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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-166

Clinical Pharmacology and Biopharmaceutics Review

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA:	**21-166**
Compound:	Estradiol, 0.06% (w/w) ESTROGEL®
Sponsor:	Unimed Pharmaceuticals, Inc.
Type of Submission:	New Drug Product; Classification, 3S
Dates of Submission:	Original: August 13, 1999; B2: January 21, 2000; B2: March 28, 2000; N000BZ: June 16, 2003; IND 29,020 serial 110: September 26, 2003; N000BL: December 10, 2003; N000BB: December 22, 2003; N-000-BM: January 15, 2004
Reviewer:	S.W. Johnny Lau, R.Ph., Ph.D.

Executive Summary

NDA 21-166 (ESTROGEL®, IND 29,020) for the topical 0.06% (w/w) estradiol in hydroalcoholic gel to treat postmenopausal symptoms was submitted on August 13, 1999 by Unimed Pharmaceuticals, Inc. Numerous companies contributed to the content of NDA 21-166. Briefly, Laboratories Besins Iscovesco, through its US agent, LaSalle Laboratories, Inc., submitted IND 29,020 and NDA [ ] for ESTROGEL® in the late 1980s. NDA [ ] for ESTROGEL® was found to be non-approvable on August 17, 1990. On July 1, 1990, IND 29,020 and NDA [ ] were transferred to Schering-Plough Corporation. On April 26, 1994, IND 29,020 and NDA [ ] were transferred from Schering-Plough Corporation to Bristol-Myers Squibb. On March 25, 1997, IND 29,020 and NDA [ ] were transferred from Bristol-Myers Squibb to LaSalle Laboratories. On July 2, 1997, Solvay Pharmaceuticals, Inc. acquired the product registration rights for ESTROGEL® from LaSalle Laboratories. On August 9, 1999, IND 29,020 was transferred to Unimed Pharmaceuticals, Inc. ESTROGEL® is marketed in Europe by Laboratories Besins Iscovesco (Paris, France) as OESTRODOSE®, OESTRACLIN®, and OESTROGEL®. The sponsor submitted the results of 10 studies (S1661002, S1661003, MKL2593, AD1245H/96OEST01, 9566.01.01, 92 OGEL 01, H832-13074-01, 97-OEST-01, CV141-001, and CV141-002) to support the Human Pharmacokinetics and Bioavailability section of NDA 21-166 on August 13, 1999.

An optional Intra-Division Clinical Pharmacology and Biopharmaceutics Briefing for NDA 21-166 was conducted on May 15, 2000. Participants included J. Hunt, A. Parekh, R. Agarwal, D. Spell-LeSane, and J. Lau. Because the sponsor was under Application Integrity Policy (AIP), NDA 21-166's review could not be continued. After the AIP removal, the sponsor responded to the reviewer's former questions via NDA 21-166 N000BZ on June 16, 2003. The sponsor submitted the reports of Studies UMD-01-078 (transfer potential/washing) and Study UMD-00-073 (application sites) via IND 29,020 serial 110 on September 26, 2003 to further support NDA 21-166. Hence, this is a revised review for the updated Human Pharmacokinetics and Bioavailability Section of NDA 21-166. The sponsor intends to market the 1.25 g [ ] of 0.06% (w/w) ESTROGEL® dispensed via both pump and tube. However, the approval of both doses will be based on the clinical safety and efficacy findings.

The unadjusted estradiol AUC and C_{max} were not dose proportional among single doses of 0.625 (1.25 g of 0.03%), 1.25, and 2.5 g of 0.06% ESTROGEL®. However, the geometric mean AUC and C_{max} for unadjusted estradiol were linearly related to the 3 single doses studied.

It appears that steady state serum estradiol concentrations were reached after 3 days of daily 2.5 g of 0.06% ESTROGEL® topical application to the 2 arms of subjects. Upon 14 days of daily topical

application of 1.25 g of 0.06% ESTROGEL® to 1 arm, the estimated mean accumulation index for the unadjusted plasma estradiol AUC_{0-24} and C_{max} were 3.33 and 3.07, respectively.

Per $AUC_{(0-24)}$ and C_{max} values for E_2, E_1, and E_1 sulfate for the naïve patients after 15 minutes contact with dosed subjects (single and multiple daily doses of 1.25 g of 0.06% ESTROGEL® for 14 days), the estradiol gel was either not transferred to naïve subjects or the transferred estradiol gel did not significantly increase the estradiol exposure from baseline in the naïve subjects. In general, washing the application site 1 hour postdose decreased about 25% of the unadjusted estradiol exposure. Per the 2.5 g ESTROGEL® application, estradiol absorption depended on application surface area.

The 0.06% estradiol gel formulation used in the 2 clinical safety and efficacy studies (CV141-001 and CV141-002) contained [ ] % w/w ethanol and the to-be-marketed formulation contains [ ]% w/w ethanol. The ethanol (excipient) difference between the 2 formulations [ ] is a Level 1 change per "Guidance for Industry, Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation." No in vivo bioequivalence documentation is recommended for the Level 1 change. The sponsor conducted a study and demonstrated the similarity in in vitro estradiol release between these 2 formulations.

The sponsor confirmed via NDA 21-166 N-000-BM on January 15, 2004 that with the exception of Study UMD-01-078, subjects were allowed to apply the gel over the inner and outer surfaces of the arm in both the pivotal and clinical pharmacology studies. Subjects in clinical pharmacology studies might apply ESTROGEL® on the inner and/or outer side of arms and might apply ESTROGEL® to varying surface areas. Hence, the different application locations on the arm and application surface areas might add more variability to the transdermal absorption of estradiol and the pharmacokinetic parameters for estradiol and estrone in the clinical pharmacology studies.

Recommendations

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation II (OCPB/DPEII) reviewed the Human Pharmacokinetics and Bioavailability section for NDA 21-166 and finds it acceptable. The sponsor should receive this comment "The sponsor is encouraged to develop an in vitro estradiol release test and define the estradiol release specifications for ESTROGEL® to evaluate potential postapproval changes."

S.W. Johnny Lau, R.Ph., Ph.D.
OCPB/DPEII

FT signed by Ameeta Parekh, Ph.D., Team Leader 2/ /2004

The following questions, based on the content of NDA 21-166, guided this review.

1. What is ESTROGEL®?

It is a clear, colorless, topical hydroalcoholic gel that contains 0.75 mg estradiol (E_2) in 1.25 g gel (0.06% w/w) and is stored in a non-aerosol, metered-dose pump. Each individually packaged pump contains 80 g of gel and is capable of delivering sixty-four 1.25 g doses.

2. What is the proposed indication for ESTROGEL®?

Treatment of:

1. moderate to severe vasomotor symptoms associated with menopause
2. vulval and vaginal atrophy
3. [redacted]

3. How does ESTROGEL® work?

ESTROGEL® functions as a continuous topical estrogen replacement therapy.

4. What are the adverse effects of E_2 (from formulation not related to ESTROGEL®)?

Prolonged and particularly continuous use of E_2 without progestins may lead to endometrial hyperplasia, and rarely, to endometrial carcinoma.

5. What are the studies that support the Human Pharmacokinetics (PK) and Bioavailability (BA) section of NDA 21-166?

ESTROGEL®	Study	Review Question
Dose response	†CV141-001, CV141-002	7
Dose proportionality	S1661002, CV141-001, CV141-002	12
Single dose PK	S1661002, S1661003	12,14
Multiple dose PK	UMD-01-078, S1661003	13
Relative PK to transdermal patch	MKL2593	15
Transfer potential & washing effect	UMD-01-078	16
Relative BA of application sites	UMD-00-073	17
Surface area of application	92 OGEL 01	18
Ineffectiveness of the lowest dose	S1661003, CV141-002	19
In vitro release	9566.01.01	21

†CV141-001 and CV141-002 are the clinical safety and efficacy studies that support NDA 21-166.
Studies H832-13074-01 and 97-OEST-01 examined the [^{14}C]-E_2 transfer potential for 0.165 g gel dose and formulations that were similar to ESTROGEL®. Study UMD-01-078 used the to-be-marketed formulation. Hence, only Study UMD-01-078's review is presented in Question 16.
Study AD1245H/96OEST01 examined E_2 topical gel formulation that was not relevant for NDA 21-166. Hence, it is not presented in this review.

6. What is the proposed dose and mode of administration for ESTROGEL®?

The initial dose is 1.25 g ESTROGEL® applied to the skin once daily. [redacted]

ESTROGEL® [redacted] The arm, from wrist to shoulder, is the recommended area of application.

7. How is the ESTROGEL® dose determined?

The sponsor investigated dose-response relationship for different ESTROGEL® doses in studies CV141-001 and CV141-002. In study CV141-001, 2.5 g ESTROGEL® dose was the most effective dose in reducing the frequency of moderate-to-severe hot flushes and alleviating the severity of all hot

flushes than placebo. The 1.25 g ESTROGEL® dose showed improvement over placebo. Study CV141-002 demonstrated that the 2.5 g ESTROGEL® dose was the most effective ESTROGEL® dose in reducing the frequency and severity of hot flushes. Thus, 1.25 g ESTROGEL® was proposed as the starting dose ; [ ] Refer to medical officer's (Dr. Phill Price) review for more dose-response details.

8. What are the bioanalytical methods used in NDA 21-166?

Bioanalytical assays including validation for E_2, unconjugated estrone (E_1), and total E_1 in plasma[¶] samples for Studies S1661002, S1661003, MKL2593, UMD-01-078, and UMD-00-073 follow:

	$E_2^{\dagger}$ pg/mL	$E_1^{\dagger}$ pg/mL	Total $E_1^{\dagger}$ pg/mL	$E_2^{\ddagger}$ pg/mL	$E_1^{\ddagger}$ pg/mL	E_2^{*} pg/mL	E_1^{*} pg/mL	$E_1SO_4^{*}$ ng/mL	E_2^{ϕ} pg/mL	E_1^{ϕ} pg/mL	$E_1SO_4^{\phi}$ pg/mL
Method											
LLOQ	[										
Linearity											
Accuracy,											
% recovery											
intra-day											
inter-day											
Precision,											
% CV											
intra-day											
inter-day											]

¶ = except Study MKL2593 for serum samples but bioanalytical method was validated with plasma samples. Study UMD-01-078 collected serum samples

† = Studies S1661002 and S1661003

‡ = Studies MKL2593

* = Study UMD-00-073

φ = Study UMD-01-078. Both E_2 and E_1 were measured via radioimmunoassay (RIA) after [ ] E_1SO_4 was measured as E_1 after incubation with sulfatase. Specificity for E_2 was evaluated via 25 structurally similar steroids. E_2 and E_1 demonstrated cross reactivity with the antibody of [ ], respectively. These compounds were separated from E_2 by chromatography. All other steroids cross reacted [ ] Specificity for E_1 was evaluated via 22 structurally similar steroids. E_2 cross reacted [ ] with the antibody, it was separated chromatographically. Equilin cross reacted [ ] and was not present in human serum. All other steroids cross reacted [ ]. Recovery for E_2 and E_1 approaches [ ] Those were inter-batch precision values for E_2, E_1, and E_1SO_4 instead.

