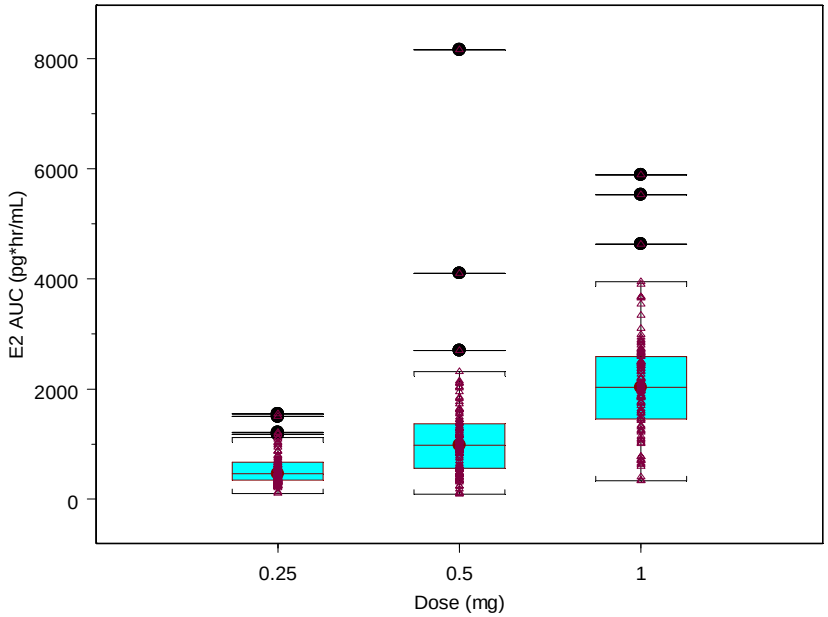

Figure 16. Box plot of individual posthoc E2 AUC following multiple administration of Divigel 0.25 mg, 0.5 mg and 1.0 mg (Data from population PK analysis).

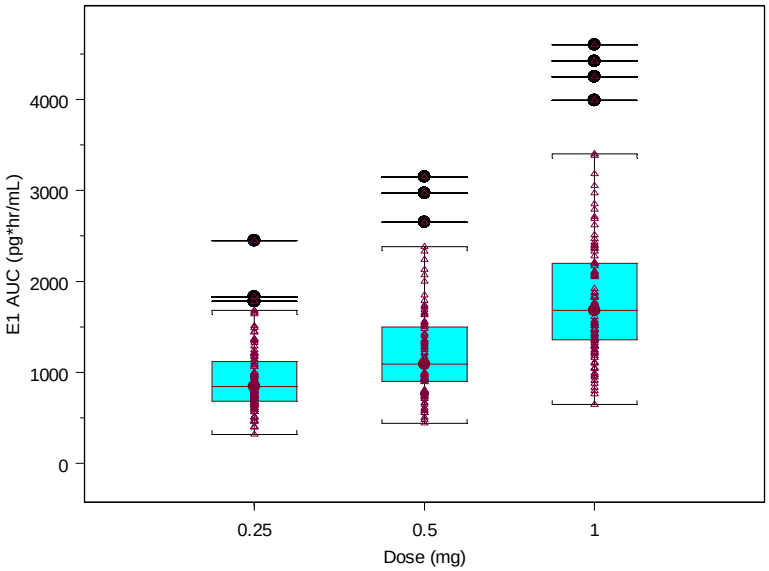

Figure 17. Box plot of individual posthoc E1 AUC following multiple administration of Divigel 0.25 mg, 0.5 mg and 1.0 mg (Data from population PK analysis).