[ ]

LLOQ = lower limit of quantitation

NA = not available

Studies CV141-001 and CV141-002 (pivotal clinical studies) used the same RIA method (with hexane:ethyl acetate [ ] extraction) as that for Study UMD-01-078 to measure E_2 and E_1. The LLOQ for E_2 and E_1 were 2 pg/tube and the standard curve was [ ] The recovery for E_2 and E_1 through the method (^{3}H-E_2 and ^{3}H-E_1) were both about [ ] For E_2: Intraassay accuracy was [ ] from [ ] mL and [ ] mL and the interassay accuracy was [ ] %. Intraassay precision was [ ] % and the interassay precision was [ ] For E_1: Intraassay accuracy was [ ] from [ ] mL and [ ] mL and the interassay accuracy was [ ] Intraassay precision was [ ] % and the interassay precision was [ ]

9. What is the clinical PK of E_2 (via formulation not related to ESTROGEL®)?

Absorption

E_2 is well absorbed in the gastrointestinal tract (*Dollery's Therapeutic Drugs* Vol. 2 Churchill Livingstone 1991 page 4). E_2 is extensively metabolized to E_1 in the gut and liver with high systemic E_1:E_2 ratio. Transdermal E_2 administration may lead to systemic E_1:E_2 ratio resembling premenopausal condition. Refer to questions 12 – 18 below for transdermal absorption of E_2 from ESTROGEL®.

Distribution

The apparent volume of distribution is 9 - 15 L, despite E_2 is a highly lipophilic drug (*Dollery's Therapeutic Drugs* Vol. 2 Churchill Livingstone 1991 page 4). E_2 is 50.3% bound to sex hormone binding globulin and 47.7% to albumin (Langley et al. *JNCI* **75**:823 1985).

Metabolism

E_2 is converted to E_1 via 17-hydroxysteroid dehydrogenase and unconjugated E_1 can be reduced back to E_2 (*Dollery's Therapeutic Drugs* Vol. 2 Churchill Livingstone 1991 page 4). E_1 is a biologically active metabolite of E_2. E_2 and E_1 and their metabolites are conjugated in the liver to yield corresponding glucuronides and sulfates (*Dollery's Therapeutic Drugs* Vol. 2 Churchill Livingstone 1991 page 4). E_1-3-sulfate is the major circulating estrogen metabolite and is readily deconjugated back to E_1. E_2 and E_1 are further metabolized to 2-, 4-, and 16α-hydroxylated metabolites via cytochrome P450 3A4 and 1A2 (Yamazaki et al. *Chem. Res. Toxicol.* **11**:659 1998). The 2- and 4-hydroxylated E_2 are catechols and can be methylated via catechol-O-methyltransferase (Zhu and Conney *Carcinogenesis* **19**:1 1998). However, the 2- and 4- hydroxylated E_2 can also undergo metabolic redox cycling and form semiquinone to react as free radicals with DNA and cellular macromolecules and lead to estrogen-induced cancers (Zhu and Conney *Carcinogenesis* **19**:1 1998). E_2 and E_1 may be metabolized to the corresponding quinols, namely 10β,17β-dihydroxy-1,4-estradiene-3-one and 10β-dihydroxy-1,4-estradiene-3,17-one, respectively via CYP1A1, CYP2B6, and CYP2E1 (Ohe et al. *Drug Metab. Dispos.* **28**:110 2000). These quinols contains α,β-unsaturated ketones that are electron deficient and Michael reaction acceptors, which can bind covalently to DNA, RNA, and other cellular macromolecules and may lead to genotoxicity and cytotoxicity.

Elimination

Upon intravenous administration, E_2 has an initial half life of about 20 min, followed by a 2nd half life of about 70 min (*Dollery's Therapeutic Drugs* Vol. 2 Churchill Livingstone 1991 page 4). Its clearance is 600 - 800 L/24 h/m^2 in premenopausal women and 500 - 600 L/24 h/m^2 in postmenopausal women.

10. What is the drug-drug interaction potential for E_2 upon transdermal administration of ESTROGEL®?

The labeling of transdermal E_2 therapeutic systems such as ALORA™, CLIMARA®, FEMPATCH®, VIVELLE™, and ESTRASORB™ do not contain statements on the drug-drug interaction potentials of E_2. The potential for E_2 to interact with other drugs is minimal since the plasma E_2 concentrations achieved upon administration of different ESTROGEL® doses would be within the physiological range. However, the potential for other drugs to interact with E_2 upon ESTROGEL® administration is uncertain.

11. What are the formulations used in the clinical safety and efficacy studies for NDA 21-166 and what are the differences as compare to the to-be-marketed formulation?
E_2 (0.06% w/w) is formulated with ethanol, carbomer 934P, trolamine and purified water as inactive components. Ethanol solubilizes E_2 and also acts as vehicle with purified water, which is further gelled with carbomer 934P and neutralized with trolamine.

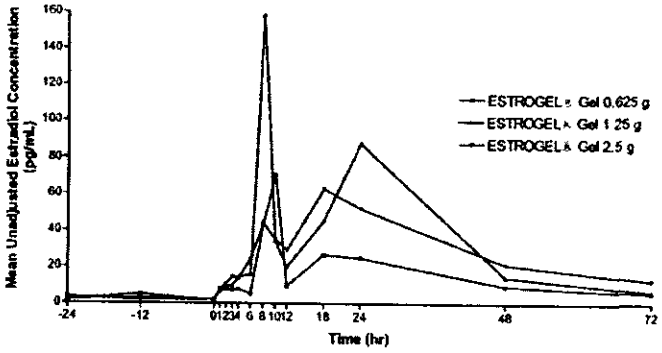
The Bristol-Meyers Squibb formulation contains [ ] w/w ethanol and was used in the 2 clinical safety and efficacy studies (CV141-001 and CV141-002) that support this NDA. The Besins Iscovesco formulation contains [ ] w/w ethanol; it is the proposed to-be-marketed formulation and was used in 5 clinical pharmacology studies (S1661002, S1661003, MKL2593, UMD-01-078, and UMD-00-073). The ethanol (excipient) difference between the 2 formulations [ ] is a Level 1 change per "Guidance for Industry, Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation" (Nonsterile Semisolid Dosage Forms Guidance). No in vivo bioequivalence (BE) documentation is recommended for Level 1 change. However, the sponsor did conduct Study 9566.01 to assess the in vitro E_2 release between the Bristol-Meyers Squibb ESTROGEL® formulation and the Besins Iscovesco ESTROGEL® formulation. See question 21 below for details.

The sponsor used glaminate tube to dispense the test formulation in pivotal Studies CV141-001 and CV141-002. However, the sponsor plans to market a pump to dispense the 0.06% ESTROGEL® formulation. The sponsor conducted an Estrogel® pump vs. Estrogel® glaminate tube comparability and consistency study. This study's data indicate that acceptance criteria and consistency for the mean dose delivered from the pump and glaminate tube were met. See chemist's (Dr. Rajiv Agarwal) review for details.

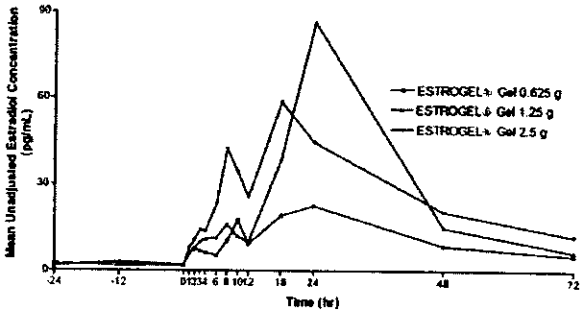
The sponsor also used a 0.03% formulation (1.25 g; 0.375 mg E_2) for the lowest dose treatment in study CV141-002. However, the sponsor only seeks approval for the 0.06% ESTROGEL® formulation.

12. Is the ESTROGEL® PK dose-proportional?
Study S1661002 examined the dose proportionality for E_2 and unconjugated E_1 PK parameters upon single dose administration of 1.25 g of 0.03% (0.375 mg E_2), 1.25 g of 0.06% (0.75 mg E_2), and 2.5 g of 0.06% (1.5 mg E_2) ESTROGEL® to 24 healthy postmenopausal women. This was a 3-period, open label, balanced, randomized, crossover study with 7-day washout between treatments. Each subject applied 1.25 g doses of 0.03% and 0.06% ESTROGEL® to the skin from shoulder to wrist of 1 arm. Each subject applied 2.5 g dose of 0.06% ESTROGEL® to the skin from shoulder to wrist of 2 arms. Prior to all applications, the sites for venipuncture were marked and covered with transparent dressings, which were removed after the gel dried. Serial blood samples were collected for the determination of plasma E_2 and unconjugated E_1 concentrations via [ ]

Mean Unadjusted Plasma Estradiol Concentration – Time Profile for the ESTROGEL® Gel 0.625, 1.25 and 2.5 g Treatment Groups



Mean Unadjusted Plasma Estradiol Concentration - Time Profiles Excluding Subject No. 60019 for the ESTROGEL® Gel 0.625, 1.25 and 2.5 g Treatment Groups

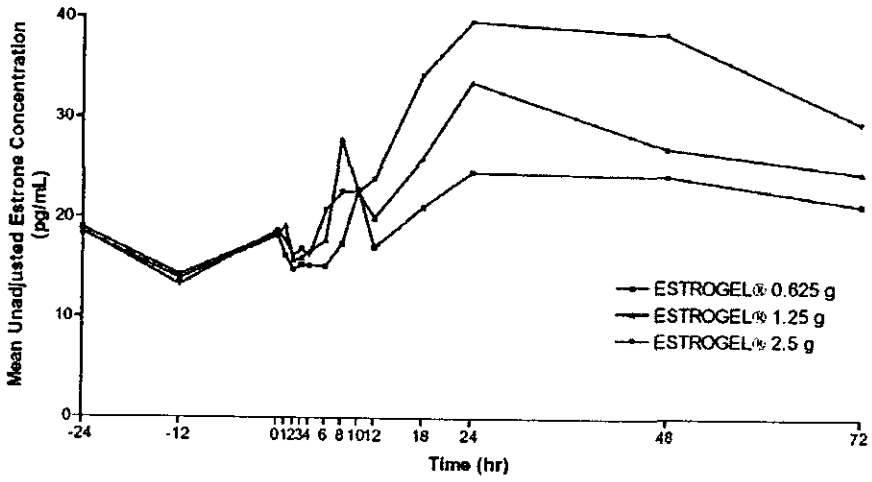


Unadjusted Plasma E_2 PK Parameters After A Single Dose Application of ESTROGEL® Gel

ESTROGEL® Gel Treatment Group	Descriptive Statistics	AUC(0-72) (pg•h/mL)	AUC(0-∞) (pg•h/mL)	Cmax (pg/mL)	Tmax (hr)	Kel (1/h)	T1/2 (hr)
0.625 g[a] (0.375 mg estradiol)	Arithmetic Mean	1133.8	1887.5	86.59	16.7	0.022	44.7
	Geometric Mean	**720.2**	**1477.7**	**30.34**	**11.8**	**0.018**	**38.2**
	SD	1512.5	1893.4	251.50	10.9	0.014	25.8
	CV%	133.4	100.3	290.5	65.2	66.1	57.7
	N	23	13	23	23	13	13
1.25 g (0.75 mg estradiol)	Arithmetic Mean	2482.2	3140.2	248.88	18.3	0.026	64.4
	Geometric Mean	**998.7**	**1812.9**	**41.57**	**15.3**	**0.019**	**36.5**
	SD	5245.7	5502.0	728.26	9.0	0.018	105.3
	CV%	211.3	175.2	292.6	49.4	69.1	163.7
	N	23	17	23	23	17	17
2.5 g (1.5 mg estradiol)	Arithmetic Mean	2171.2	2650.3	95.32	19.1	0.031	26.3
	Geometric Mean	**1665.4**	**2130.2**	**60.93**	**16.1**	**0.029**	**24.1**
	SD	1882.9	2131.3	119.5	10.9	0.012	11.9
	CV%	86.7	80.4	125.4	57.0	39.5	45.3
	N	23	19	23	23	19	19

[a] The 0.625 g described is equivalent to 1.25 g of 0.03% gel administered.

Mean Unadjusted Plasma Free Estrone Concentration – Time Profiles for the ESTROGEL® Gel 0.625, 1.25 and 2.5 g Treatment Groups



Unadjusted Plasma E_1 PK Parameters after a Single Dose Application of ESTROGEL® Gel

ESTROGEL® Gel Treatment Group	Descriptive Statistics	AUC(0-72) (pg•h/mL)	AUC(0-∞) (pg•h/mL)	Cmax (pg/mL)	Tmax (hr)	Kel (1/hr)	T1/2 (hr)
0.625 g[a] (0.375 mg estradiol)	Arithmetic Mean	1582.2	3999.5	31.16	33.0	0.010	92.1
	Geometric Mean	**1472.7**	**3677.4**	**27.36**	**24.5**	**0.009**	**79.5**
	SD	553.8	1654.4	23.50	20.7	0.004	64.4
	CV%	35.0	41.4	75.4	62.8	40.9	69.9
	N	23	6	23	23	6	6
1.25 g (0.75 mg estradiol)	Arithmetic Mean	1891.6	4225.4	45.98	35.1	0.015	67.5
	Geometric Mean	**1793.2**	**3799.3**	**35.91**	**27.9**	**0.013**	**55.4**
	SD	622.4	1895.5	50.30	18.5	0.009	46.2
	CV%	32.9	44.9	109.4	52.7	57.7	68.5
	N	23	9	23	23	9	9
2.5 g (1.5 mg estradiol)	Arithmetic Mean	2369.7	6396.6	44.60	30.2	0.015	83.3
	Geometric Mean	**2279.4**	**5227.5**	**42.53**	**27.5**	**0.012**	**56.35**
	SD	641.1	5446.3	12.90	13.2	0.009	115.6
	CV%	27.1	85.1	28.9	43.7	58.1	138.7
	N	23	15	23	23	15	15

[a] The 0.625 g described is equivalent to 1.25 g of 0.03% gel administered.

Per discussion with Dr. He Sun (pharmacometrician), the sponsor's analysis of (log PK parameter)/dose vs. dose and log PK parameter vs. dose are not appropriate approaches to determine dose proportionality and dose linearity. The log PK parameter vs. log dose and PK parameter vs. dose are appropriate approaches to assess dose proportionality and dose linearity instead. Hence, log geometric mean AUC vs. log dose and geometric mean AUC vs. dose, log geometric mean C_{max} vs. log dose, and geometric mean C_{max} vs. dose were analyzed. The regression results indicated that the PK parameters were not dose proportional among the 3 doses studied (all analyzes have an intercept). The geometric mean AUC and C_{max} for unadjusted E_2 were linearly related to the 3 doses administered in this study. The geometric mean AUC for adjusted E_2 was not linearly related to the 3 doses administered in this study. However, the geometric mean C_{max} for adjusted E_2 was linearly related to the 3 doses administered in this study.

Study CV141-001 was a double-blind, randomized, placebo-controlled, multi-center safety and efficacy study. 221 menopausal women received either placebo (2 arms), 1.25 g of 0.06% ESTROGEL® (1 arm and placebo gel on another arm), or 2.5 g of 0.06% ESTROGEL® (2 arms) daily for 3 months. ESTROGEL® or the matching placebo gel was applied to the skin in an area approximately equal to 750 cm^2, which corresponded to the area of the entire arm extending from the shoulder to the wrist. Serum E_2 and E_1 concentrations were measured at baseline and Week 12 via RIA.

Serum E_2 and E_1 Concentrations for Study CV141-001

Treatment Group		Estradiol (pg/mL)		Estrone (pg/mL)	
		Baseline	Week 12	Baseline	Week 12
Placebo	Arithmetic Mean	4.8	14.4	24.5	32.5
	Median	5.0	5.0	24.0	23.0
	SD	5.09	31.93	8.63	32.90
	Min	(			)
	Max				
	N	73	67	73	67
ESTROGEL® Gel 1.25 g (0.75 mg estradiol)	Arithmetic Mean	4.0	106.0	25.8	58.5
	Median	0.0	33.5	26.0	49.0
	SD	6.32	243.74	10.91	40.98
	Min	(			)
	Max				
	N	74	66	73	66
ESTROGEL® Gel 2.5 g (1.5 mg estradiol)	Arithmetic Mean	5.5	123.2	25.9	75.0
	Median	5.0	65.0	25.0	58.0
	SD	4.77	151.10	10.37	58.98
	Min	(			)
	Max				
	N	71	67	71	67

Mean unadjusted serum E_2 and E_1 concentrations upon active treatments are not interpretable due to the extensive variability and outliers, especially in the E_2 results. However, median serum E_2 concentrations appear to be linear to dose in the study. The median serum E_1 concentrations also increased with dose but were not linear.

The median serum E_2 concentration of 65.0 pg/mL for the 2.5 g ESTROGEL® dose is consistent with the 66.1 pg/mL average plasma E_2 concentration observed for the 2.5 g ESTROGEL® dose in the multiple dose PK study MKL2593 (question 15 below).

Study CV141-002 was a randomized, double-blind (ESTROGEL® treatments), active-control, multi-center safety and efficacy study. 361 menopausal women each received daily for 3 months either 1.25 g of 0.03% (expressed as 0.625 g of 0.06% in the following table) ESTROGEL® (on 1 arm and placebo gel on another arm), 1.25 g of 0.06% ESTROGEL® (on 1 arm and placebo gel on another arm), 2.5 g of 0.06% ESTROGEL® (on 2 arms), or 12.5 cm^2 CLIMARA® transdermal patch (0.05 mg E_2/day every 7 days for 3 months). ESTROGEL® or the matching placebo gel was applied to the skin in an area approximately equal to 750 cm^2, which corresponded to the area of the entire arm extending from the shoulder to the wrist. The open label transdermal patch was to be applied on a clean dry area of the abdomen, avoiding the waistline area. The patch application sites were to be rotated with an interval of at least 1 week between particular sites. Serum E_2 and E_1 concentrations were measured at baseline, Week 4, Week 8, and Week 12 via RIA.

Median serum E_2 and E_1 concentrations were relatively stable at Weeks 4, 8 and 12 for all treatments. Serum E_2 and E_1 concentrations increased with dose and were nearly linear (median) at Week 12 for the 1.25 g and 2.5 g doses, as observed in Study CV141-001.

Serum E_2 and E_1 Concentrations for Study CV 141-002

Treatment Group		Estradiol (pg/mL)				Estrone (pg/mL)			
		Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12
ESTROGEL® Gel 0.625 g (0.375 mg estradiol)	Arithmetic Mean	5.2	93.8	96.1	93.9	26.3	46.6	46.6	46.0
	Median	5.5	23.5	29.5	25.0	26.0	38.0	37.0	39.0
	SD	6.49	254.55	215.28	203.64	8.97	31.50	34.80	30.52
	Min	[							]
	Max								
	N	92	86	80	77	92	86	80	77
ESTROGEL® Gel 1.25 g (0.75 mg estradiol)	Arithmetic Mean	4.2	72.5	71.4	67.1	24.2	50.9	50.1	51.6
	Median	0.0	42.0	31.0	32.0	24.0	45.5	44.0	41.0
	SD	5.31	81.51	125.86	87.07	9.72	28.18	33.63	35.90
	Min	[							]
	Max								
	N	93	87	81	81	93	88	81	81
ESTROGEL® Gel 2.5 g (1.5 mg estradiol)	Arithmetic Mean	4.1	174.2	161.0	180.7	23.1	78.8	78.9	77.4
	Median	5.0	70.0	59.5	60.0	22.0	60.0	52.5	62.5
	SD	4.23	314.68	501.52	532.25	9.56	67.17	83.50	80.10
	Min	[							]
	Max								
	N	89	80	74	76	89	80	74	76
CLIMARA Patch (50 µg/day estradiol)	Arithmetic Mean	4.1	37.5	44.4	45.8	24.6	38.9	44.1	43.7
	Median	0.0	31.0	39.0	38.5	23.5	34.0	37.0	40.0
	SD	4.93	29.70	28.76	39.93	9.81	21.57	31.66	27.06
	Min	[							]
	Max								
	N	86	86	82	82	86	86	82	82

Median serum E_2 concentrations for ESTROGEL® 1.25 g and 2.5 g treatments at Week 12 were comparable between studies (CV141-001: 33.5 pg/mL and 65.0 pg/mL, respectively; CV141-002: 32.0 pg/mL and 60.0 pg/mL, respectively). Median serum E_2 concentrations for the CLIMARA® treatment ranged from 31.0 to 39.0 pg/mL and were similar to the median serum E_2 concentrations (range from 31.0 to 42.0 pg/mL) for 1.25 g ESTROGEL® at Weeks 4, 8, and 12. Based on median serum E_2 and E_1 concentrations, the 1.25 g ESTROGEL® and CLIMARA® treatments were similar at Week 12 (E_2: 32.0 pg/mL vs. 38.5 pg/mL, respectively; E_1: 41.0 pg/mL vs. 40.0 pg/mL, respectively).