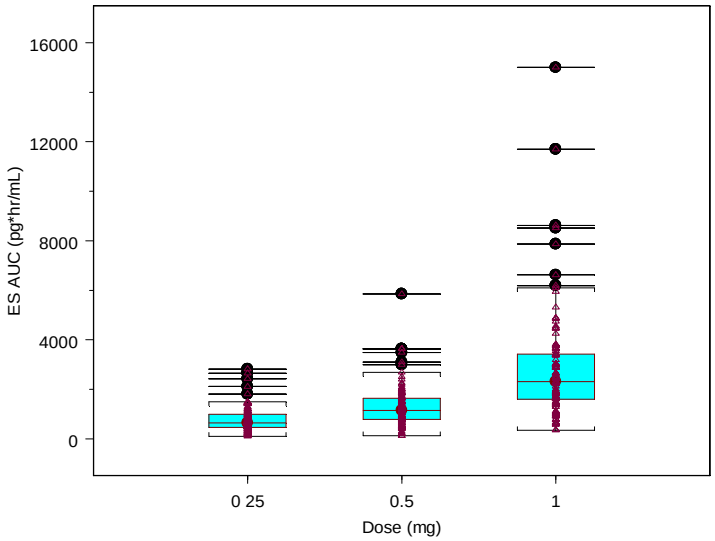

Figure 18. Box plot of individual posthoc ES AUC following multiple administration of Divigel 0.25 mg, 0.5 mg and 1.0 mg (Data from population PK analysis).

Table 12. Comparison of model predicted E2 and E1 average concentrations and steady state AUCs following multiple administration of Divigel 0.25, 0.5 and 1.0 mg/day to postmenopausal women

Variable	Mean reported by sponsor	Mean calculated by this reviewer
	0.25 mg estradiol (n =109)	
CL/F of El (L/hr)	964	961
CL/F of E2 (L/hr)	597	596
AUC of El (hr*pg/mL)	922.2	931
AUC of E2 (hr*pg/mL)	613.2	616
Cavg of E1 (pg/mL)	38.4	38.8
Cavg of E2 (pg/mL)	25.6	26
Cavg of E2/ Cavg of E1	0.68	0.67
	0.5 mg estradiol (n =106)	
CL/F of El (L/hr)	989	987
CL/F of E2 (L/hr)	768	765
AUC of El (hr*pg/mL)	12056	1229
AUC of E2 (hr*pg/mL)	1188.1	1193
Cavg of E1 (pg/mL)	50.2	51.22
Cavg of E2 (pg/mL)	50	50
Cavg of E2/ Cavg of E1	0.96	0.99
	1.0 mg estradiol (n =112)	
CL/F of El (L/hr)	955	953
CLIF of E2 (L/hr)	637	637
AUC of El (hr*pg/mL)	1839.7	1843
AUC of E2 (hr*pg/mL)	2155	2163
Cavg of E1 (pg/mL)	77	77
Cavg of E2 (pg/mL)	89.8	90
Cavg of E2/ Cavg of E1	1.23	1.17