Note the high variability (100-300%), which may be due to:

- The area that was applied to the arm (inner and/or outer side of the arm).
- The surface area of application.
- Contamination of sampling sites from dose application.

More recent studies did not seem to have such high variability.

13. What is the ESTROGEL® PK upon multiple administrations?

Per Study MKL2593 (Question 15) below, it appears that steady state serum E_2 concentrations were reached after 3 days of daily 2.5 g of 0.06% ESTROGEL® topical application to the 2 arms (forearm and shoulder) of subjects.

Per Study UMD-01-078 (Question 16) below, the estimated mean accumulation index for the unadjusted plasma E_2 AUC_{0-24} was 3.33 (678.74/203.98) and the estimated mean accumulation index for the unadjusted plasma E_2 C_{max} was 3.07 (46.38/15.1) upon 14 days of daily topical application of 1.25 g of 0.06% ESTROGEL® to 1 arm of the subjects, whose gel application site were not washed. In the same study for the "washed" subjects, the estimated mean accumulation index for the unadjusted plasma E_2 AUC_{0-24} was 3.38 (522.6/154.6) and the estimated mean accumulation index for the unadjusted plasma E_2 C_{max} was 3.36 (34.96/10.4) upon 14 days of daily topical application of 1.25 g of 0.06% ESTROGEL® to 1 arm per subject. The mean accumulation index for the unadjusted plasma E_2 AUC was 3.19 and the accumulation index for the unadjusted plasma E_2 C_{max} was 2.19 (see the 1st

table below for this question) upon 14 days of daily topical application of 0.625 g of 0.06% ESTROGEL® to 1 arm of the subjects.

Study S1661003 assessed the multiple dose PK upon daily administration of 1.25 g 0.03% (0.375 mg) or 0.625 g 0.06% (0.375 mg) ESTROGEL® for 14 days. This open-label, balanced, randomized, 2-period, crossover study (Study Number S1661003) in 48 healthy, hypoestrogenic postmenopausal women was conducted to evaluate the bioequivalence of E_2, unconjugated E_1 and total E_1 after multiple dose administration of 2 ESTROGEL® formulations, 0.03% and 0.06%. The study was conducted to confirm the equivalence of both gel strengths used in the clinical efficacy program. Each subject received 2 treatments, either 0.03% ESTROGEL® 1.25 g or 0.06% ESTROGEL® Gel 0.625 g.

The gel was percutaneously applied to the surface of the same arm once daily on Day 1, and Days 3 to 15 of each treatment period. A 14-day washout separated each treatment period. Baseline blood samples were collected at -24, -12, 0 hours prior to ESTROGEL® applications on Day 1. In addition, blood samples were collected at regular intervals for 48 hours after ESTROGEL® application on Day 1, just prior to ESTROGEL® application on Days 13, 14 and 15, and for 24 hours after ESTROGEL® application on Day 15 of each treatment period. Plasma samples were obtained from the arm that was not used for ESTROGEL® applications, minimizing the possibility of needle contamination with E_2. Plasma E_2, unconjugated E_1 and total E_1 concentrations were determined via [illegible]

Steady-State PK Parameters for Unadjusted Plasma E_2

Treatment Group	Descriptive Statistic	AUC(0-24sd) (pg•h/mL)	Cmax(sd) (pg/mL)	AUC(0-24ss) (pg•h/mL)	Cmax(ss) (pg/mL)	Cave (pg/mL)	AI AUC	AI Cmax
0.03% ESTROGEL® Gel (0.375 mg estradiol)	Arithmetic Mean	184.3	14.77	513.1	32.06	21.37	4.02	2.64
	SD	108.5	9.35	267.6	18.53	11.17	5.57	1.42
	CV%	58.8	63.3	52.2	57.8	52.3	138.7	53.6
	Geometric Mean	**160.4**	**12.69**	**440.1**	**27.44**	**18.28**	**2.97**	**2.24**
	N	45	44	45	45	45	43	44
0.06% ESTROGEL® Gel (0.375 mg estradiol)	Arithmetic Mean	187 9	16.25	458.1	27.82	19.08	3.19	2.19
	SD	104.4	10.96	194.7	11.96	8.13	3.36	1.01
	CV%	55.6	67.4	42.5	43.0	42.6	105.4	46.3
	Geometric Mean	**170.6**	**13.67**	**406.4**	**25.17**	**16.76**	**2.58**	**1.92**
	N	45	43	45	45	45	43	43

Steady-State PK Parameters for Unadjusted Plasma E_1

Treatment Group	Descriptive Statistic	AUC(0-24sd) (pg•h/mL)	Cmax(sd) (pg/mL)	AUC(0-24ss) (pg•h/mL)	Cmax(ss) (pg/mL)	Cave (pg/mL)	AI AUC	AI Cmax
0.03% ESTROGEL® Gel (0.375 mg estradiol)	Arithmetic Mean	561.6	34.95	1,284.7	65.81	53.53	2.88	1.93
	SD	237.8	13.58	634.3	31.92	26.43	3.81	0.71
	CV%	42.3	38.8	49.4	48.5	49.4	132.0	36.6
	Geometric Mean	**515.0**	**32.78**	**1,133.4**	**58.80**	**47.22**	**2.30**	**1.79**
	N	45	45	45	45	45	44	45
0.06% ESTROGEL® Gel (0.375 mg estradiol)	Arithmetic Mean	570.8	36.47	1,193.6	63.54	49.71	2.23	1.91
	SD	213.7	13.69	517.2	25.69	21.59	0.75	0.73
	CV%	37.4	37.5	43.3	40.4	43.4	33.6	38.1
	Geometric Mean	**507.1**	**34.23**	**1,066.6**	**58.84**	**44.00**	**2.11**	**1.76**
	N	45	44	45	45	45	42	44

Steady-State PK Parameters for Unadjusted Plasma Total E_1

Treatment Group	Descriptive Statistics	AUC(0-24sd) (pg•h/mL)	Cmax(sd) (pg/mL)	AUC(0-24ss) (pg•h/mL)	Cmax(ss) (pg/mL)	Cave (pg/mL)	AI AUC	AI Cmax
0.03% ESTROGEL® Gel (0.375 mg estradiol)	Arithmetic Mean	6,608.6	469.03	18,731.4	1,049.71	780.48	3.54	2.21
	SD	4,223.3	355.76	15,894.6	958.07	662.27	5.17	0.93
	CV%	63.9	75.9	84.9	91.3	84.9	146.1	41.8
	Geometric Mean	**5,256.8**	**385.31**	**14,007.1**	**759.96**	**583.63**	**2.66**	**1.97**
	N	45	45	45	45	45	45	45
0.06% ESTROGEL® Gel (0.375 mg estradiol)	Arithmetic Mean	6,371.9	422.61	15,948.8	864.42	664.53	2.71	2.10
	SD	4,583.9	275.45	11,286.6	617.53	470.28	1.11	0.80
	CV%	71.9	65.2	70.8	71.4	70.8	40.8	37.9
	Geometric Mean	**5,043.9**	**360.57**	**12,733.4**	**706.99**	**530.56**	**2.52**	**1.96**
	N	45	45	45	45	45	45	45

For bioequivalence assessment, 0.03% formulation was the reference and 0.06% formulation was the test treatment. Ratios of least-squares means with 90% confidence intervals for $AUC_{(0\text{-}24,ss)}$ and $C_{max(ss)}$ at steady-state are in Table 6.5.2.1.4.

Ratios of Least-Squares Means with 90% Confidence Intervals for AUC and Cmax Parameters

Data Set	AUC(0-24ss)	Cmax(ss)
Unadjusted Plasma Estradiol	92.0% (82.1-103.1%)	91.4% (82.3-101.6%)
Unadjusted Plasma Estrone	93.9% (86.7-101.7%)	99.9% (92.9-107.4%)
Unadjusted Plasma Total Estrone	90.5% (80.8-101.4%)	92.7% (83.9-102.4%)
Adjusted Plasma Estradiol	79.1% (68.0-92.0%)	81.8% (72.1-92.9%)
Adjusted Plasma Estrone	84.3% (73.1-97.1%)	90.7% (80.5-102.1%)
Adjusted Plasma Total Estrone	75.1% (63.9-88.2%)	79.9% (70.2-91.0%)

For the 0.06% and 0.03% ESTROGEL® treatments, the 90% confidence intervals of the least-squares means ratio for AUC and C_{max} derived from unadjusted E_2, unconjugated E_1, and total E_1 concentrations at steady-state, were within the 80% to 125% range. However, the 90% confidence intervals for all adjusted E_2, unconjugated E_1 and total E_1 concentrations PK parameters at steady-state were not within the 80% to 125% range, except the adjusted E_1 $C_{max(ss)}$. At steady-state, the unadjusted average plasma E_2 concentrations for both treatments ranged from 19.08 to 21.37 pg/mL, which is below the average serum E_2 concentration of 27 pg/mL upon administration of 1 FEMPATCH® (0.025 mg E_2/day) 7-day (PDR 1998 ed. p2100). The large intersubject variability in plasma E_2 concentrations observed in the single dose proportionality study S1661002 was not observed in the single dose or multiple dose sampling intervals in study S1661003. Sampling from the arm not used for gel application may reduce the variability as observed study S1661002.

14. Is the 0.03% ESTROGEL® bioequivalent to the 0.06% ESTROGEL® per equal E_2 amount?

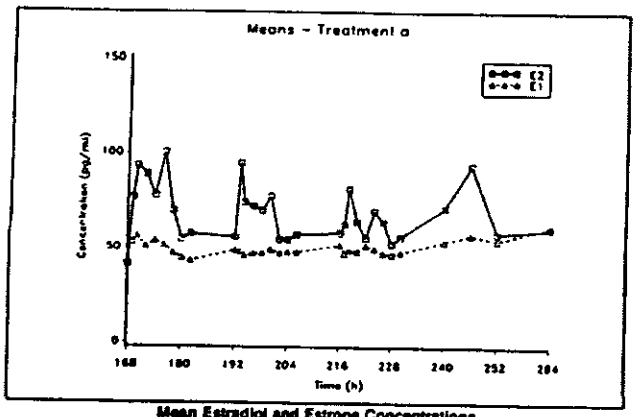
Study S1661003 above (Question 13) was originally conducted to determine the multiple dose PK of 1.25 g of 0.03% (0.375 mg E_2) and 0.625 g of 0.06% (0.375 mg E_2) ESTROGEL®. Per OCPB NDA 21-166 filing request (originally requested in End of Phase II meeting), the sponsor provided the single dose bioequivalence assessment for 1.25 g of 0.03% and 0.625 g of 0.06% ESTROGEL® in the March 29, 2000 NDA 21-166 B2 amendment. 0.03% formulation was the reference and 0.06% formulation was the test treatment. The 90% confidence intervals of the least-squares means ratio (test over reference) for all $AUC_{0\text{-}48}$ and C_{max} derived from unadjusted plasma E_2, unconjugated E_1, and total E_1 concentrations were within the 80% to 125% range. However, the 90% confidence intervals of the least-squares means ratio (test over reference) for all adjusted plasma E_2, unconjugated E_1 and total E_1

AUC_{0-48}, $AUC_{0-\infty}$, and C_{max} were not within the 80% to 125% range. These observations are consistent with the bioequivalence assessment via the steady state approach in question 13 above. This lack of BE between the 2 formulations is not an issue for NDA 21-166, since the sponsor only seeks approval for the 0.06% ESTROGEL®.

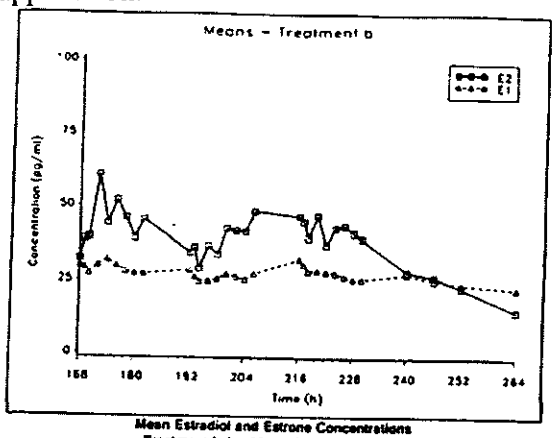
15. What is the relative PK of ESTROGEL® versus E_2 transdermal therapeutic system?
Study MKL2593 was an open-label, randomized, 2-way crossover, multiple dose study in 24 healthy postmenopausal women to assess the relative PK of ESTROGEL® to ESTRADERM®. A > 24-day washout separated the treatments. The E_2 gel used in Study MKL2593 was the to-be-marketed formulation (per NDA 21-166 N 000 BZ, June 16, 2003).

Treatment A: 2.5 g of 0.06% ESTROGEL® (1.5 mg E_2) was applied to the 2 arms (forearm and shoulder) of each subject once daily for 11 consecutive days. Treatment B: an ESTRADERM® (0.05 mg E_2/day) patch was placed at the dorsal region of the hip of each subject on Day 1 and a replacement patch was placed on Day 5 and Day 8. On Day 12 the ESTRADERM® patch was removed from each subject. Serial blood samples were collected daily, especially frequent sampling from Day 8 to Day 11 to determine serum E_2 and E_1 concentrations via [redacted] However, the sponsor's bioanalytical report for study MKL12593 was validated for plasma samples. 1 subject had a relatively strong skin reaction to ESTROGEL®, which resulted in erratic concentration time profiles (per discussion with Dr. Phill Price this kind of skin reaction is not uncommon and may be due to the alcohol in the gel). The data from this subject was excluded from the PK evaluations and statistical analyses. The mean PK parameters for plasma unadjusted E_2 and E_1 are in the tables below.

Upon the gel application, mean trough serum E_2 concentrations were 41.7 pg/mL at 72 h, 41.4 pg/mL at 96 h, 40.8 pg/mL at 120 h, 37.2 pg/mL at 144 h, 41.2 pg/mL at 168 h and did not show increasing trend. Per these observations, it appears that steady state serum E_2 concentrations were reached after 72 h of daily 2.5 g of 0.06% ESTROGEL® topical application.



Mean Estradiol and Estrone Concentrations
Treatment a (n=23, subject 23 excluded)



Mean Estradiol and Estrone Concentrations
Treatment b (n=23, subject 23 excluded)

Unadjusted PK Parameters (mean ± SD) for E_2 and E_1 After Multiple Doses of ESTROGEL® Gel and ESTRADERM® Patch Treatments

PK Parameters	Treatments	
(Unadjusted)	ESTROGEL® Gel 2.5 g (1.5 mg estradiol)	ESTRADERM® Patch (50 µg/day estradiol)
AUCγ8 (pg/mL*h)	1644 ± 808	1036 ± 373
AUCγ9 (pg/mL*h)	1527 ± 864	989 ± 381
AUCγ10 (pg/mL*h)	1521 ± 612	944 ± 282
AUCγ11 (pg/mL*h)	1657 ± 1123	533 ± 318
AUCγ8-11 (pg/mL*h)	6348 ± 2979	3502 ± 1186
Cavγ8-11 (pg/mL)	66.1 ± 31.0	36.6 ± 12.3
Cmax (pg/mL)	209.2 ± 127.9	74.9 ± 27.2
Cmin(pg/mL)	23.6 ± 9.7	14.6 ± 10.0
PTF	2.881± 1.908	1.692 ± 0.640
Tmax (h)	36.96 ± 29.41	27.13 ± 24.36

AUCγ8 : AUC for Day 8 using unadjusted serum estradiol concentrations
AUCγ9 : AUC for Day 9 using unadjusted serum estradiol concentrations
AUCγ10 : AUC for Day 10 using unadjusted serum estradiol concentrations
AUCγ11 : AUC for Day 11 using unadjusted serum estradiol concentrations
AUCγ8-11 : Total AUC for Days 8 to 11 using unadjusted serum estradiol concentrations
Cavγ8-11 : Mean average serum estradiol concentration for Days 8 to 11
Cmax : Mean maximal serum estradiol concentration for Days 8 to 11
Cmin : Mean minimum serum estradiol concentration for Days 8 to 11
PTF : The peak trough fluctuation for Days 8 to 11, calculated as (Cmax-Cmin)/(Cavγ8-11)

PK Parameters	Treatments	
(Unadjusted)	ESTROGEL® Gel 2.5 g (1.5 mg estradiol)	ESTRADERM® Patch (50 µg/day estradiol)
AUCγ8 (pg/mL*h)	1161 ± 508	682 ± 363
AUCγ9 (pg/mL*h)	1165 ± 469	650 ± 327
AUCγ10 (pg/mL*h)	1187 ± 463	665 ± 320
AUCγ11 (pg/mL*h)	1366 ± 579	581 ± 324
AUCγ8-11 (pg/mL*h)	4879 ± 1919	2578 ± 1296
Cavγ8-11 (pg/mL)	50.8 ± 20.0	26.9 ± 13.5
Cmax (pg/mL)	74.1 ± 24.0	38.1 ± 17.1
Cmin(pg/mL)	35.1 ± 17.1	17.8 ± 9.6
PTF	0.848 ± 0.400	0.797 ± 0.258
Tmax (h)	54.22 ± 36.84	38.47 ± 27.79

AUCγ8 : AUC for Day 8 using unadjusted serum estrone concentrations
AUCγ9 : AUC for Day 9 using unadjusted serum estrone concentrations
AUCγ10 : AUC for Day 10 using unadjusted serum estrone concentrations
AUCγ11 : AUC for Day 11 using unadjusted serum estrone concentrations
AUCγ8-11 : Total AUC for Days 8 to 11 using unadjusted serum estrone concentrations
Cavγ8-11 : Mean average serum estrone concentration for Days 8 to 11
Cmax : Mean maximal serum estrone concentration for Days 8 to 11
Cmin : Mean minimum serum estrone concentration for Days 8 to 11
PTF : The peak trough fluctuation for Days 8 to 11, calculated as (Cmax - Cmin)/(Cavγ8-11)

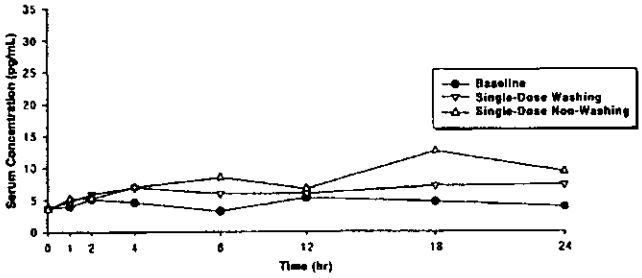
The intrasubject coefficients of variation for unadjusted E_2 AUC and C_{max} range from 30% to 56%. Mean average unadjusted serum E_2 concentration on Days 8 to 11 was 66.1 pg/mL for the 2.5 g ESTROGEL® treatment and 36.6 pg/mL for the ESTRADERM® treatment. Mean minimum serum E_2 concentration on Days 8 to 11 was 23.6 pg/mL for ESTROGEL® treatment. Mean average and minimum unadjusted serum E_1 concentrations on Days 8 to 11 were 50.8 pg/mL and 35.1 pg/mL, respectively for the 2.5 g ESTROGEL® treatment. Hence, E_2/E_1 ratio of approximately 1 was maintained for Days 8 to 11. This study used the point estimate of the ratio E_2 $AUC_{D8\text{-}D11}$ for ESTROGEL® versus that for ESTRADERM®, 1.925, to estimate the E_2 delivery rate for 2.5 g of ESTROGEL® as 96.25 µg/day (1.925 x 50 µg/day). This estimation does not consider the BA and E_2 dose difference between ESTROGEL® and ESTRADERM®. Hence, this is a rough estimated relative E_2 delivery rate for ESTROGEL®, which is not recommended to be included in the labeling.

16. Will E_2 be transferred from an ESTROGEL®-dosed woman to others upon skin contact? What is the effect of washing the application site on the transfer potential?