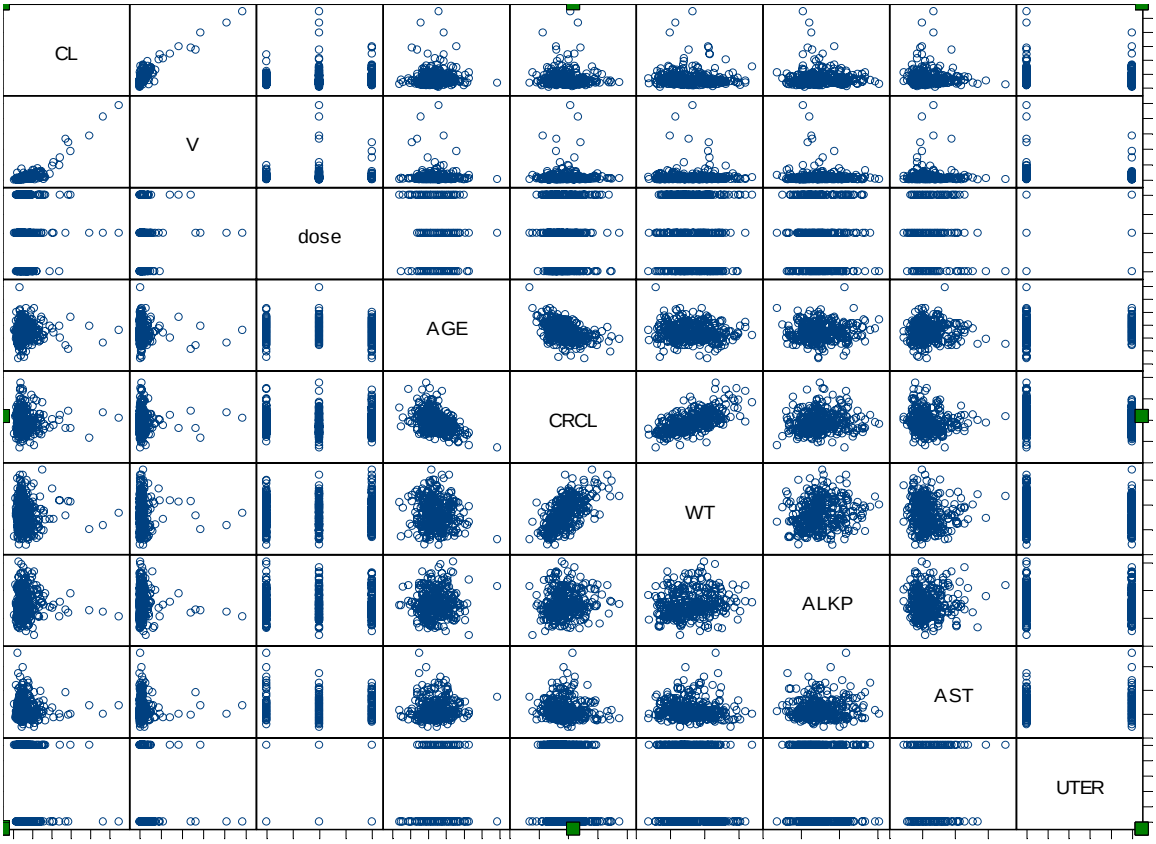

Figure 19. Matrix plots of E2 CL (L/hr) versus demographic variables: AGE (years), WT (kg), Dose (mg), CrCL (mL/min), presence/absence of Uterus, Hepatic function (ALKP, AST), and Race (Caucasian, Black, Asian, Other).

SUMMARY OF FINDINGS

1. The population PK models were successfully fitted to data from the Phase 3 study. The plots of observed versus IPRED and PRED versus WRES plots were satisfactory. No apparent bias can be found in these diagnostic plots.
2. Large between-subject variability and intra-subject variability (ISV) in CL/F values were observed for E2 and its metabolites.
3. Uterus status, SHBG, and FSH levels at screening, estradiol dose, race, age, body weight, BMI, renal and hepatic functions and concomitant medications were evaluated as covariates in the population PK analysis. None of the covariates evaluated had a significant effect on the CL/F estimates of E2 and its metabolites.
4. There were no significant differences in the model-predicted baseline E2 or E1 concentrations or E2/E1 ratio between dose groups (Divigel 0.25, 0.5 and 1mg). The model predicted E2 concentrations at baseline were 3.3 to 3.5 pg/mL and the model predicted E2/E1 ratio at baseline was 0.13 across dose groups.
5. The model-predicted average E2/E1 reported by the sponsor varied from 0.68 to 1.17.
6. The predicted unadjusted E2 AUC increased proportionally to the dose of Divigel. Two-fold increase in the Divigel dose resulted in a two fold increase in systemic exposure of E2.
7. The predicted E1 AUC increased proportionally to the dose of Divigel. Two-fold increase in the Divigel dose resulted in a two-fold increase in systemic exposure of E1.

8. The predicted E2 (range: 616 to 2163 pg*hr/mL) and E1 (931 to 1843 pg*hr/mL) AUC values were higher than those E2 (range: 712-1421 pg*hr/mL) and E1 (555 to 1122 pg*hr/mL) AUC24hrs values derived by non-compartmental analysis in pharmacokinetic studies in phase I studies. The uncorrected AUCss values from population PK analysis were similar to those AUC_{72hrs} reported in Study P04-003.