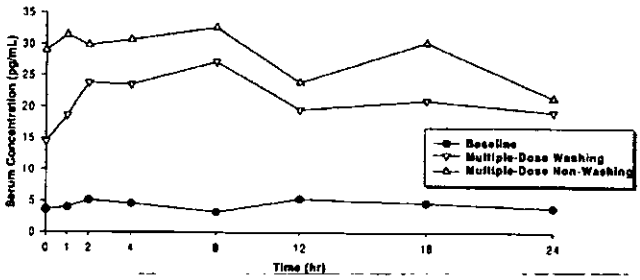
Study UMD-01-078 examined the transfer potential of ESTROGEL® and the effect of washing the application site on the systemic E_2 exposure in this open-label, 2-period, crossover, and multiple-dose study. Forty eight randomized healthy postmenopausal women participated in either Group A or Group B (sequence of washing or not washing the application site prior to contact) and participated as either dosed (gel application) subjects or naïve (secondary exposure) subjects. In Group A, 12 subjects applied 1.25 g of 0.06% E_2 gel on the posterior surface of 1 arm from wrist to shoulder daily for 14 days. Another 12 subjects rubbed against the application site of the dosed subjects daily for 14 days. The 15 minutes skin-to-skin contact between the dosed and naive subjects was 1 hour postdose. The dosed subject and naïve subject rubbed their posterior forearms for 1 minute, posterior upper arms for 1 minute, and posterior forearms again for 1 minute with side-to-side and up-and-down motion. The treated and contact arms of the dosed and naive subjects were gently bound together for 12 more minutes. After a washout of 14 days, the Group A subjects repeated the previous procedures except that the gel application site was washed 1 hour postdose. In Group B, another 24 subjects participated in the same procedures as Group A except that the 12 dosed subjects washed the application site in the 1st period and did not wash the application site in the 2nd period. Serial 24-hour serum samples were

collected from all subjects on Days 1, 14, 29, and 42 for the determination of E_2, E_1 and E_1 sulfate concentrations. Prior to venipuncture, the sampling sites were washed with soap and water and dried thoroughly. Alcohol wipes were not used to cleanse the sampling sites. The phlebotomist put on fresh gloves before collecting blood samples from subjects.

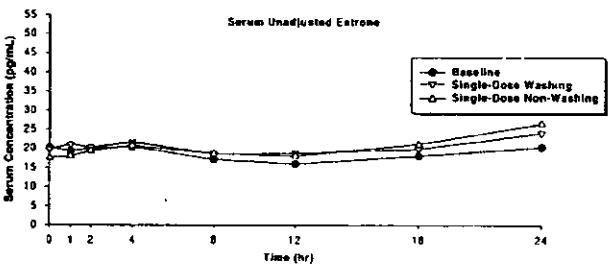
Mean Concentration-Time Profiles for Unadjusted Estradiol in Dosed Subjects After a Single Gel Application

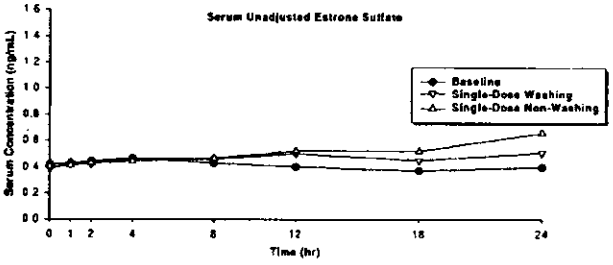


Mean Concentration-Time Profiles for Unadjusted Estradiol in Dosed Subjects After Multiple Gel Applications for 14 Days

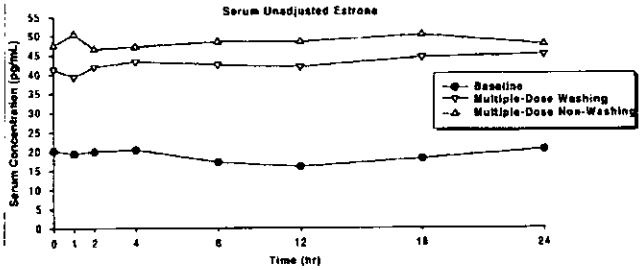


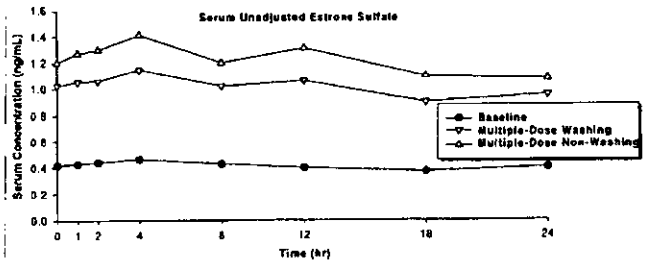
Mean Concentration-Time Profiles for Unadjusted Estrone and Estrone Sulfate in Dosed Subjects After a Single Gel Application





Mean Concentration-Time Profiles for Unadjusted Estrone and Estrone Sulfate in Dosed Subjects After Multiple Gel Applications for 14 Days





Pharmacokinetic Results After Single-Dose Administration by Washing Status: Dosed Subjects

Parameters	Unadjusted (N=24)		Baseline Adjusted (N=24)	
	Washed	Not Washed	Washed	Not Washed
Estradiol				
AUC_{0-24} (pg•hr/mL)				
Mean±SD	154.60±62.81	203.98±121.79	48.32±59.47	97.7±112.27
Median	159.75	158.75	44.63	55.00
Range (min-max)	[			]
C_{avg} (pg/mL)				
Mean±SD	6.44±2.62	8.50±5.07	2.01±2.48	4.07±4.68
Median	6.66	6.61	1.86	2.29
Range (min-max)	[			]
C_{max} (pg/mL)				
Mean±SD	10.40±4.85	15.10±12.54	7.00±4.73	11.42±12.39
Median	10.50	10.00	6.75	7.50
Range (min-max)	[			]
Estrone				
AUC_{0-24} (pg•hr/mL)[a]				
Mean±SD	484.63±171.56	490.02±143.42	45.54±137.33	50.83±100.10
Median	522.00	477.00	20.50	68.25
Range (min-max)	[			]
C_{avg} (pg/mL)				
Mean±SD	20.19±7.15	20.42±5.98	1.90±5.72	2.11±4.17
Median	21.75	19.88	0.85	2.84
Range (min-max)	[			]

Pharmacokinetic Results After Single-Dose Administration by Washing Status: Dosed Subjects

Parameters	Unadjusted (N=24)		Baseline Adjusted (N=24)	
	Washed	Not Washed	Washed	Not Washed
C_{max} (pg/mL)				
Mean±SD	29.58±8.59	30.13±7.56	12.71±6.95	12.71±7.18
Median	31.00	30.00	12.50	12.00
Range (min-max)	[			]
Estrone sulfate				
AUC_{0-24} (ng•hr/mL)				
Mean±SD	11.18±3.89	12.13±4.44	1.36±1.84	2.30±2.44
Median	10.53	10.59	1.68	1.77
Range (min-max)	[			]
C_{avg} (ng/mL)				
Mean±SD	0.47±0.18	0.51±0.19	0.06±0.08	0.10±0.10
Median	0.44	0.44	0.07	0.07
Range (min-max)	[			]
C_{max} (ng/mL)				
Mean±SD	0.56±0.20	0.70±0.38	0.18±0.09	0.31±0.30
Median	0.48	0.60	0.18	0.24
Range (min-max)	[			]

[a] N=23 for the washed group. Value not calculated for Subject 00106 - missing serum concentration at 24 hr after dosing.

Pharmacokinetic Results After Multiple-Dose Administration by Washing Status: Dosed Subjects

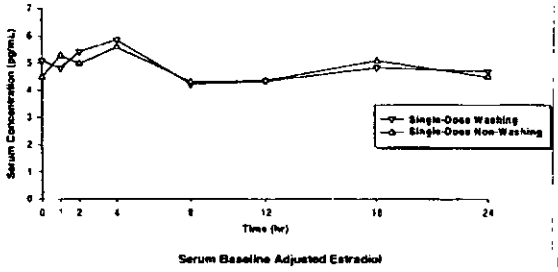
Parameters	Unadjusted (N=24)		Baseline Adjusted (N=24)	
	Washed	Not Washed	Washed	Not Washed
Estradiol				
AUC_{0-24} (pg•hr/mL)				
Mean±SD	522.60±276.42	678.74±351.37	416.32±277.74	572.46±349.04
Median	469.38	608.00	337.75	527.00
Range (min-max)	[			]
C_{avg} (pg/mL)				
Mean±SD	21.78±11.52	28.28±14.64	17.35±11.57	23.85±14.54
Median	19.56	25.33	14.07	21.96
Range (min-max)	[			]
C_{max} (pg/mL)				
Mean±SD	34.96±21.03	46.38±26.98	30.92±21.06	42.83±26.95
Median	26.00	41.50	24.00	39.00
Range (min-max)	[			]
Estrone				
AUC_{0-24} (pg•hr/mL)				
Mean±SD	1031.10±333.75	1165.29±471.45	591.71±325.35	725.90±446.09
Median	993.25	1142.25	537.50	648.00
Range (min-max)	[			]

Pharmacokinetic Results After Multiple-Dose Administration by Washing Status: Dosed Subjects

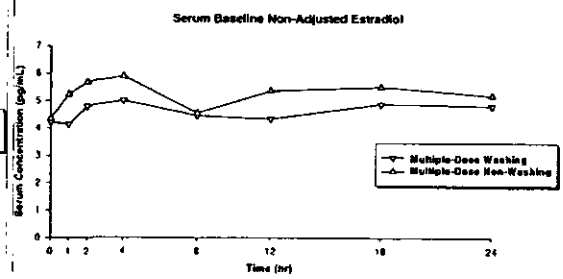
Parameters	Unadjusted (N=24)		Baseline Adjusted (N=24)	
	Washed	Not Washed	Washed	Not Washed
C_{avg} (pg/mL)				
Mean±SD	42.96±13.91	48.56±19.64	24.65±13.56	30.25±18.59
Median	41.39	47.59	22.40	27.00
Range (min-max)	[			]
C_{max} (pg/mL)				
Mean±SD	55.58±18.67	64.21±28.44	36.79±16.39	47.85±25.59
Median	52.00	59.00	36.00	40.50
Range (min-max)	[			]
Estrone sulfate				
AUC_{0-24} (ng•hr/mL)				
Mean±SD	24.25±11.01	29.18±18.00	14.42±8.86	19.35±16.51
Median	21.19	24.18	12.20	14.96
Range (min-max)	[			]
C_{avg} (ng/mL)				
Mean±SD	1.01±0.46	1.22±0.75	0.60±0.37	0.81±0.69
Median	0.88	1.01	0.51	0.62
Range (min-max)	[			]
C_{max} (ng/mL)				
Mean±SD	1.27±0.57	1.59±1.25	0.86±0.46	1.15±1.18
Median	1.07	1.20	0.78	0.78
Range (min-max)	[			]

After 14-day multiple dosing, the % reductions in $AUC_{(0-24)}$ for the application with washing versus that application without washing was 23% for unadjusted E_2, 12% for unadjusted E_1, and 17% for unadjusted E_1 sulfate. After 14-day multiple dosing, the % reductions in C_{max} were 25% for unadjusted E_2, 13% for unadjusted E_1, and 20% for unadjusted E_1 sulfate. In general, washing the application site 1 hour postdose decreases about 25% of the unadjusted E_2 exposure.

Mean Concentration-Time Profiles for Baseline Non-Adjusted and Adjusted Estradiol in Non-Dosed Subjects After a Single Gel Application



Mean Concentration-Time Profiles for Baseline Non-Adjusted and Adjusted Estradiol in Non-Dosed Subjects After Multiple Gel Applications for 14 Days



Pharmacokinetic Results After Single-Dose Administration: Non-dosed Subjects

Parameters	Baseline (N=24)	Unadjusted (N=24)		Baseline Adjusted (N=24)	
		Washed	Not Washed	Washed	Not Washed
Estradiol					
$AUC_{0\text{-}24}$ (pg•hr/mL)					
Mean±SD	126.57±61.79	114.64±58.15	114.83±60.89	-11.73±32.72	-11.74±30.93
Median	128.38	92.13	108.50	0.00	-4.25
Range[a]	[				]
C_{max} (pg/mL)					
Mean±SD	7.38±3.55	7.48±3.48	6.92±3.42	2.98±2.36	2.71±2.53
Median	8.00	8.00	7.00	2.75	2.50
Range[a]	[				]
Estrone					
$AUC_{0\text{-}24}$ (pg•hr/mL)					
Mean±SD	510.46±262.69	506.58±270.47	502.06±211.97	-0.06±122.21	-8.69±123.42
Median	502.75	467.75	425.13	-4.75	-11.50
Range[a]	[				]

Pharmacokinetic Results After Single-Dose Administration: Non-dosed Subjects

Parameters	Baseline (N=24)	Unadjusted (N=24)		Baseline Adjusted (N=24)	
		Washed	Not Washed	Washed	Not Washed
C_{max} (pg/mL)					
Mean±SD	29.75±13.81	30.79±12.25	29.92±8.90	11.08±7.77	10.81±9.73
Median	28.50	28.00	28.50	10.50	8.50
Range[a]	[				]
Estrone sulfate					
$AUC_{0\text{-}24}$ (ng•hr/mL)					
Mean±SD	11.45±4.01	10.87±4.05	10.94±3.89	-0.57±1.80	-0.50±1.68
Median	10.97	10.28	10.16	-0.95	-0.16
Range[a]	[				]
C_{max} (ng/mL)					
Mean±SD	0.59±0.21	0.55±0.21	0.56±0.19	0.10±0.08	0.10±0.07
Median	0.55	0.53	0.52	0.11	0.10
Range[a]	[				]

[a] Range = min-max

Pharmacokinetic Results After Multiple-Dose Administration: Non-dosed Subjects

Parameters	Baseline (N=24)	Unadjusted (N=24)		Baseline Adjusted (N=24)	
		Washed	Not Washed	Washed	Not Washed
Estradiol					
$AUC_{0\text{-}24}$ (pg•hr/mL)					
Mean±SD	126.57±61.79	112.02±54.54	127.52±66.60	-14.55±31.17	0.95±35.75
Median	128.38	107.75	125.75	-10.25	5.88
Range[a]	[				]
C_{max} (pg/mL)					
Mean±SD	7.38±3.55	6.69±3.46	7.85±3.28	2.17±2.01	3.48±2.64
Median	8.00	7.00	8.00	2.00	4.00
Range[a]	[				]
Estrone					
$AUC_{0\text{-}24}$ (pg•hr/mL)					
Mean±SD	510.46±262.69	475.93±231.53	529.00±225.27	-32.84±93.47	20.08±147.65
Median	502.75	447.00	535.00	-20.13	-3.75
Range[a]	[				]
C_{max} (pg/mL)					
Mean±SD	29.75±13.81	27.52±12.10	32.21±10.19	9.00±7.54	13.77±9.27
Median	28.50	25.50	30.50	7.50	12.00
Range[a]	[				]
Estrone sulfate					
$AUC_{0\text{-}24}$ (ng•hr/mL)					
Mean±SD	11.45±4.01	10.80±3.74	11.44±4.29	-0.64-2.10	-0.00-2.30
Median	10.97	9.93	11.18	-0.26	-0.01
Range[a]	[				]

Pharmacokinetic Results After Multiple-Dose Administration: Non-dosed Subjects

Parameters	Baseline (N=24)	Unadjusted (N=24)		Baseline Adjusted (N=24)	
		Washed	Not Washed	Washed	Not Washed
C_{max} (ng/mL)					
Mean±SD	0.59±0.21	0.55±0.17	0.58±0.21	0.10±0.11	0.11±0.10
Median	0.55	0.53	0.52	0.10	0.11
Range[a]	[				]

[a] Range = min-max

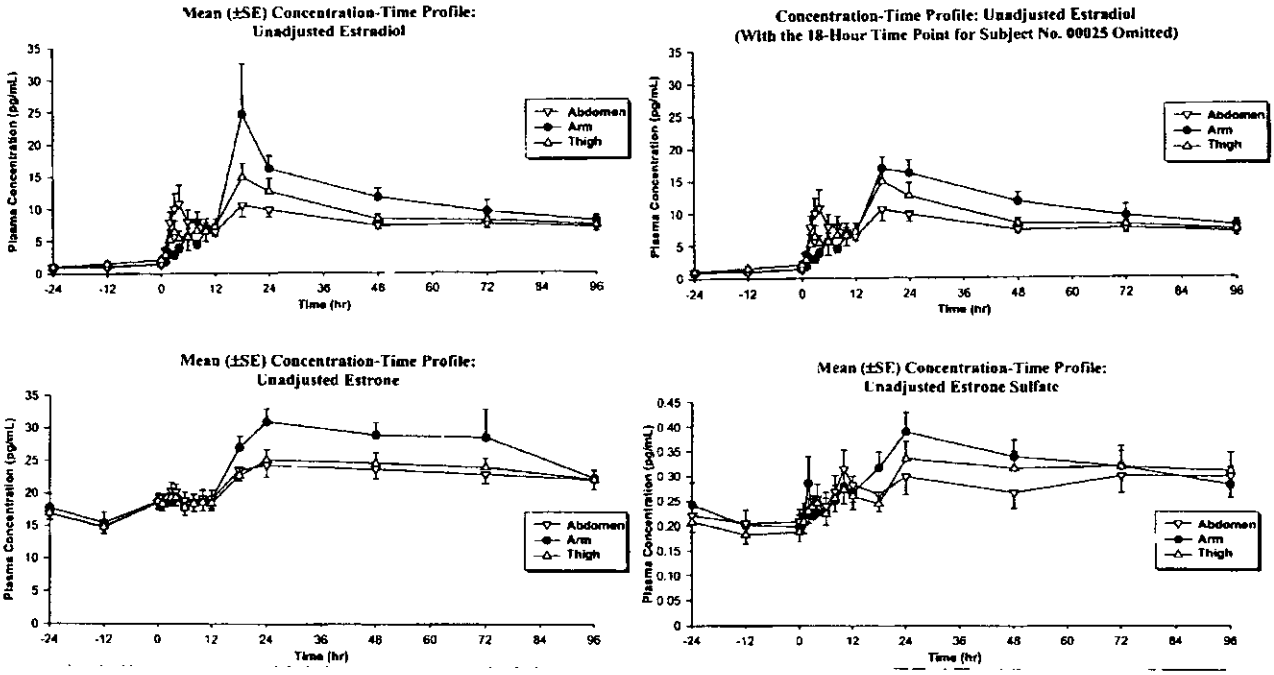
None of the $AUC_{(0\text{-}24)}$ values for E_2, E_1, and E_1 sulfate for the naïve patients after single and multiple dosing were statistically increased from baseline value. None of the corresponding C_{max} values for the naïve patients were statistically different from the baseline value with the exception of the value for E_1 sulfate during the non-washed period, where the difference from baseline was negative. These results suggest that there was either no transfer of the E_2 gel or the transferred E_2 gel did not significantly

increase the E_2 exposure from baseline in the non-dosed subjects after 15 minutes skin-to-skin contact with dosed subjects.

The sponsor confirmed via NDA 21-166 N-000-BM on January 15, 2004 that the formulation used in Study UMD-01-078 was the to-be-marketed formulation.

17. What are the relative BA of ESTROGEL® from different application sites?

Study UMD-00-073 examined the relative BA of the topical E_2 gel from 3 application sites in this single-dose, 3-period, crossover study. Forty eight randomized healthy postmenopausal women percutaneously applied 1.25 g of 0.06% E_2 gel to the surface of: 1 arm (wrist to shoulder; reference), lower abdomen (waistline to proximal pubic bone and from side to side to the hip bones), and 1 inner thigh (knee to top of thigh) in 3 different occasions. A 14-day washout separated each occasion. Serial plasma samples were collected for 96 hours postdose to determine E_2 and E_1 and E_1 sulfate concentrations. Prior to venipuncture, the sampling sites were washed with soap and water and dried thoroughly. Alcohol wipes were not used to cleanse the sampling sites. The phlebotomist put on fresh gloves before collecting blood samples from subjects.



The sponsor did not calculate the PK parameters for E_2, E_1, and E_1 sulfate due to the number of plasma drug concentrations that were below the LOQ. As an example, the following table shows the time points at which plasma E_2 concentrations were above the LOQ in 2-third of the subjects for all samples.

Summary of Unadjusted Estradiol by Application Site and Time Point: Time Points With >32 Subjects With Values >LOQ

Application Site	Time Point (Hour)	N	Unadjusted Estradiol (pg/mL) Mean±SD	Median	Range	CV (%)
Arm	18	45	26.221±56.198	14.10	[	214.32
	24	44	17.447±13.881	12.50		79.56
	48	40	12.876±9.749	10.05		75.71
	72	32	10.155±10.410	7.34		102.51
Abdomen	18	36	12.588±12.222	8.19		97.10
	24	33	11.301±6.705	9.20		59.33
Inner thigh	18	38	15.304±13.490	9.96	]	88.14

Application of 1.25 g 0.06% E_2 gel to the arm, abdomen, and inner thigh resulted in small elevation of plasma E_2 concentrations from baseline. Application to the arm resulted in higher elevation of plasma E_2 concentrations than those applying to abdomen and inner thigh. Study UMD-00-073's observed plasma E_2 concentrations were lower than those for Study S1661002 (Question 12 above).

Study UMD-01-078 used the to-be-marketed E_2 gel formulation. The formulation used in Study UMD-00-073 was the same formulation used in Study UMD-01-078 (same strength, batch, lot, and expiration date).

The sponsor conducted Study UMD-00-073 to seek alternative application sites. The pivotal Studies CV141-001 and CV141-002 used the arm from wrist to shoulder as the application site. The application site on the proposed product labeling is also the arm from wrist to shoulder.