CONCLUSIONS

- Based on population PK analysis the POSHOC predicted unadjusted E2 and E1 AUC increased proportionally to the dose.
- None of the demographic characteristics: age, uterus status, and body weight, estradiol dose, FSH and SHBG levels at screening had a significant effect on the pharmacokinetics of E2, E1 or ES following multiple dose administration of Divigel 0.25 mg, 0.5 mg, or 1 mg/day.
- No effect of race on the PK of the drug was also observed. However, NO effect of race should be interpreted with caution since there were only there were 287 White subjects and only 40 Non-White patients (31 Black, 4 Asian, and 5 others).
- NO effect of severe renal function and severe hepatic function should also be interpreted with caution since no subjects with severe renal or hepatic impairment were enrolled in the study.
- There were 50 concomitant medications taken by at least 6 patients each. E2 undergoes extensive hepatic metabolism involving the CYP450 3A4 isoenzyme (CYP3A4). The median CL/F of E2 for patients on miconazole (n = 7) was about 30% lower than the median of the whole population. These results are in disagreement with the findings for fluconazole, another CYP3A4 inhibitor. The median CL/F of E2 for patients on fluconazole (n = 6) was about the same as the median of the whole population. Therefore, no final conclusions on the effect of concomitant administration can be made from this population PK analysis.

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	22-038	**Brand Name**	Divigel® (estradiol gel 0.1%)
OCPB Division (I, II, III)	3	**Generic Name**	estradiol
Medical Division	DRUP	**Drug Class**	**Estrogen (hormone)**
OCPB Reviewer	**Sandra Suarez-Sharp**	**Indication(s)**	**Treatment of Vasomotor Symptoms** (b) (4)
OCPB Team Leader	**Ameeta Parekh**	**Dosage Form**	Topical gel
PM Reviewer		**Dosing Regimen**	0.25, 0.5, or 1 mg/day
Date of Submission	May 1, 2006	**Route of Administration**	Topical (skin)
Estimated Due Date of OCPB Review	**December 2006**	**Sponsor**	Upsher-Smith Laboratories
PDUFA Due Date	March 4, 2007	**Priority Classification**	**Standard**
Division Due Date	**January, 2007**		

3 Clin. Pharm. and Biopharm. Information

	"X" if included at filing	**Number of studies submitted**	**Number of studies reviewed**	**Critical Comments If any**
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	**X**			
Tabular Listing of All Human Studies	**X**			
HPK Summary	**X**			
Labeling	**X**			
Reference Bioanalytical and Analytical Methods	**X**			Analytical method reports are in electronic submission as part of individual study reports.
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	**x**			**Study P04-002:** Transferability of estrogen gel during skin-skin contact to partner (electronic submission).
multiple dose:				
Patients-				
single dose:	**x**	**2**		**Study P04-005:** 3-Way Crossover Study of the Washability of estrogen gel (electronic submission). **Study P04-015:** Single dose, open label study for analytical method development to determine residual levels of estrogen remaining in the skin before and after washing the application site in postmenopausal women (3) (paper submission, vol. 1.22).
multiple dose:	**x**	**2**		**Study P04-003:** 3-Way Cross-over PK Study Evaluating Three Dose Levels of estrogen gel (electronic submission) and **Study P04-001** which was the pivotal efficacy and safety study. The PK data from this study was analyzed using a population PK method (paper submission, vol. 1.37).
Dose proportionality -	**x**	**1**		**Study P04-003:** 3-Way Cross-over PK Study Evaluating Three Dose Levels of estrogen gel (electronic submission).
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				

Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:	**x**	**1**		Population PK analysis of estradiol, estrone and estrone sulfate following once daily administration of estrodiol gel in postmenopausal women (data from Study P01-001)
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:	**x**	**1**		**Study FR00.037.2:** assessed the BE of two newly developed estradiol gel, 0.1% formulations (EF107 and EF108) versus the original formulation.
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
QTC STUDIES (PHASE 1)				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**6**		

Filability and QBR comments

	"X" if yes	**Comments**
Application filable ?	**X**	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm ?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.