18. Why would about 2000 cm^2 of skin area (wrist to shoulder) be needed for ESTROGEL® administration?

Study 92 OGEL 01 compared the effect of different surface area of OESTROGEL® topical application on E_2 BA. Twelve healthy postmenopausal women participated in this randomized, multiple dose crossover study. Each woman applied daily 2.5 g OESTROGEL® for 7 days on 3 areas with at least 1-week washout separating each treatment:

A. 750 cm^2 precisely measured and clearly identified on the upper part of both arms delimiting an area of 375 cm^2 per arm.
B. 1,500 cm^2 precisely measured and clearly identified on both arms delimiting an area of 750 cm^2 per arm.
C. Application on both arms, forearms, and shoulders without precisely delimiting the surface area in advance. The actual surface area of application was measured to be 2,310 ± 174 cm^2 (mean ± SD).

Study 92 OGEL 01's report stated that surface area C was about 2250 cm^2 throughout the report, volume 1.12 of 1.83. However, the sponsor's Human Pharmacokinetics and Bioavailability summary on study 92 OGEL 01 stated that surface area C was 2,310 ± 174 cm^2, volume 1.11 of 1.83.

The E_2 gel used in Study 92 OGEL is the to-be-marketed formulation (per NDA 21-166 N 000 BZ, June 16, 2003). Plasma E_2 and E_1 concentrations were measured via RIA after [ ] Study 92 OGEL 01's report did not state whether the results in the table below was baseline adjusted or unadjusted.

Influence of the Skin Surface Area on E_2 Transdermal Absorption

Parameter	Skin Surface Area		
	A 750 cm^2	B 1500 cm^2	C ≈ 2310 cm^2
Cmax[a] (pg/mL)	172.7 ± 186.6	206.8 ± 168.3	250.3 ± 167.3
AUC(0-24)[b] (pg•hr/mL)	1519.2 ± 878.7	1911.0 ± 1128.7	2674.0 ± 1500.9
Cave[c] (pg/mL)	63.3 ± 36.6	79.6 ± 47.0	111.4 ± 62.5
CV of AUC(0-24)	57.8%	59.1%	56.1%
Estradiol/Estrone	0.69	0.77	1.00

[a] Cmax = Maximum plasma concentration
[b] AUC(0-24) = Area under the plasma concentration vs time curve, from time 0 to 24 hours
[c] Cave = Mean average plasma concentration over a dosing interval

Since 2.5 g OESTROGEL® was used for all 3 treatment groups, the thickness of the gel on skin was inversely related to the surface area of application. Based on the E_2 AUC(0-24)s, the maximum E_2 BA was associated with the largest surface area of application. Decreasing the surface of application by 35% to 1,500 cm^2 resulted in no statistically significant decrease ($p > 0.05$) in BA. However, a statistically significant decrease ($p < 0.05$) in mean AUC(0-24)s was observed when the surface of application was reduced from surface area C (2,310 cm^2) to A (750 cm^2). No statistically significant difference ($p > 0.05$) in BA was observed when the surface area was decreased from 1,500 to 750 cm^2. Hence, ESTROGEL® absorption depended on application surface area. E_2 inter-individual coefficient of variation was about 57% and E_2 intra-individual coefficient of variation was about 50%, irrespective of the application surface area.

19. Why does the lowest ESTROGEL® dose not work?

Study S1661003 (question 13 above) showed that average plasma E_2 concentrations at steady-state ranged from 19.08 to 21.37 pg/mL upon the administration of 1.25 g of 0.03% or 0.625 g of 0.06% ESTROGEL®. This average plasma E_2 concentration range for the lowest ESTROGEL® dose (0.375 mg) tested is below the average serum E_2 concentration of 27 pg/mL upon administration of 1 low dose E_2 transdermal therapeutic system (FEMPATCH®, 0.025 mg E_2/day 7-day, PDR 1998 ed. p2100). Moreover, study CV141-002 (question 12 above) showed that median serum E_2 concentrations at Weeks 4 to 12 ranged from 23.5 to 29.5 pg/mL for 1.25 g of 0.03% ESTROGEL®, 31.0 to 42.0 pg/mL for 1.25 g of 0.06% ESTROGEL®, and 59.5 to 70.0 pg/mL for 2.5 g of 0.06% ESTROGEL®. The sponsor claimed that unexpectedly high serum E_2 concentrations were observed in study CV141-002.

20. How does the PK of ESTROGEL® differ in special populations?

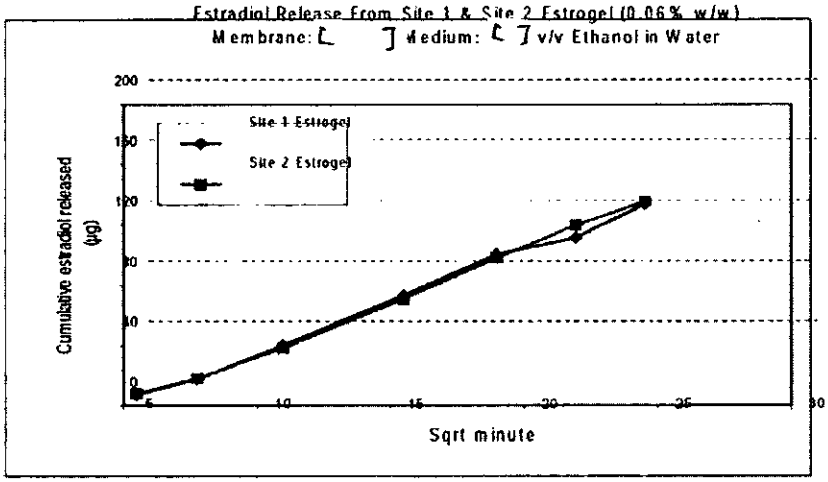
The sponsor did not study ESTROGEL® in any special populations such as obese, ethnic, renally and hepatically impaired patients. However, the sponsor attempted to use non-linear mixed effect modeling (NONMEM) techniques to analyze the serum E_2 and E_1 concentrations data for Studies CV141-001 and CV141-002. The observed high serum E_2 concentrations (> 500 pg/mL) prevented further NONMEM analysis or stratification with selected demographic variables.

21. What are the proposed in vitro method and specifications for ESTROGEL®?

The sponsor did not propose any in vitro E_2 release method nor specifications for ESTROGEL® per NDA 21-166 N 000 BZ on June 16, 2003. Per End of Phase II meeting request, the sponsor conducted Study 9566.01.01 to compare the in vitro release characteristics between the Bristol-Meyers Squibb ESTROGEL® formulation (0.06% E_2, [ ] ethanol; lot B94D001-1; designated as site 1 Estrogel®) and the Besins Iscovesco ESTROGEL® formulation (0.06% E_2, [ ] ethanol; lot 324; designated as site 2 Estrogel®). In vitro release method for Study 9566.01.01 follows:

Apparatus: [redacted]

Receptor: 7 mL at 32 ± 2°C
Receptor medium: [redacted] ethanol in water
[redacted] rpm (per NDA 21-166 N 000 BZ, June 16, 2003)
Weight of ESTROGEL® tested: about 350 mg
Amount of E_2 released to the receptor medium was analyzed via validated HPLC method. The sponsor plotted the mean cumulative E_2 released (µg) versus square root of time (minute$^{1/2}$).



Individual slopes of these plots were estimated and provided in the January 21, 2000 NDA 21-166 B2 amendment. The 8th and 29th ordered individual ratios of the slope for the Bristol-Meyers Squibb ESTROGEL® formulation over the slope for Besins Iscovesco ESTROGEL® formulation were the lower and upper limits, respectively, of the 90% confidence interval for the ratio of the median in vitro release rate (slope) for the Bristol-Meyers Squibb ESTROGEL® formulation over the median in vitro release rate (slope) for Besins Iscovesco ESTROGEL® formulation. The confidence interval was 92.8 - 110.3% for the slope estimate that included 3 time points (10.95, 15.49, and 18.97 min$^{1/2}$) and 85.1 - 106.6% for the slope estimate that included 5 time points (10.95, 15.49, 18.97, 21.91, and 24.49 min$^{1/2}$). Both confidence intervals fell within the 75 - 133.33% limits per Nonsterile Semisolid Dosage Forms Guidance. Therefore, the in vitro E_2 release rate for the Bristol-Meyers Squibb ESTROGEL® formulation was similar to the in vitro E_2 release rate for Besins Iscovesco ESTROGEL® formulation.

The sponsor determined the E_2 solubility in [redacted] v/v ethanol aqueous solution as 309.1 µg/mL. The amount of ESTROGEL® applied on the [redacted] membrane of each [redacted] cell was about 350 mg. Therefore, 210 µg E_2/350 mg (0.06% w/w E_2) ESTROGEL® would be the maximal theoretical E_2 amount released to the receptor medium. The receptor volume was 7 mL. Hence, the maximal theoretical E_2 concentration in the receptor medium would be 30 µg/mL. Sink condition ([redacted] of E_2 solubility in receptor medium; 309.1 µg/mL x 0.2 = 61.8 µg/mL) needed to be maintained in order for the in vitro release system to be valid. Per Nonsterile Semisolid Dosage Forms Guidance, in vitro hydroalcoholic receptor release medium for sparingly water soluble drugs is appropriate. Solubility of E_2 is [redacted] mg/dL in 0.05 I phosphate buffer at 37°C and [redacted] g/dL in [redacted] v/v ethanol at 40°C (Analytical Profiles of Drug Substances, **15**:283 1985). Although the [redacted] v/v ethanol does not resemble physiological medium, the sponsor's justification (page 035 of the January 21, 2000 NDA 21-166 B2 amendment) for the [redacted] v/v ethanol in water as the receptor medium to assess the

sameness of E_2 release between the Bristol-Meyers Squibb ESTROGEL® formulation and the Besins Iscovesco ESTROGEL® formulation is acceptable.

Lack of proposed in vitro E_2 release test and specifications for ESTROGEL® is acceptable per Nonsterile Semisolid Dosage Forms Guidance (the development and validation of an in vitro release test are not required for semisolid drug products' NDA approval). However, the sponsor is encouraged to develop an in vitro estradiol release test and define the estradiol release specifications for ESTROGEL® postapproval changes evaluation.

22. What are the ESTROGEL® Clinical Pharmacology section labeling comments?

The following comments are based on the proposed labeling (relevant for clinical pharmacology) submitted on December 10, 2003, the 1E Rev 11/2003 version. Strikethrough text means recommended deletion. Single underscore text means recommended addition. Double underscore text means annotation for the recommendation and does not need to be communicated with the sponsor.

3 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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/s/

S.W. Johnny Lau
2/6/04 01:28:26 PM
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