QBR questions (key issues to be considered)	**1. Dose-Response for efficacy and safety** **2. Sunscreen and other topical product effect on the systemic exposure of estradiol gel** **3. Transferability to partner** **4. Effect of washing on the systemic exposure of estradiol gel**

Other comments or information not included above	
Primary reviewer Signature and Date	
Secondary reviewer Signature and Date	

CC: NDA 20-907, HFD-580 (L

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

/s/

Sandra Suarez
5/29/2007 03:04:00 PM
BIOPHARMACEUTICS

Myong-Jin Kim
5/29/2007 05:51:07 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION		Clinical Pharmacology Tracking/Action Sheet for Formal/Informal Consults	
From: **Sandra Suarez-Sharp**		**To: DOCUMENT ROOM (LOG-IN and LOG-OUT)** **Please log-in this consult and review action for the specified IND/NDA submission**	
SUBMISSION DATE: April 6, 2007	**NDA No.:** 22-038 **Serial No.:**	BLA No.	**DATE OF REVIEW OF DOCUMENT:** May 22, 2007
NAME OF DRUG: Divigel (Estradiol Gel 0.1%)	**PRIORITY CONSIDERATION:** **S or P**		**Date of informal/Formal Consult**: May 22, 2007

NAME OF THE SPONSOR: UPSHER-SMITH

TYPE OF SUBMISSION

CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS RELATED ISSUE

☐ PRE-IND	☐ DISSOLUTION/IN-VITRO RELEASE	☐ FINAL PRINTED LABELING
☐ ANIMAL to HUMAN SCALING	☐ BIOAVAILABILITY STUDIES	☒ LABELING REVISION
☐ IN-VITRO METABOLISM	☐ IN-VIVO WAIVER REQUEST	☐ CORRESPONDENCE
☐ PROTOCOL (SPA)	☐ SUPAC RELATED	☐ DRUG ADVERTISING
☐ PHASE II PROTOCOL	☐ CMC RELATED	☐ ADVERSE REACTION REPORT
☐ PHASE III PROTOCOL	☐ PROGRESS REPORT	☐ ANNUAL REPORTS
☐ DOSING REGIMEN	☐ SCIENTIFIC INVESTIGATIONS	☐ FAX SUBMISSION
☐ PK/PD- POP PK ISSUES	☐ MEETING PACKAGE	☐ OTHER *(SPECIFY BELOW):*
☐ PHASE IV RELATED		

REVIEW ACTION

☐ NAI (No action indicated)	☐ Oral communication with	☒ Formal Review/Memo (attached)
☐ E-mail comments to:	Name: [ ]	☐ See comments below
☐ Medical ☐ Chemist ☐ Pharm-Tox	☐ Comments communicated in meeting/Telecon.	☐ See submission cover letter
☐ Micro ☐ Pharmacometrics ☐ Others	see meeting minutes dated: [ ]	☐ OTHER *(SPECIFY BELOW)*:
(Check as appropriate and attach e-mail)		[Please see attached memo]

REVIEW COMMENT(S)

☒ **NEED TO BE COMMUNICATED TO THE SPONSOR** ☐ **HAVE BEEN COMMUNICATED TO THE SPONSOR**

COMMENTS/SPECIAL INSTRUCTIONS:

Summary

Divigel® (estradiol gel 0.1%) is a smooth, clear to opalescent gel (alcohol-based) in which the active ingredient, estradiol (E2), is dissolved. E2 has been widely used as hormone replacement therapy in postmenopausal women. Divigel® is being proposed for once daily topical administration to skin (right or left upper thigh) of postmenopausal women with/without uterus for the treatment of moderate to severe vasomotor symptoms (VMS) **(b) (4)**

The sponsor's proposed starting dose is 0.5 g (equivalent to 0.5 mg of E2) daily. The dose can be increased to 1.0 g (eq. to 1 mg of E2) /day or decreased to 0.25 g (eq. to 0.25 mg of E2)/day depending on clinical response, in order to achieve the lowest effective dose.

The present submission contains an update of the package insert proposed for Divigel. In this version, the sponsor is proposing to include the amount of estradiol systemically delivered in the package insert under

the description and absorption sections as follows (underlined and red font):

DESCRIPTION

DIVIGEL® (Estradiol Gel) 0.1% is a clear, colorless gel, which is odorless when dry. It is designed to deliver sustained circulating concentrations of estradiol when applied once daily to the skin. The gel is applied to a small area (200 cm^2) of the thigh in a thin, quick-drying layer. DIVIGEL is available in three doses of 0.25, 0.5, and 1.0 g for topical application (corresponding to 0.25, 0.5, and 1.0 mg estradiol, respectively). The 0.25, 0.5, and 1.0 mg estradiol dose provides systemic delivery of 0.003, 0.009, and 0.027 mg of estradiol daily, respectively.

A. Absorption

Estradiol diffuses across intact skin and into the systemic circulation by a passive absorption process, with diffusion across the stratum corneum being the rate-limiting factor.

In a 14-day, Phase 1, multiple-dose study, DIVIGEL demonstrated linear and dose-proportional estradiol pharmacokinetics at steady state for both AUC_{0-24} and C_{max} following once daily dosing to the skin of either the right or left upper thigh (Table 1). Steady-state serum concentration of estradiol are achieved by day 12 following daily application of Divigel to the skin of the upper thigh. The mean (SD) serum estradiol levels following once daily dosing at day 14 are shown in Figure 1. The delivery rates of estradiol using the baseline-corrected average serum concentrations from pharmacokinetic studies using 0.25, 0.5, and 1.0 g/day provides systemic delivery of 0.003, 0.009, and 0.027 mg/day of estradiol, respectively.

Data Submitted in the present submission to support the additions to the label in terms of daily delivered rate

The sponsor is relying on pharmacokinetic (PK) data obtained from Study P-04-003 for calculation of daily delivery rates of Divigel. A detailed review of this study was included in the Clinical Pharmacology review for original submission of NDA 22-038. Study P-04-003 was a Phase 1, randomized, open-label, multiple-dose PK study conducted according to a 3-way crossover design. Twenty-one subjects were randomized to 1 of 3 treatment sequences in which each subject received the following treatments over 3 study periods: Treatment A: 0.25 g of estradiol gel 0.1% (0.25 mg E2) applied to a 200-cm^2 area of the thigh once daily for 14 days; Treatment B: 0.5 g of estradiol gel 0.1% (0.5 mg E2) applied to a 200-cm^2 area of the thigh once daily for 14 days; Treatment C: 1.0 g of estradiol gel 0.1% (1.0 mg E2) applied to a 200-cm^2 area of the thigh once daily for 14 days.

In the present submission, the amount of estradiol delivered/day was calculated using the following formula:

Amount of estradiol delivered/day (µg) = (Cavg *CL)/1000, where:

Cavg (pg/mL) = AUC0-24h/24hr, was derived from data obtained from Study P04-003.

The metabolic clearance (CL) of estradiol in postmenopausal women is based on the value reported in literature (1,240 L/day). The OCP has previously accepted this method of calculation of estradiol delivered/day for other estradiol related products[1].

It should be noted that the sponsor did not submit the baseline-corrected multiple-dose PK data (e.g AUC) (Study P04-003) to the original NDA (see Original CP review for this NDA) and the present submissions. The present submission included the mean/median baseline-corrected Cavg concentrations of Estradiol (Table 1) but not the AUC and raw data. According to the sponsor, subjects who had "sufficient" baseline-corrected serum concentration data to calculate AUC_{0-24h} were included in the analysis that 6

[1] CP review for NDA 21-813 DFSed by Dr. Tran on 10/5/06

subjects were excluded. The Cavg values were extremely high ranging from 99% to 193%. According to the sponsor, one subject had an extremely high estradiol concentration (137.86 pg/mL) in the Divigel 0.5 g treatment group, which skewed the mean Cavg. Therefore, the sponsor stated that the median Cavg is a more appropriate measure of the central tendency than the mean for the 0.5g dose, as well as for the overall assessment of the amount of systemically delivered estradiol.

Table 1. Summary of Baseline-Corrected Cavg Concentrations of Estradiol After the Last Dose of Divigel on Day 14 (based on the sponsor's analysis)

Parameter (unit)	Divigel 0.25 g (N = 20)	Divigel 0.5 g (N = 20)	Divigel 1.0 g (N = 19)
	Caverage (pg/mL)		
N*	16	18	19
Mean (%CV) Median (Min, Max)	3.13 (99) 2.20 (0.07, 10.18)	16.34 (193) 7.10 (0.96,137.86)	25.42 (99) 21.88 (0.76, 102.65)

*after excluding subjects with insufficient baseline-corrected E2 concentration

The mean and median amounts of estradiol delivered per day after the last dose of multiple daily applications of Divigel on Day 14 are summarized in Table 2. According to the sponsor, the high estradiol concentration (137.86 pg/mL) for 1 subject that skewed the mean Cavg for the 0.5-g dose is reflected in the calculation of the amount of estradiol delivered per day. Therefore, the sponsor stated that the median amount of estradiol delivered per day is a better measure of the central tendency than the mean.

Table 2. Amount of Estradiol Delivered Per Day After the Last Dose of Multiple Daily Applications of Divigel on Day 14

Divigel Treatment	**Mean Amount of Estradiol Delivered/Day (µg)**	**Median Amount of Estradiol Delivered/Day (µg)**
0.25 g (0.25-mg estradiol)	3.88	2.72
0.5 g (0.5-mg estradiol)	20.26	8.80
1 g (1.0-mg estradiol)	31.2	27.13

Table 3 shows the estimates of estradiol delivery rates for Elestrin®, an estradiol gel recently approved for the same indication as Divigel. It is noted that the variability in baseline-adjusted Cavg at steady state was smaller than that observed for Divigel. Also, it should be noted that the reported mean delivery rates for Divigel are about 200% higher for the 0.5 mg dose and 50% lower for the 1 mg dose compared to the ones reported for Elestrin® equivalent dosed (0.52 mg and 1.02 mg, respectively).

Table 3. Estradiol in vivo delivery rate estimates (Elestrin*)[1]

Study	Dose of gel applied (dose of estradiol)	Baseline-adjusted Cave at steady state (pg/mL) Mean ± SD	Nominal in vivo estradiol delivery (mg/24 h)
EST007	0.87 g (0.52 mg)	9.2 ± 5.5	0.012
	1.7 g (1.02)	31.9 ± 23.1	0.041
EST003a	1.25 g (0.75 mg)	18.4 ± 9.3	0.023
	2.5 g (1.5 mg)	49.8 ± 21.3	0.064
EST008 (group 1 and 2 combined on day 15)	2.6 g (1.56 mg)	60.0 ± 38.4	0.077

a In study EST003 (shaded rows), Elestrin was applied to the front and inner thigh instead of the upper arms that was used in all other studies

*the approved doses of Elestrin are 0.87 g and 1.7 g.

Comments to Sponsor:

The proposed additions to the package insert of Divigel in terms on daily delivery rate of estradiol are not supported by the data included in the April 6, 2007 submission to NDA 22-038. The following deficiencies are identified:

- The baseline-corrected mean and individual AUC $_{0\text{-}24hr}$ values at steady-state (day 14) were not submitted.
- There were 6 subjects excluded from the calculation of baseline-corrected Cavg.
- The derived baseline-corrected Cavg values show extremely high variability (%CV range from 98 % to 193%). Therefore, the reported values are uncertain.
- Median values are being considered in the package insert instead of mean values.

Recommendation

The Division of Clinical Pharmacology 3 (DCP3) has reviewed the sponsor's submission to NDA 22-038 dated April 6, 2007. The inclusion of the daily delivery rates of Divigel doses in the package insert is not acceptable. The above comments should be conveyed to the sponsor.

SIGNATURE OF REVIEWER: Sandra Suarez-Sharp, Ph.D. _____ **SIGNATURE OF TEAM LEADER:** Myong-Jin Kim, Pharm.D. _____	Date _____ . Date _____
CC.: HFD # [ ]; TL: [ ]; DD: [ ]	**Project Manager:_____ Date _____**

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

/s/

Sandra Suarez
5/24/2007 01:03:14 PM
BIOPHARMACEUTICS

Myong-Jin Kim
5/24/2007 01:18:20 PM
PHARMACOLOGIST